DOWN-REGULATION OF PERIPHERAL α_2-PRESYNAPTIC RECEPTORS ON CHRONIC ANTIDEPRESSANT THERAPY

J.P.M. Finberg & A. Tal, Rappaport Medical Research Center, Department of Pharmacology, Faculty of Medicine, Technion, P.O.Box 9649, Haifa, Israel.

Chronic administration of tricyclic antidepressants or monoamine oxidase (MAO) inhibitors in rats reduces binding site density for ^{3}H-clonidine and ^{3}H-yohimbine in cerebral cortex (Smith et al, 1981; Campbell & McKernan, 1982). Clonidine induced responses of behavioural hypoactivity and reduction in central amine turnover are also decreased by chronic tricyclic antidepressant treatment (Green et al, 1982; Sugrue, 1981). Down-regulation of platelet α_2 receptors in humans treated chronically with tricyclic antidepressants has been reported by Smith et al (1982).

The present work was undertaken to study functional responsiveness to clonidine in a peripheral noradrenergic system following chronic antidepressant treatment. Rats were injected daily for 21 days with desmethylimipramine (10mg/kg), tranylcypromine (5mg/kg), clorgyline (2mg/kg) or deprenyl (1mg/kg). The doses of clorgyline and deprenyl chosen were those which have been shown to selectively inhibit MAO type A and B respectively (Waldmeier et al, 1981). Tranylcypromine at the dose indicated fully inhibits both forms of MAO.

The animals were killed 24h after the last dose and the vasa deferentia suspended in organ baths for electrical field stimulation (0.1 Hz, 1msec, 0.22A). Cumulative dose response curves to clonidine were obtained on the stimulated organ, after which non-cumulative dose-response curves to noradrenaline were determined on the non-stimulated organ. Control EC_{50} values for clonidine were 6.8±0.78 (SD) nM and for noradrenaline 3.1±0.9μM. Clonidine EC_{50} was increased by chronic desmethylimipramine to 16.0±6.1, by tranylcypromine to 12.3±3.3 and by clorgyline to 17.8±5.2. All these changes are highly significant ($P<0.001$). No significant change occurred in clonidine EC_{50} following chronic deprenyl treatment, or in noradrenaline EC_{50} values following any of the chronic treatments. There were no changes in responsiveness to either agonist 24h after a single dose of desmethylimpramine or clorgyline, whereas a significant increase in clonidine EC_{50}, with no change in noradrenaline EC_{50}, was seen 24h after a single injection of tranylcypromine. These results provide further evidence for a similarity between the pharmacological effects of monoamine oxidase inhibitors and tricyclic antidepressants on aminergic neurotransmission, and also correlate with clinical data showing greater antidepressant effectiveness of type A as opposed to type B monoamine oxidase inhibitors (Youdim & Finberg, 1982).

Campbell, I.C. & McKernan, R.M. (1982) Br.J.Pharmac. 75, Proc. Suppl., 100P.
Green, A.R. et al (1982) Br.J.Pharmac. 75, Proc. Suppl., 33P.
Smith, C.B. et al (1981) Brain Res. 210, 413-418.
Smith, C.B. et al (1982) In: Briley, M et al "New Vistas in Depression" pergamon Press, Oxford.
Sugrue, M.F. (1981) Br.J.Pharmac. 74, 760P-761P.
Waldmeier, P.C. et al (1981) In: Youdim, M.B.H. & Paykel, E.S. "Monoamine Oxidase Inhibitors - The State of the Art" Wiley, Chichester.
Youdim, M.B.H. & Finberg, J.P.M. (1982) In: Grahame-Smith, D.G. et al "Psychopharmacology, Excerpta Medica, Amsterdam.

HOW SELECTIVE IS RAUWOLSCINE FOR α_2-RECEPTORS IN VIVO?

P.E. Hicks and C. Waldron*, Department of Pharmacology, Materia Medica and Therapeutics, University of Manchester M13 9PT

Rauwolscine is believed to be a selective α_2-adrenoceptor antagonist (Wietzell et al, 1979; Timmermans et al, 1980). Recent evidence suggests that both yohimbine and rauwolscine possess significant α_1-adrenoceptor blocking activity, in vivo (Shepperson et al, 1981). Furthermore, we have been unable to demonstrate selectivity of these drugs at postsynaptic α_2-receptors. This has prompted us to examine the antagonist time course of rauwolscine and yohimbine in the rat.

Male Sprague Dawley (SD) or Wistar (W) rats (250-350 g, n = 6-10) were pithed under pentobarbitone anaesthesia (60 mg/kg; i.p.) and respired with room air. Single submaximal doses of the α_1-receptor agonists phenylephrine (PE 10 µg/kg; i.v. ΔDBP 70±7 mmHg) or methoxamine (ME, 50 µg/kg; i.v. ΔDBP 56±5 mmHg) or the α_2-receptor agonist TL99 (10 µg/kg; i.v. ΔDBP 52±4 mmHg), were given before and at 5, 30, 60 and 90 min after administration of the antagonist (1 mg/kg i.v.) or vehicle. At this dose, rauwolscine was a competitive antagonist against TL99 and PE. α-adrenoceptor blockade, expressed as % inhibition of control DBP responses, by rauwolscine is shown (Fig. 1). Vasoconstriction induced by angiotensin II (0.1 µg/kg i.v.) was unaffected by rauwolscine. Recovery of the pressor responses to α_1-receptor agonists was significantly faster (P=0; analysis of variance) than responses to TL99, but in vehicle treated rats, pressor responses to TL99 were significantly (P<0.05) enhanced after 90 min. Yohimbine (1 mg/kg; i.v. 5 min) antagonised TL99 and PE induced pressor responses by 73±2 and 64±3% respectively, however the recovery of these pressor effects was significantly slower (P<0.005) than after rauwolscine.

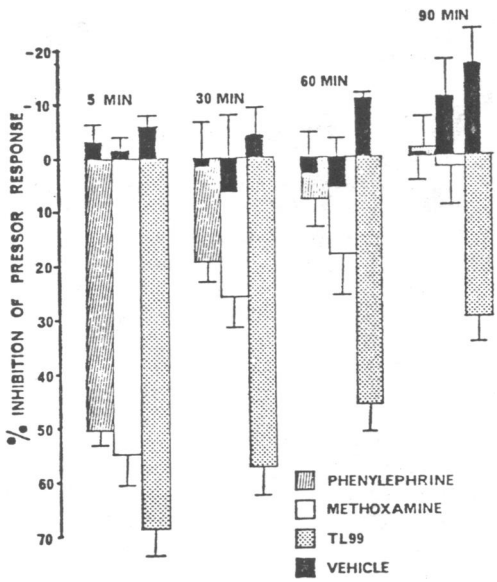

Figure 1. Antagonist effects of rauwolscine (1 mg/kg; i.v.) in Sprague-Dawley rats against α-receptor agonists.

Neither the pressor responses to α_1 or α_2-receptor agonists, nor the antagonist effects of rauwolscine(5 min) were significantly different in W or SD rats, although recovery of the responses to PE, but not to ME or TL99 was significantly (P=0) slower in W rats.

These results demonstrate that both yohimbine and rauwolscine possess significant postsynaptic α_1- and α_2-receptor blocking activity, however, the degree of selectivity of rauwolscine at postsynaptic α_2-receptors, in vivo can vary with the antagonist contact time.

Shepperson, N.B. et al (1981) J. Pharm. Exp. Ther. 219, 540-546
Timmermans, P.B.M.W.M. et al (1980) J. Auton. Pharmac. 1, 53-60
Wietzell, R. et al (1979) Naunyn Schmiedebergs Arch. Pharmacol. 308, 127-136

MODULATION OF NORADRENALINE RELEASE IN THE RABBIT THROUGH α-ADRENOCEPTORS

L. Hedler, H. Majewski*, L.C. Rump & K. Starke, Pharmakologisches Institut, University of Freiburg, Hermann-Herder-Strasse 5, D-7800 Freiburg, Federal Republic of Germany

Experiments with rabbit isolated tissues have unequivocally shown that noradrenaline release from sympathetic nerves is modulated through inhibitory presynaptic alpha-adrenoceptors (Starke, 1977). Experiments with conscious rabbits (Hamilton et al, 1982) have shed some doubt as to whether such a mechanism operates in vivo. In the present study selective $alpha_1$- and $alpha_2$-adrenoceptor blocking drugs (corynanthine HCl and yohimbine HCl/rauwolscine HCl respectively, each in a dose of 1 mg/kg i.v.) were used to determine whether noradrenaline release in vivo is, as in vitro, modulated by noradrenaline activation of inhibitory presynaptic $alpha_2$-adrenoceptors. In all experiments the noradrenaline release rate into the plasma was determined according to Majewski et al (1982). The effect of drugs was determined 20 min after drug administration in the case of conscious and anaesthetized rabbits and after 30 min in pithed rabbits.

In the conscious rabbit yohimbine and rauwolscine enhanced the noradrenaline release rate to 256.9 + 46.5% (n=6) and 424.2 + 120.1% (n=4) of control respectively. Corynanthine had no effect (98.2 + 13.3%, n=5, of control). Rauwolscine and yohimbine increased blood pressure whereas corynanthine had no effect. These results are consistent with noradrenaline release being modulated through inhibitory presynaptic $alpha_2$-adrenoceptors but central effects of the drugs cannot be excluded. The animals exhibited discomfort after drug administration.

In order to reduce some of the side-effects of the drugs, the experiments were repeated in the pentobarbitone anaesthetized rabbit. In this model corynanthine, and to a lesser extent rauwolscine, produced falls in blood pressure; yohimbine having no effect. Yohimbine enhanced the noradrenaline release rate to 187.8 + 34.5% (n=7) of control. The two other drugs were compared to equihypotensive doses of the vasodilators hydralazine HCl (0.1-1.25 mg/kg) and sodium nitroprusside (1-10 ug/kg per min), to take into account baroceptor effects due to the fall in blood pressure. Rauwolscine increased the noradrenaline release rate to 160.3 + 22.0% (n=8) of the vasodilator controls whereas corynanthine slightly inhibited release to 68.4 + 7.1% (n=6) of the vasodilator controls.

The pithed rabbit preparation was then used to exclude possible central effects of the drugs. The rabbits were pithed and the brain destroyed. The spinal sympathetic outflow at approximately T8 was stimulated continuously at 3 Hz with square wave pulses of 0.5 ms duration and supramaximal current for increasing blood pressure (70-150 mA). Gallamine triethiodide (3 mg/kg i.v.) was used to prevent skeletal muscle contraction. Yohimbine and rauwolscine enhanced the noradrenaline release rate to 146.8 + 20.5 (n=9) and 217.2 + 46.8% (n=5) of control respectively. Corynanthine had no effect (101.0 + 10.2%, n=6, of control).

In all three models the $alpha_2$-adrenoceptor blocking drugs enhanced the noradrenaline release rate, presumably due to the blockade of noradrenaline activation of inhibitory presynaptic $alpha_2$-adrenoceptors at sympathetic nerve endings. This suggests that presynaptic alpha-adrenoceptors have functional significance in vivo.

Dr. Majewski is a C.J. Martin Research Fellow of the National Health & Medical Research Council of Australia.

Hamilton, C.A. et al (1982) Br. J. Pharmac. 75, 417-424.
Majewski, H. et al (1982) Naunyn-Schmiedeberg's Arch. Pharmac. 321, 20-27.
Starke, K. (1977) Rev. Physiol. Biochem. Pharmac. 77, 1-124.

SUBCLASSIFICATION OF SMOOTH MUSCLE α-ADRENOCEPTORS OF THE RAT TAIL ARTERY

S.Z. Langer and I.C. Medgett*, Department of Biology, Synthélabo-L.E.R.S., 58, rue de la Glacière, 75013 Paris, France.

The subclassification of vascular smooth muscle α-adrenoceptors requires rigorous in vitro pharmacodynamic analysis. The present communication describes the effects of RX781094, prazosin and corynanthine on responses to exogenous noradrenaline (NA) and periarterial electrical stimulation in rat isolated perfused tail arteries.

Sprague-Dawley rats weighing 170-220g were anaesthetised with pentobarbitone; the proximal 2-3 cm of the tail artery was removed, cannulated at both ends and perfused and superfused with oxygenated Krebs' solution at 37°C (Medgett and Rand, 1981). Cocaine (4 μM) and propranolol (1 μM) were present throughout. Three sets of concentration-response curves to the vasoconstrictor effects of NA (i.e. increases in perfusion pressure at constant flow), alternating with frequency-response (FR) curves to periarterial electrical stimulation (0.3ms pulses, 15V, 1-60 Hz to maximum response), were subsequently obtained. In each case a non-cumulative technique was used ; in control experiments all responses were highly reproducible. Maximal responses to NA and electrical stimulation were not significantly different to those of KCl (60mM : 195 ± 14 mmHg, n=35).

The pD_2 value of NA was 6.5 ± 0.1 (n=45) ; the frequency for half-maximal response to stimulation was between 3 and 10 Hz. RX781094 (10nM - 100 μM), prazosin (0.1 nM - 1 μM) and corynanthine (0.1 - 100 μM) each caused parallel, concentration-dependent rightward shifts of the NA curve, with no depression of the maximum response. For corynanthine the antagonism was competitive throughout the 1,000-fold concentration range used ; the pA_2 value was 6.6 ± 0.2 (n=6). For RX781094, the antagonism was competitive from 1 - 100 μM (pA_2 = 6.5 ± 0.1, n=6) whereas the shifts produced by 10 and 100 nM RX781094 yielded $-\log K_B$ values of 7.6 ± 0.3 (n=8) and 7.1 ± 0.1 (n=6), respectively.

For prazosin, the antagonism was competitive from 10 nM - 1 μM (pA_2 = 8.8 ± 0.1, n=6) whereas the effects of 0.1 and 1 nM prazosin yielded $-\log K_B$ values of 9.4 ± 0.3 (n=6) and 9.3 ± 0.2 (n=6), respectively. In addition, each of the three antagonists caused concentration-dependent unsurmountable rightward shifts of the FR curve. The response to stimulation was exquisitely sensitive to prazosin : 1 nM reduced the response (measured as the area under the FR curve) by 82 % but only caused a 0.3 log unit shift of the NA curve, whereas 1 μM corynanthine reduced the response to stimulation by 81 % but caused a 0.8 log unit shift of the NA curve. In contrast, RX781094 selectively antagonised the NA response : 10 μM RX781094 was required to reduce the response to stimulation by 83 % however, there was a 1.7 log unit shift of the NA curve ; the relative resistance of the response to electrical stimulation may result from the counteracting effect of blockade of prejunctional α_2-adrenoceptors (Medgett and Rand, 1981).

From these results it may be postulated that exogenous NA activates three subtypes of smooth muscle α-adrenoceptors in rat tail artery in vitro. The predominant subtype resembles an α_1-adrenoceptor : the $-\log K_{aff}$ values of prazosin, corynanthine and RX781094 are, respectively, 8.8, 6.6 and 6.5. A second distinct subpopulation of α_1-adrenoceptors may be identified by a $-\log K_{aff}$(prazosin) > 9.4 ; possibly endogenous NA preferentially activates this subtype. A third subpopulation of α-adrenoceptors may be identified by a $-\log K_{aff}$(RX781094) > 7.6 ; such a value (Chapleo et al., 1981) would suggest an α_2-adrenoceptor.

Chapleo, C.B. et al, (1981), Br. J. Pharmacol. 74, 842P.
Medgett, I.C. & Rand, M.J. (1981), Clin. Exp. Pharmacol. Physiol., 8, 503-507.

PRAZOSIN EXHIBITS α_1, BUT NOT POSTSYNAPTIC, SELECTIVITY IN THE PITHED RAT

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

In the pithed rat, pressor responses are mediated by both alpha$_1$- and alpha$_2$-adrenoceptors (Docherty & McGrath,1980). Cardiac presynaptic alpha-receptors are presumed to be entirely alpha$_2$, although the alpha$_1$-selective antagonist prazosin has some potency as a presynaptic antagonist (Cavero et al.,1979; Docherty & McGrath,1980). The purpose of the present investigation is to determine whether this presynaptic action of prazosin is due to loss of selectivity when used in higher doses, or indicative of the presence of presynaptic alpha$_1$-receptors, as suggested previously by Kobinger & Pichler (1980).

In pithed rats, presynaptic alpha-agonism was assessed against the cardioaccelerator response to a single stimulus pulse, and postsynaptic alpha-agonism as the rise in diastolic blood pressure (DBP) produced by the drug. All agonists examined produced dose-dependent inhibition of the cardioaccelerator response and dose-dependent pressor responses. Postsynaptic/presynaptic dose ratios (dose producing rise in DBP of 50mmHg divided by dose producing 50% inhibition of the cardioacceleration to a single pulse) were 40.3, 18.9, 5.3 & 0.57 for xylazine, clonidine, cirazoline and amidephrine, respectively; xylazine showed greatest presynaptic, and amidephrine greatest postsynaptic, selectivity.

Prazosin (0.1 & 1mg/kg) produced large shifts in the pressor dose/response curves of amidephrine and cirazoline, a smaller shift in that of clonidine, and failed to shift the dose/response curve of xylazine. Yohimbine (1mg/kg) produced shifts in the pressor dose/response curves of all agonists, but yohimbine (0.1mg/kg) failed to shift the pressor dose/response curves of amidephrine and cirazoline. Yohimbine (1mg/kg) produced large shifts in the cardio-inhibitory dose/response curves of xylazine and clonidine but only small shifts in those of cirazoline and amidephrine. Prazosin (1mg/kg) produced a small shift in the cardio-inhibitory dose/response curve of clonidine, larger shifts in those of cirazoline and amidephrine, but failed to shift the dose/response curve of xylazine. The combination of yohimbine and prazosin produced a larger shift in the cardio-inhibitory dose/response curve of cirazoline than did either alone.

Amidephrine, cirazoline and, to a lesser extent, clonidine produced small rises in basal heart rate, and this rise was prevented by prazosin (1mg/kg). To minimise the influence of drug-induced rises in basal heart rate on the cardio-inhibitory effects of the drug, further experiments were carried out employing stimulation with 10 pulses at a frequency of 1Hz which produced a cardioacceleration of $82 \pm 8.6 min^{-1}$ as compared to $24 \pm 1.4 min^{-1}$ to a single stimulus pulse. In this situation, the cardio-inhibitory effects of xylazine were antagonised by yohimbine (1mg/kg) but not by prazosin (1mg/kg), whereas prazosin (1mg/kg) antagonised the cardio-inhibitory effects of amidephrine and cirazoline.

In conclusion, no evidence was found for alpha$_2$-antagonism by prazosin, since it did not alter responses to xylazine, but prazosin did exhibit presynaptic potency against alpha$_1$-agonists. Hence, the lack of postsynaptic selectivity of prazosin in these experiments can be explained by the presence of presynaptic alpha$_1$-adrenoceptors.

Cavero,I. et al. (1979) Br.J.Pharmac. 67, 283-292
Docherty,J.R. & McGrath,J.C. (1980) Naunyn-Schmiedeberg's Arch. Pharmac. 312, 107-116
Kobinger,W. & Pichler,L. (1980) Eur.J.Pharmac. 65, 393-402

CIRCADIAN VARIATION IN PAIN SENSITIVITY IN RATS MAY BE RELATED TO ALTERATIONS IN OPIATE RECEPTOR DENSITY

CA Hendrie*, V Nohria[1] and RJ Rodgers
Postgraduate Schools of Studies in Psychology and Pharmacology[1], University of Bradford, Bradford, BD7 1DP, UK.

Recently, there have been reports of circadian variation of, naloxone-induced hyperalgesia (Fredrickson et al, 1977), tolerance to painful stimuli, alterations in brain enkephalin levels (Wesche and Fredrickson, 1981) and density of rat brain opiate receptors (Naber et al 1980). There have also been reports of changes in the pain sensitivity of rats from a.m. to p.m. (Rosenfeld and Rice, 1979). However, none of these studies have attempted to correlate changes in the pain sensitivity of rats with alterations in opiate receptors per se.

The present study investigated whether a) pain latencies varied in a circadian manner and b) if so, whether alterations in opiate receptor followed the same pattern.

For experiment 1, 45 male hooded-Lister rats (310-380g) from Bradford University colony were housed in groups of 5 (lights on 0800/off 20.30 hrs), for 21 days prior to the start of experimentation. On test days animals were assayed for tail-flick latency (radiant-heat method) according to an independent measures design, so that data was obtained every 3 hrs over a 24 hr period. Results indicated there to be an upward trend in tail flick latency from 2100 to 0900 hrs and a downward trend from 0900 to 2100 hrs, there being a significant ($t(df=32)=3.18, p<0.05$) difference between these points.

For experiment 2, 10 male hooded-Lister rats (300-350g) from the same source were housed under exactly similar conditions to those used in experiment 1. On test days animals were sacrificed A.M. (55 min intervals, starting 8 am) or P.M. (55 min intervals, starting 8 pm) (n=5/gp). Whole brains(- cerebellum) were homogenized in 15mls of 50mM TRIS sucrose(0.32M) buffer, centrifuged (10,000 rpm at 4°C for 30min) and the pellet resuspended in TRIS buffer (25 vols). 0.5ml aliquots (in triplicate) of homogenate were incubated with ^{3}H-naloxone (0.41-6.6 nM) in the absence or presence of levorphan (10^{-6} M) for 20min at 25°C. Final incubation volume was 1ml. Bound radioactivity was separated from free using the rapid filtration method and determined using liquid scintillation procedure. Specific binding was defined as Total binding - binding in the presence of levorphan. Data for each animal were analysed by Scatchard method. Statistical analysis revealed there to be a significant difference ($t(df=8)=6.16$, $p<0.001$) between Bmax at AM (10.84±0.14pmols/g tissue) and PM(3.52±0.33pmols/g tissue), whilst the K_d was unchanged($t(df=8)=0.2$, N.S.; K_d (AM)=1.88 ± 0.52nM/K_d (PM)=2.0±0.27nM).

These data, together, clearly demonstrate that the time period when animals were most sensitive to noxious stimulation was also the time period when opiate receptor density was at its lowest. Whilst the mechanisms of circadian variation in pain sensitivity have yet to be elucidated, these data suggest that fluctuations in pain sensitivity may be related to alterations in opiate receptor density.

C.A.H. is supported by an MRC Research Studentship, V.N. gratefully acknowledges the support of the Parkinsons Disease Society.

Fredrickson RCA et al (1977), SCIENCE 198 756-758
Naber D et al (1980), NEUROSCI. LETT. 21 45-50
Rosenfeld JP and PE Rice (1979), PHYSIOL. BEHAV. 23 419-420
Wesche DL and RCA Fredrickson (1981), LIFE SCI 29 2199-2055

FURTHER INVESTIGATIONS INTO THE PROPERTIES OF AGONISTS FOR THE K OPIATE RECEPTOR

A. G. Hayes, R. G. Hill†, M. Skingle* & M. B. Tyers, Neuropharmacology Department, Glaxo Group Research Ltd., Ware, Hertfordshire.

Certain opiate analgesics (e.g. nalorphine, pentazocine and ketocyclazocine) thought to be agonists for the K receptor are more effective in rodent tests utilising pressure or chemical noxious stimuli than they are in tests utilising thermal noxious stimuli (Tyers, 1980). This is in contrast to the properties of known μ receptor agonists such as morphine and codeine which are effective in all three situations. We have extended these observations and have compared the putative K ligands BL 5572M, butorphanol, bremazocine and pentazocine with the μ ligands morphine and codeine.

The methods used were essentially those described by Skingle et al (1982) but, briefly, the paw pressure test was conducted in weanling rats (35-80g), and the acetylcholine-induced abdominal constriction and hot plate (55°C) tests were performed in the same adult mice (18-22g) so that direct comparisons could be made. The mouse rotarod test was used to determine the degree of motor-incapacitation produced by each drug. In all tests the operators were unaware of the drug treatments that the animals had received.

Antinociceptive potencies of opioid analgesic drugs against non-heat and heat induced nociception

Compound	Antinociceptive activity ED_{50} mg/kg s.c. (95% conf lim)			
	Rat Paw Pressure	Mouse ACh abdominal constriction test	Mouse Hot-plate test	Mouse Rotarod test
Bremazocine	0.007 (0.002-0.025)	0.008 (0.003-0.028)	3.2 (0.02-U.L)	0.74 (0.34-1.64)
BL 5572M	0.015 (0.001-0.143)	0.012 (0.003-0.038)	>90	83 (26-824)
Butorphanol	0.014 (0.001-0.13)	0.077 (0.024-0.24)	35.3 (25-48)	26 (12-64)
Pentazocine	1.0 (0.1-10.8)	1.4 (0.5-4.0)	28.1 (1.5-56)	40.2 (21-112)
Codeine	3.6 (2.1-5.2)	6.7 (2.2-21.1)	15.9 (4.3-47.7)	82.4 (36-206)
Morphine	0.38 (0.20-0.69)	0.45 (0.23-0.85)	1.7 (1.1-2.2)	14.2 (6.7-42.1)

It is clear that both μ- and K- agonists were effective in rat paw pressure and mouse abdominal constriction tests at doses many times lower than those needed to produce motor incapacitation. However, only the μ agonists, morphine and codeine, were clearly effective in the mouse hot plate test. Thus the observation that K agonists are more effective as antinociceptive agents against pain produced by mechanical and chemical noxious stimuli is likely to be generally applicable. This selectivity of action may stem from differences in the mechanisms by which the brain perceives pain originating from different sensory modalities.

Skingle, M. et al (1982) Life Sciences 11, 1123-1132
Tyers, M.B. (1980) Br.J.Pharmac., 69, 503-512

† Permanent address:- Department of Pharmacology, University of Bristol.

FURTHER IN VIVO EVALUATION OF ICI 154129, AN OPIATE δ-RECEPTOR ANTAGONIST

T. Priestley,* M.J. Turnbull and E. Wei[1], Department of Bioscience, ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG and [1]Department of Biomedical & Environmental Health Sciences, School of Public Health, University of California, U.S.A.

We have previously reported that ICI 154129 (N,N-bisallyl-Tyr-Gly-Gly-Ψ-(CH_2S)-Phe-Leu-OH) is a selective and competitive antagonist at the opiate δ-receptor in vitro, based on its ability to reverse the effects of opiate agonists on the mouse vas deferens and guinea-pig ileum preparations (Shaw et al., 1982). We have also shown that ICI 154129 is active as an antagonist of etorphine in a striatally-evoked rat head-turn test (Gormley et al., 1982), a system which has been proposed as an in vivo model of opiate δ-receptor function (Wheeler, 1982). The present experiments were undertaken to repeat the head-turn study using a peptide agonist and to demonstrate the selectivity of ICI 154129 in vivo for the opiate δ-receptor.

In our experiments with the head-turn model, we have evaluated the effect of ICI 154129 against the selective δ-receptor agonist, D-Ala^2, D-Leu^5-enkephalin (BW180C). The methodology used was exactly as described previously by Slater et al. (1980) and Wheeler (1982). When injected into the globus pallidus, BW180C (0.5μg) slowed the head-turn evoked by electrical stimulation of the striatum (pre-drug 2.8 ± 0.4; post-drug 6.3 ± 0.8 sec.(mean ± s.e.m.) $P < 0.05$). This was completely prevented by s.c. injection of ICI 154129 (30mg/kg) (pre-drug 3.1 ± 0.3; post-drug 3.4 ± 0.5 sec.).

In our studies on the selectivity of action we considered it important to demonstrate that ICI 154129 was inactive in test situations considered to be mediated via the opiate μ-receptor. In the rat tail flick and mouse 55°C hot-plate analgesic assays, ICI 154129 (30mg/kg s.c.) was inactive as an agonist and as an antagonist against morphine (hot plate) and etorphine (tail flick), whereas naloxone (0.5mg/kg s.c.) was an effective antagonist. ICI 154129 was also inactive as an antagonist against etorphine-induced locomotor activity in C57BL mice; again, naloxone prevented the effect of etorphine.

In a standard 2-lever operant drug discrimination procedure, in which animals were trained to recognise morphine (10mg/kg i.p.) from saline, ICI 154129 (50mg/kg s.c.) induced saline-appropriate responding and did not antagonise the morphine cue. Naloxone (1mg/kg i.p.) significantly antagonised morphine-induced cueing.

In morphine dependent rats (75mg morphine pellet for 3 days) ICI 154129 (30mg/kg s.c.) did not precipitate any signs of withdrawal (score = 0 when animals assessed according to the method of Wei (1973)). In contrast, naloxone (0.5mg/kg i.p.) induced the characteristic signs of opiate abstinence (Score 2.1; $P < 0.005$ in comparison with ICI 154129 treated rats).

Thus our results show that ICI 154129 is an opiate δ-receptor antagonist in the rat head-turn model. We have been unable to detect any antagonism at opiate μ-receptors in a number of other test situations.

Gormley, J.J. et al. (1982) Life Sciences (in the press)
Shaw, J.W. et al. (1982) Life Sciences (in the press)
Slater, P. et al. (1980) J. Pharmac. Methods 3, 39-49
Wei, E. (1973) Psychopharmac. 28, 35-44
Wheeler, H. (1982) Neuropharmac. (in the press)

PROSTAGLANDIN RELEASE IN PULMONARY ANAPHYLAXIS: PGD_2 A MARKER OF MAST CELL ACTIVATION IN SITU?

I.A. Blair, C.T. Dollery, M. Ennis, J.R.S. Hoult[1], C. Robinson* & K.A. Waddell, Department of Clinical Pharmacology, Royal Postgraduate Medical School, London W12 OHS and [1]Department of Pharmacology, King's College, London WC2R 2LS.

Anaphylactic reactions in guinea-pig lung result in the oxidative conversion of arachidonate to an array of potent mediators (Becker et al, 1981). Some of these, e.g. thromboxane A_2 are generated secondary to the release of others such as the leukotrienes (Piper & Samhoun, 1981) but PGD_2, the major mast cell-derived prostaglandin (Becker et al, 1981) may be released as an initial event in anaphylaxis. We have investigated whether PGD_2 could serve as an index of mast cell activation in situ under commonly employed experimental manipulations.

Lungs from ovalbumin-sensitized guinea-pigs (Robinson & Hoult, 1980) were artificially ventilated and perfused via the pulmonary artery at 10 ml/min with Krebs' solution. Anaphylaxis was induced by a bolus injection of 200 µg ovalbumin and the perfusate collected on ice for 9 min. Cyclooxygenase metabolites were extracted from aliquots of perfusate. Keto functions were converted to methoximes then carboxy and hydroxy functions derivatised to pentafluorobenzyl esters and trimethylsilyl ethers respectively. Analysis was performed by combined capillary GC negative chemical ionisation mass spectrometry (Waddell et al, 1982). Quantitative determinations were made by comparison of peak areas of the respective $[M-C_7H_2F_5]^-$ ion with that of an appropriate deuterated internal standard, viz: $[^2H_4]$-$PGF_{2\alpha}$ for $PGF_{2\alpha}$, $[^2H_4]$-PGE_2 for PGE_2 and PGD_2 and $[^2H_4]$-6-keto-$PGF_{1\alpha}$ for 6-keto-$PGF_{1\alpha}$, TXB_2 13,14-dihydro-15-keto-TXB_2 (TXDK) and 6,15-diketo-13,14-dihydro-$PGF_{1\alpha}$ ($K_2H_2F_{1\alpha}$). Histamine was quantified fluorimetrically. Results are expressed as ng per 90 ml sample (means ± s.e.mean).

Table 1 Prostaglandin release in pulmonary anaphylaxis

	$PGF_{2\alpha}$	PGD_2	PGE_2	TXB_2	6-$KF_{1\alpha}$	TXDK	$K_2H_2F_{1\alpha}$	Hist	n
Prechallenge	1.5± 0.5	1.9± 0.7	3.0± 0.8	20.3± 2.7	45.1± 7.3	54.6± 15.5	33.8± 7.4	78.9± 21.9	10
Anaphylaxis	7.7± 3.5	52.8± 16.1*	5.0± 1.6	500± 147*	132.3± 24*	1417± 204*	126± 37*	1951± 606*	10

(*P<0.05-0.001). FPL 55712†(10 µM) significantly reduced (n=5) 6-keto-$PGF_{1\alpha}$ release by 75%*; TXDK by 93%*; $K_2H_2F_{1\alpha}$ by 85%*and histamine by 71%*. TXB_2 release was down by 78% but PGD_2 release was unaffected. With a lower concentration of FPL 55712 (500 nM) TXDK release was down by 63%*, histamine by 83%* and TXB_2 by 77% (not significant). Again PGD_2 was unaffected. Mepyramine (10 µM) only significantly affected the release of $PGF_{2\alpha}$ and PGE_2; they rose by 186% and 176% respectively (5 lungs). In the presence of mepyramine and FPL 55712 together (both 10 µM), 6-keto-$PGF_{1\alpha}$ was decreased by 83%* and TXDK by 87%*.

The principal conclusions from this study are (a)anaphylaxis results in large increases in the synthesis and release of PGD_2, prostacyclin and thromboxane A_2 as well as of histamine;(b)thromboxane A_2 and prostacyclin release appear to be secondary to the release of leukotrienes as they are much reduced after treatment with FPL 55712;(c)histamine is not an important stimulus for arachidonate metabolism in guinea-pig anaphylaxis;(d)PGD_2 formation may be a useful index of mast cell activation in situ.

† FPL 55712, a recognised leukotriene antagonist

Becker, E.L. et al (1981) Biochemistry of the acute allergic reactions, A.R. Liss New York.
Piper, P.J. & Samhoun, M.N. (1981) Prostaglandins 21, 793-803.
Waddell, K.A. et al (1982) Biochem. Soc. Trans. In press.

PRODUCTION OF PROSTAGLANDINS I_2, $F_{2\alpha}$ AND E_2 BY THE AORTA AND VENA CAVA OF NORMOTENSIVE AND HYPERTENSIVE RATS

E Anne Lennon and N.L. Poyser, Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ.

Prostaglandin (PG) I_2, a vasodilator and inhibitor of platelet aggregation, has been implicated in the maintenance of vascular homeostasis. It has been shown to be synthesised by blood vessels *in vitro* (Moncada *et al*, 1977) and by aortic endothelial and smooth muscle cells in culture (Weksler *et al*, 1977). The production of other prostaglandins and their role in the vasculature is less well-defined.

The object of this study was to investigate the overall profile of prostaglandin production by the aorta and vena cava of the New Zealand genetically hypertensive rat (GH). PGI_2, (as 6-oxo-$PGF_{1\alpha}$), $PGF_{2\alpha}$ and PGE_2 were measured using radioimmunoassay (RIA).

Total synthetic capacity was determined in homogenates of blood vessels from male Wistar rats and age-matched male GH rats. After incubation for 90 min. with 2 μg/ml arachidonic acid (AA), prostaglandin production was in the order of 6-oxo-$PGF_{1\alpha}$ > $PGF_{2\alpha}$ >> PGE_2 for the aorta, but in the order of $PGF_{2\alpha}$ > 6-oxo-$PGF_{1\alpha}$ ≡ PGE_2 for the vena cava of the GH rats. There was a significant increase in $PGF_{2\alpha}$ production by the aorta ($p < 0.05$, t-test) in GH rats compared to Wistar rats.

To localise the site of the increase in $PGF_{2\alpha}$ production, aortas were separated into adventitia, smooth muscle and endothelium (as described by Moncada *et al*, 1977). The adventitia and smooth muscle layer were homogenised, and the endothelium was left as a cell suspension. Each layer was incubated for 60 min. with 2 μg/ml AA. Adventitia from GH rats produced less 6-oxo-$PGF_{1\alpha}$ than controls but no difference was found for $PGF_{2\alpha}$ and PGE_2 production. The smooth muscle layer from GH rats showed a decrease in 6-oxo-$PGF_{1\alpha}$ synthesis ($p < 0.05$) and a marked increase in $PGF_{2\alpha}$ synthesis ($p < 0.01$). 6-oxo-$PGF_{1\alpha}$ production by the endothelium showed no difference between the 2 groups but $PGF_{2\alpha}$ production was again greater in GH rats ($p < 0.001$) compared to Wistar rats.

The basal output of prostaglandins from the isolated, perfused aorta (5 ml/min, Krebs' soln) of Wistar and GH rats was determined. After a 30 min. equilibration period, perfusate was collected for 30 min, was extracted and was assayed by RIA. The profile of prostaglandin release from the aorta of Wistar rats was 6-oxo-$PGF_{1\alpha}$ > PGE_2 > $PGF_{2\alpha}$, while the profile from the aorta of GH rats was 6-oxo-$PGF_{1\alpha}$ > PGE_2 ≡ $PGF_{2\alpha}$. $PGF_{2\alpha}$, but not 6-oxo-$PGF_{1\alpha}$ and PGE_2, release was significantly greater ($p < 0.01$) from the aorta of GH rats compared to Wistar rats.

In conclusion, the aorta and vena cava of the GH rat showed a greater capacity to synthesise $PGF_{2\alpha}$ and this was reflected in an increased release of $PGF_{2\alpha}$ from the aorta. The finding that the smooth muscle layer of the aorta showed a decreased ability to synthesise PGI_2, a vasodilator, and a concomitant increase in the synthesis of $PGF_{2\alpha}$, a vasoconstrictor (Ducharme *et al*, 1968) suggests that there may be a disruption in the local control of vascular tone in the GH rat. Whether this contributes to, or is a result of the hypertension requires further investigation.

* E.A.L. was supported by an MRC studentship.

Ducharme, D.W. *et al* (1968) J. Pharmacol. Exp. Ther. 160: 1-10.
Moncada, S. *et al* (1977) Thromb. Res. 11:323-344.
Weksler, B.B. *et al* (1977) Proc. Natl. Acad. Sci. 74: 3922-3926.

THE EFFECT OF DRUGS ON THE SYNTHESIS OF 6 OXO PROSTAGLANDIN E_1

R.J. Griffiths*, F.J. Lofts & P.K. Moore, Dept. of Pharmacology, Chelsea College, Manresa Road, London SW3 6LX.

6 oxo prostaglandin E_1 (6 oxo PGE_1) is a biologically active metabolite of prostacyclin (PGI_2) produced by the enzyme prostaglandin 9 hydroxydehydrogenase (PG-9HDH) in kidney, liver and platelets. There have been few reports of the effect of drugs on the conversion of PGI_2 to 6 oxo PGE_1. Chang & Tai (1982) have shown that rutin inhibited 6 oxo PGE_1 formation by pig kidney. We have now studied the effect of rutin and two other flavone derivatives, quercetin and naringenin as well as indomethacin and sulphasalazine on the enzymes of prostaglandin synthesis and breakdown.

Enzymes tested were the PG-9HDH of rabbit kidney cortex (converts PGI_2 to 6 oxo PGE_1 and $PGF_{2\alpha}$ to PGE_2) and human platelet rich plasma (converts PGI_2 to 6 oxo PGE_1). Enzyme activity was estimated by determining the loss of extracted radioactivity following incubation with 9β ^{3}H labelled substrate. PG-9HDH activity of rat kidney (converts 13,14 dihydro 15 oxo $PGF_{2\alpha}$ to 13,14 dihydro 15 oxo PGE_2) and rabbit colon 15-hydroxydehydrogenase (15-PGDH) were measured by radiochromatography. Prostaglandin biosynthesis was measured by incubation of arachidonic acid with resuspended bovine seminal vesicle microsomes (BSVM) and reduced glutathione as cofactor. Prostaglandins synthesised were bioassayed on the rat stomach strip. These enzyme assays have been described in detail previously (Moore & Hoult, 1978, 1982).

Rutin, naringenin and quercetin inhibited 6 oxo PGE_1 synthesis in rabbit kidney cortex (IC_{50} = 82.7 $\pm$ 2.5 µM, n=8 ; 45.4 $\pm$ 1.4 µM, n=8, and 751.9 $\pm$ 26.0 µM, n=8, respectively). In addition, rutin inhibited 6 oxo PGE_1 formation by human platelet rich plasma (IC_{50} = 51.8 $\pm$ 6.2 µM, n=8). Conversion of $PGF_{2\alpha}$ to PGE_2 was also inhibited with IC_{50} values of 2.57 $\pm$ 0.11 µM, n=6 (rutin), 0.56 $\pm$ 0.02 µM, n=6 (naringenin) and 104.5 $\pm$ 6.3 µM, n=8 (quercetin). None of these drugs affected metabolism of 13,14 dihydro 15 oxo $PGF_{2\alpha}$ to 13,14 dihydro 15 oxo PGE_2 by rat kidney PG-9HDH at concentrations up to 300 µM. In contrast, sulphasalazine and indomethacin (both 50 µM) potentiated rabbit kidney cortex 6 oxo PGE_1 synthesis (by 51.6 $\pm$ 6.2%, n=8 and 51.6 $\pm$ 2.9%, n=8, respectively) but had no effect on platelet formation of this prostaglandin at concentrations up to 1mM. Neither sulphasalazine nor indomethacin (up to 1mM) influenced metabolism of 13,14 dihydro 15 oxo $PGF_{2\alpha}$ by rat kidney PG-9HDH.

As expected, sulphasalazine (IC_{50} = 48.2 $\pm$ 1.6 µM, n=6) and indomethacin (IC_{50} = 241.1 $\pm$ 12.2 µM, n=6) inhibited rabbit colon 15-PGDH while rutin, naringenin and quercetin had no effect on this enzyme at concentrations up to 1mM. High concentrations of rutin (1mM) produced a small potentiation of prostaglandin formation by BSVM (35.1 $\pm$ 10.1%, n=8) while the same concentration of naringenin and quercetin inhibited synthesis (by 33.1 $\pm$ 10.1%, n=8, and 71.0 $\pm$ 6.8%, n=8, respectively). Both indomethacin and sulphasalazine inhibited prostaglandin synthesis by BSVM with IC_{50} values of 0.21 $\pm$ 0.03 µM, n=8, and 1391 $\pm$ 46.2 µM, n=8, respectively.

Rutin and naringenin are potent and selective inhibitors of PG-9HDH. As such they may be useful experimental tools to study the physiological and pathophysiological roles of 6 oxo PGE_1.

We would like to thank the MRC for financial support.

Chang, D.G-B. & Tai, H.H. (1982) Prost. leuk. med. 8, 11
Moore, P.K. & Hoult, J.R.S. (1978) Biochem. pharmacol. 27, 1839
Moore, P.K. & Hoult, J.R.S. (1982) Biochem. pharmacol. 31, 969

EFFECT OF LIPID BILAYER FLUIDISING AGENTS ON CYCLO-OXYGENASE ACTIVITY

J.R.S. Hoult & S.H. Peers,* Department of Pharmacology, King's College, Strand, London WC2R 2LS.

The initial steps in the biosynthesis of prostaglandins may be triggered by mechanical, chemical or immunological stimuli, and involve (a) release of arachidonic acid (AA) from membrane phospholipids due to the action of phospholipase(s), and (b) conversion of AA to prostaglandin endoperoxides by membrane bound cyclo-oxygenase. It is to be expected that either or both steps may be influenced by the dynamic state of the surrounding membrane lipids. We have investigated the effects of aliphatic alcohols on cyclo-oxygenase activity, since these drugs are known to fluidise membrane lipids, to influence the haemolytic susceptibility of red blood cells and to affect the activity of other membrane-bound enzymes.

The microsomes prepared from sheep seminal vesicles (SSV) were resuspended in pH 7.4 'Tris' buffer, or in buffer containing 2% v/v Tween-20. Stirring of the latter on ice for 45 minutes afforded partial solubilisation of SSV enzyme activity: 60 arbitrary units (u) of activity were not precipitable after 45 minutes centrifugation at 150,000 x g, and 40 u remained in the microsomal pellet, compared to 5 u solubilised and 80 u microsomal in preparations stirred without detergent.

After incubation at 37°C for 45 minutes with 10 µg/ml AA containing 0.05 µCi/ml [^{14}C]-AA and 3 mM GSH, SSV microsomes and solubilised enzyme both converted 71% AA to PGE_2, whereas without cofactors overall utilisation of AA, although similar, was directed towards material co-chromatographing with 6-keto $PGF_{1\alpha}$, TXB_2 and $PGF_{2\alpha}$ (36% in native and 47% in solubilised enzyme, both preparations containing ca. 7.7 mg/ml protein). Despite these similarities, the solubilised enzyme was more susceptible to inhibition by indomethacin (ID_{50} 6.7 µM versus 79.4 µM by radio-t.l.c. or ID_{50} 2.7 µM versus 27.7 µM by bioassay). We have noted other differences in drug effects on cyclo-oxygenase when comparing bioassay and radio-t.l.c. methods of analysis; these may be due in part to differences between the access to enzyme and conversion of *endogenous* AA (the major source for bioassayable PG's) versus that of *exogenous* AA (the sole source for radiochemically-detected PG's).

Ethanol, butanol, hexanol, octanol and benzyl alcohol all produced monophasic concentration-dependent reductions in SSV microsomal cyclo-oxygenase activity (ID_{50} values > 3.5 M, 480 mM, 18 mM, 0.8 mM and 160 mM respectively; these are in the haemolytic range). Effects were very similar on solubilised SSV enzyme, and a similar wide range of lipid-solubility-dependent inhibitory potencies was observed using acetone-powder (obtained from Miles), and rabbit kidney medulla microsomes. Inhibition was reversible (dilution of enzyme preparations pre-treated with the maximally inhibitory doses afforded preparations with 'native' activity), and appeared to be non-competitive (eg control SSV microsomal enzyme Km 198 ± 32.6 µM, V_{max} 3520 ± 373 pmol/ml/min; after 200 mM benzyl alcohol Km 184 ± 18.0 µM, V_{max} 2197 ± 168 pmol/ml/min; $P < 0.001$). There was no evidence for activation of cyclo-oxygenase at low concentrations of alcohols.

Our results suggest that the activity of cyclo-oxygenase may be affected in a non-specific manner by hydrophobic interactions of fluidising concentrations of alcohols, but show that this is not an expression of a bulk-phase effect on membrane lipids as it occurs using the soluble enzymes. These findings do not prejudice the possibility that the phospholipase steps of the arachidonate cascade might be strongly dependent upon the membrane lipid microenvironment.

SHP is a MRC scholar.

THE EFFECTS OF PROSTAGLANDINS AND AN ENDOPEROXIDE ANALOGUE (U-46619) ON LOWER SEGMENT HUMAN MYOMETRIUM IN VITRO

J.K. Clayton[1], A.Y. Massele* & Judith Senior, School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP, and [1]The Royal Infirmary, Bradford, BD9 6RJ

Prostaglandins (PG) of the F series are uterine stimulants in vitro, whereas E series PG show mainly an inhibition of spontaneous activity on myometrium in Kreb's solution but a stimulatory effect in the low calcium de Jalon's solution (Massele and Senior, 1981). U-46619 (15S)-hydroxy-11, 9-(epoxymethano) prosta-5Z, 13E-dienoic acid) (Upjohn) was investigated for direct or indirect activity on human myometrial tissue in vitro, and any oxytoxic activity was compared to the effects of PGE_1, PGE_2 and $PGF_{2\alpha}$.

Uterine muscle strips were obtained from the lower segment of patients, at term but not in labour, during Caesarian section. The myometrial strips were set up and superfused with either Kreb's or de Jalon's solution as previously described (Massele and Senior, 1981), at least five strips were used for each comparison.

$PGF_{2\alpha}$ (Upjohn) always caused an increase in tension in the myometrial strip whether superfused with Kreb's or de Jalon's solution but the sensitivity of the strips in Kreb's varied enormously whereas superfusion with de Jalon's solution gave reproducible results. PGE_1 and PGE_2 caused an increase in tension in de Jalon's solution but in Kreb's exhibited a biphasic response, an increase in tension followed by inhibition of spontaneous activity.

U-46619 was tested for activity and produced a stimulatory effect in both solutions. U-46619 was significantly ($P < 0.001$) more potent than $PGF_{2\alpha}$ at higher concentrations. The thromboxane antagonist EP 045 (3×10^{-6}M) (Dong and Jones,1982), in the presence of flurbiprofen (2.5×10^{-5}M), caused a parallel shift of the dose-response relationship to U-46619 in both solutions used. The responses to PGE_1, PGE_2 and $PGF_{2\alpha}$ were not inhibited by the presence of EP 045 in the bathing solution.

It is suggested that lower segment human myometrium contains thromboxane-like receptors in addition to prostaglandin receptors.

We thank Dr. R.L. Jones for the supply of EP 045 and the Upjohn Company, U.S.A. for the gift of prostaglandins. A.Y. Massele is supported by the British Council.

Dong, Y.J. & Jones, R.L. (1982) Br.J.Pharmac. 76, 149-155
Massele, A.Y. & Senior, J. (1981) Br.J.Clin.Pharmac. 12, 285P

LEVELS OF HAPTOGLOBIN AND PROSTAGLANDIN 'RCF' IN THE MOUSE DURING TURPENTINE-INDUCED INFLAMMATION

C.N. Berry*, M.E.J. Billingham[1] & J.R.S. Hoult, Department of Pharmacology, King's College, Strand, London WC2R 2LS and [1]ICI Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire.

'Reciprocal coupling factor' (RCF) designates plasma components which inhibit microsomal prostaglandin (PG) synthesis and activate cytosolic PG breakdown (Moore & Hoult, 1980; Hellewell et al, 1980). Levels of RCF are changed in certain human and animal disease states (Moore & Hoult, 1980; Berry et al, 1982) and the factor may play a role in the regulation of the PG system. We describe the effect of turpentine-induced inflammation on RCF and serum haptoglobin levels in mouse.

Male ABU mice were injected intramuscularly with 0.05 ml turpentine into each hind-leg and plasma samples collected 14 days post-injection. Serum haptoglobin levels were assayed by radial immunodiffusion. RCF activity was measured in terms of (a) inhibition at 5% v/v serum of BSV PG synthetase, and (b) activation at 10% v/v serum of rat colon 100,000 g cytosolic supernatant $PGF2_{\alpha}$ breakdown by radiochromatography. The effects of purified mouse haptoglobin on PG synthesis and breakdown were also studied.

Serum haptoglobin levels and RCF activity were increased at 1 day (Table 1); with the exception of the activation of PG breakdown, the increases were sustained throughout the study period, although the haptoglobin levels fell after 7 days. Purified haptoglobin inhibited BSV PG synthetase with an IC50 of 100 µg/ml. Although the inhibition is substantial it cannot account fully for that found in the 'turpentine-treated' plasmas as the final concentration of haptoglobin in the synthesis assays was 0.08-0.17 mg/ml (i.e. ≤ IC50), whereas inhibition was 81.5-96.8% (Table 1). Moreover, haptoglobin up to 1 mg/ml did not enhance cytosolic PG breakdown.

Table 1 The effect of i-m turpentine on mouse RCF & haptoglobin activities

Days post-turpentine	Serum haptoglobin mg	Serum haptoglobin percent	Percent inhibition of PG synthesis	Percent activation of PG breakdown
0	16.5 ± 2.9 (7)		15.5 ± 3.4 (14)	21.0 ± 6.1 (14)
1	322 ± 17 (5)		91.1 ± 1.3 (10)	60.3 ± 6.1 (10)
2	340 ± 12 (5)		92.8 ± 1.8 (10)	34.3 ± 1.9 (10)
3	300 ± 18 (5)		94.4 ± 1.0 (10)	31.4 ± 2.0 (10)
4	321 ± 27 (5)		96.8 ± 1.3 (10)	30.0 ± 7.6 (10)
7	267 ± 12 (5)		94.0 ± 1.8 (10)	18.0 ± 5.9 (10)
10	184 ± 21 (5)		92.0 ± 1.6 (10)	26.4 ± 11.8 (10)
14	150 ± 32 (5)		81.5 ± 5.1 (10)	28.3 ± 10.5 (10)

We conclude that the changes in the synthesis-inhibitory component of RCF activity after turpentine injection may be explained at least in part by the increase in serum haptoglobin. However, our results also indicate that some other components (perhaps other acute phase proteins) may contribute to the elevated RCF activity.

Berry, C.N. et al (1982) Br. J. Pharmac. 75, Proc. Suppl. 7P.
Hellewell, P.G. et al (1980) Eur. J. Pharmacol. 68, 509-511.
Moore, P.K. & Hoult, J.R.S. (1980) Nature 288, 271-273.

COMPARATIVE EFFECTS OF CARPROFEN AND BENOXAPROFEN ON CARRAGEENAN-INDUCED PLEURISY IN THE RAT

D. Bradshaw,* P.H. Franz & S.J. Greenham, Department of Pharmacology, Roche Products Limited, Welwyn Garden City, Hertfordshire, AL7 3AY.

We have recently reported that indomethacin inhibits both the cellular and oedematous responses in the early (4 hours) stage of a carrageenan pleurisy but causes inhibition of cell accumulation with potentiation of exudation at a later stage (24 hours) (Bradshaw et al, 1982). The present communication describes the effects of two other non-steroidal anti-inflammatory drugs, carprofen and benoxaprofen, in the same experimental model.

Female Alderley Park strain rats weighing 220 - 250g were injected intrapleurally under ether anaesthesia with 0.2 ml of a 1% carrageenan suspension in saline. Animals were treated orally with carprofen or benoxaprofen 1 hour before injection of Viscarin carrageenan (4 hour pleurisy) or 1 hour before and 5 hours after injection of lambda carrageenan (24 hour pleurisy). Pleural exudates were collected from animals killed 4 hours or 24 hours after injection and the pleural cavity was washed out with 2 ml of phosphate-buffered saline. The exudate volume was recorded and cell counts were determined using an haemocytometer or Coulter automatic cell counter. Differential cell counts were performed for the 24 hour pleurisy.

In the 4 hour pleurisy, carprofen (10, 30 and 100 $mg.kg^{-1}$) inhibited the formation of pleural exudate by up to 46% (statistically significant, $P<0.001$) although the effect was not dose-dependent. Benoxaprofen (10, 30 and 100 $mg.kg^{-1}$) caused a dose-dependent inhibition of exudate formation (36% at 100 $mg.kg^{-1}$, $P<0.05$). Both compounds inhibited cell accumulation in a manner which was not dose-dependent. The maximum observed inhibition was 38% for carprofen ($P<0.001$) and 31% for benoxaprofen ($P<0.001$).

In the 24 hour pleurisy both carprofen (10, 30 and 100 $mg.kg^{-1}$) and benoxaprofen (30 and 100 $mg.kg^{-1}$) increased the amount of exudate recovered from the pleural cavity. At 100 $mg.kg^{-1}$ the exudate volumes represented a 6.2-fold increase for carprofen and a 2.6-fold increase for benoxaprofen over corresponding control values. Neither carprofen nor benoxaprofen had any significant effect on either the total number of polymorphonuclear leucocytes (PMN) or mononuclear cells (MN) at any of the dose levels used. Our results differ, therefore, from those previously reported in which benoxaprofen had no effect on exudate volume but selectively inhibited MN accumulation in a pleurisy induced by Seakem carrageenan (Meacock et al, 1979; Meacock and Kitchen, 1979). We attribute this to the different types of carrageenan used since we have previously observed clear differences, in the response to indomethacin, of 24 hour pleurisies induced by Viscarin carrageenan and lambda carrageenan (Bradshaw et al, 1982).

Taken in conjunction with our previous results for indomethacin, the present results indicate a similar effect of the three compounds on the exudative component of a 4 hour carrageenan pleurisy with the order of potency, indomethacin > carprofen > benoxaprofen. In the 24 hour pleurisy all three compounds increased the exudate volume with the same order of potency. These relative potencies correspond to those observed for inhibition of cyclo-oxygenase by the compounds and suggest that the effects on the oedematous response to pleural inflammation reported here may relate to inhibition of prostaglandin synthesis.

Bradshaw, D. et al (1982) Communication to the British Pharmacological Society, September 1982.
Meacock, S.C.R. et al (1979) Eur. J. Rheumatol. Inflamm. 2, 23 - 28.
Meacock, S.C.R. and Kitchen, E.A. (1979) J. Pharm. Pharmac. 31, 366 - 370.

ACTIVITY OF A RETINOID Ro 11-1430(MOTRETINIDE) IN SOME IMMUNOLOGICAL MODELS

S.A. Jones & A.J. Kennedy*, Department of Pharmacology, Roche Products Limited, Welwyn Garden City, Hertfordshire, AL7 3AY.

Vitamin A and its metabolites are involved in a variety of biological activities including growth, tissue differentiation, the visual cycle and in glycosylation reactions. There is also some evidence that vitamin A may have immuno-modulatory activity (Lotan, 1980). In this study a synthetic retinoid Ro 11-1430 (all-trans--N-ethyl-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenamide) has been examined in a group of immunological models. Ro 11-1430 (3 $mg.kg^{-1}$ p.o.) inhibited the delayed hypersensitivity footpad swelling in mice sensitized and challenged with methylated bovine serum albumin (M.B.S.A.) (Cashin et al, 1979). This activity was greatest when the retinoid was dosed at or shortly after the time of sensitization with M.B.S.A. The activity was not accompanied by significant reduction in the bone marrow cell count of the femur and tibia. Ro 11-1430 dosed at the time of challenge with M.B.S.A. did not reduce footpad swelling significantly. Ro 11-1430 had no significant activity in 4 (IgM) or 10 day sheep red cell antibody tests. The retinoid displayed equivocal activity in the oxazolone hypersensitivity test in mice. Inhibition of inflammation was noted when the interval between sensitization and challenge was 14 days but not when shorter intervals were employed (3 or 8 day). Ro 11-1430 did not delay the onset of experimental allergic encephalomyelitis in rats. In summary Ro 11-1430 exhibits a distinctive profile of immunological activity in these tests. It potently and selectively inhibited a delayed hypersensitivity reaction but did not impair antibody production. The absence of a direct effect on bone marrow cell counts suggests that the observed activity is not due simply to a generalized cytostatic action which can be measured for several immunosuppressive agents.

Table 1. Activity of Ro 11-1430 in M.B.S.A. delayed hypersensitivity

Treatment Group	Dose mg/kg	24hr paw volume (% increase)	% reduction response
Water Control	-	73.7 ± 8.7	-
Arachis Oil Control	-	84.4 ± 11.9	-
Ro 11-1430	3	53.3* ± 5.9	36.6
Ro 11-1430	10	35.7** ± 4.8	57.7
Ro 11-1430	30	40.7** ± 4.6	51.8

*P<0.05; **P<0.01; Students t test on differences from Arachis oil control. Ro 11-1430 was dosed orally in Arachis oil.

Cashin, C.H. et al (1979) Agents Actions 9, 553 - 559.
Lotan, R. (1980) Biochim. Biophys. Acta. 605, 33 - 91.

(^{3}H)-IMIPRAMINE BINDING IN PLATELETS: A STATE-DEPENDENT OR INDEPENDENT BIOLOGICAL MARKER IN DEPRESSION?

Gay C., Langer S.Z., Loo H., Raisman R.*, Sechter D. and Zarifian E., Department of Biology, Laboratoires d'Etudes et de Recherches Synthélabo, 58, rue de la Glacière, 75013 Paris, France; Hôpital Sainte-Anne, 7 rue Cabanis, 75014 Paris; Hôpital Saint-Jacques, place Henri Dunant, 63003 Clermont-Ferrand, France.

The demonstration of a specific high-affinity binding site for ^{3}H-imipramine (^{3}H-IMI) in human platelets and post-mortem human brains has provided a new potential biological marker in depression (Langer et al., 1981).

Longitudinal studies of severely depressed patients indicate that the density of ^{3}H-IMI binding sites are lower than in matched control volunteers and do not change during treatment with tricyclic antidepressant drugs and the subsequent recovery from depression (Raisman et al., 1982). However, it is possible that the continued presence of tricyclic antidepressants prevents a return of the Bmax value to the higher control levels because both in brain and in platelets, chronic tricyclic antidepressant treatment has been shown to result in a significant reduction of the Bmax for ^{3}H-IMI binding (Langer et al., 1981). To test this hypothesis we carried out a longitudinal study of ^{3}H-IMI binding in platelets obtained from both male and female untreated severely depressed patients before and during electroshock treatment (ECT). We also studied ^{3}H-IMI binding in platelets from untreated depressed patients before and during therapy with the selective noradrenaline uptake inhibitor, maprotiline.

The Bmax values of ^{3}H-IMI binding in platelets from 12 depressed patients were lower than in normal control volunteers [Controls : Age = 50.2 $\pm$ 4.3 years ; Bmax = 539 $\pm$ 40 fmoles/mg protein ; Kd = 1.2 $\pm$ 0.2 nM, n=23]. Untreated severely depressed patients : Age = 64 $\pm$ 2.9 years ; HDRS (Hamilton depression rating score) = 62.3 $\pm$ 3.5 ; Bmax = 293.8 $\pm$ 23.6 fmoles/mg protein ($p<0.001$ when compared with the controls), Kd = 1.3 $\pm$ 0.3 nM, n=12]. After at least 6 sessions of ECT the Bmax of ^{3}H-IMI binding in platelets remained low and did not vary significantly throughout the study (Bmax = 368.8 $\pm$ 47 fmoles/mg protein, Kd = 1.5 $\pm$ 0.5 nM, n=12) in spite of the clinical improvement reflected at the level of the HDRS = 32.2 $\pm$ 3 ($p<0.001$ when compared to the value before ECT).

In a group of 10 severely depressed patients who were treated for 15 days with maprotiline (150 - 200 mg/day), the Bmax of ^{3}H-IMI binding in the platelets was practically the same as that obtained before treatment (Untreated depressed patients : Age = 50.4 $\pm$ 3.9 years ; HDRS = 48.8 $\pm$ 4.1, Bmax = 307.7 $\pm$ 45.9 fmoles/mg protein, Kd = 1.69 $\pm$ 0.5 nM, n=10). After 15 days of treatment with maprotiline the HDRS was significantly reduced to 30.5 $\pm$ 0.8 while the parameters of ^{3}H-IMI binding in platelets remained unchanged (Bmax = 358.6 $\pm$ 51.2 fmoles/mg protein ; Kd = 1.19 $\pm$ 0.3 nM, n=10).

These results suggest that the lower density of ^{3}H-IMI binding sites in the platelets of depressed patients could be a genetically determined parameter reflecting a susceptibility to depression.

The binding of ^{3}H-IMI in human platelets may be a reflection of that in the brain. Recently it was demonstrated that the Bmax of ^{3}H-IMI binding in the post-mortem brain of suicides was significantly lower than that of appropriate controls (Stanley et al., 1982). It is concluded that ^{3}H-IMI binding in human platelets is a useful biological marker in depression. Additional studies are required to establish whether it is a state independent marker in depression.

Raisman R et al (1982) Psychopharmacology 75, 368.
Langer S.Z. et al (1981) Life Sci. 29, 211.
Stanley M. et al (1982) Science 216, 1337.

HISTAMINE SECRETION AND THE EFFECT OF ANTI-ALLERGIC COMPOUNDS ON PERITONEAL MAST CELLS OF THE RAT, MOUSE AND HAMSTER

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

Recent investigations in our and other laboratories (for references see Pearce, 1982) have emphasized that mast cells from different locations are functionally heterogeneous and may vary in their response to both histamine liberators and anti-allergic drugs. As part of this study, we here wish to compare the properties of peritoneal mast cells from the rat, mouse and hamster.

Cells were obtained by direct lavage and histamine release in response to various secretagogues was determined as previously reported (Pearce et al, 1981). Some representative results are shown in Table 1. Rat peritoneal cells were significantly more reactive than those from the mouse and hamster on stimulation with the basic agents compound 48/80 and peptide 401 (MCD peptide from bee venom). These differences were less marked in the case of polylysine. Murine mast cells were more responsive to ATP than the rat cells whereas those from the hamster were unreactive. The latter were, however, most responsive to stimulation with concanavalin A. The release induced by the lectin was potentiated by phosphatidylserine in the case of the rat and mouse but not the hamster (data not shown). All three cell types responded in comparable fashion to the ionophore A23187.

Table 1 Histamine release from peritoneal mast cells of various species

Secretagogue	Histamine release (%)		
	Rat	Mouse	Hamster
Compound 48/80 (1 µg/ml)	86.9 ± 2.0	21.6 ± 4.2	46.2 ± 8.0
Peptide 401 (1 µg/ml)	80.5 ± 1.2	9.2 ± 1.9	25.8 ± 5.1
Polylysine (10 µg/ml)	64.5 ± 1.5	30.1 ± 6.0	51.8 ± 2.6
ATP (1 mM)	33.9 ± 10.7	72.3 ± 2.6	3.1 ± 1.5
Concanavalin A (10 µg/ml)	39.5 ± 2.0	27.5 ± 10.8	73.0 ± 3.0
Ionophore A23187 (1 µM)	84.6 ± 2.8	75.0 ± 2.8	76.8 ± 2.8

Values are means ± s.e. mean for 4-8 experiments.

Histamine release induced by concanavalin A was similarly inhibited in each species by theophylline (ID_{50} 1-5 mM) and quercetin (ID_{50} 10-20 µM) although the murine cells were somewhat less responsive to the former agent. Dibutyryl cAMP (ID_{50} 1 mM) and AH 9679 (ID_{50} 100 nM) each produced a comparable inhibition in the rat and hamster but were much less effective in the mouse (ID_{50}'s 10 mM and 100 µM, respectively). Disodium cromoglycate was most potent in the rat (ID_{50} 1 µM), moderately effective in the hamster (ID_{50} 30 µM) and inactive in the mouse (ID_{50} > 1 mM).

Thus, these results emphasize the functional heterogeneity of mast cells from different sources and stress that data on the mechanism and modulation of histamine secretion cannot be immediately extrapolated from one species to another.

This work was supported by grants from Fisons Pharmaceuticals, MRC, NATO and SERC.

Pearce, F.L. (1982) Klinische Wchschrft., in press.
Pearce, F.L. (1981) Agents and Actions 11, 51-54.

THE INTERACTION OF CIMETIDINE WITH PROPRANOLOL IN THE PITHED RAT

Barber HE, Petrie JC and Smith KJ*. Department of Pharmacology and Department of Therapeutics and Clinical Pharmacology, University of Aberdeen, Medical School Buildings, Foresterhill, Aberdeen. AB9 2ZD.

Propranolol undergoes extensive first-pass metabolism in the liver. Hepatic clearance of propranolol following intravenous administration is determined by liver blood flow (Wilkinson and Shand, 1975). Following oral administration, clearance is dependent upon the intrinsic ability of the liver enzymes to metabolise the drug, and the degree of protein binding of the drug.

Cimetidine impairs the elimination of orally administered drugs, including propranolol, theophylline, warfarin, chlordiazepoxide and diazepam (Somogyi and Gugler, 1982). Inhibition of hepatic mixed function oxidase enzyme activity has usually been implicated as the principal mechanism involved. The effect of cimetidine on the elimination of propranolol administered both orally and intravenously has been studied to further elucidate the mechanism responsible.

Male Sprague-Dawley rats were pithed under halothane anaesthesia and ventilated with 100% oxygen (Gillespie et al, 1970). The pithing rod which acts as an electrode was positioned to produce optimal continuous stimulation of the sympathetic outflow to the heart (C6-T1, 0.05 ms, 1-2Hz, 30-60V). The pithed rat model allows simultaneous monitoring of propranolol blood concentrations and the pharmacological effect on an electrically induced tachycardia. Cimetidine ($5mg.kg^{-1}$) was infused over 30 mins into a side branch of the hepatic portal vein to simulate oral administration of the drug. {^{3}H}-Propranolol ($0.2mg.kg^{-1}$) was injected over 15 secs, either by the same route or intravenously via the jugular vein. Blood samples were removed from the carotid artery for analysis of parent drug, basic metabolites, acidic metabolites and conjugates (Barber et al, 1982).

Cimetidine produced an increase in the area under the blood concentration versus time curve (AUC) for propranolol given intravenously (1597 ± 168 ng ml^{-1} mins compared with 538 ± 49 ng ml^{-1} mins, $P < 0.001$). This was associated with a significant increase in percentage inhibition of tachycardia (89.9% ± 2.9% compared with 69.0% ± 3.8%, $P < 0.01$). A corresponding reduction in the proportion of acidic metabolites and conjugates in the blood was also seen ($P < 0.05$).

No significant effect was seen on the AUC of propranolol following oral administration, although the proportion of acidic metabolites in the blood was significantly reduced by cimetidine ($P < 0.025$).

These results suggest that at this intraportal dose of cimetidine the major mechanism responsible for the interaction is a decrease in liver blood flow rather than altered metabolism or protein binding. This is in agreement with the findings from a previous study where cimetidine was administered into the jugular vein (Smith et al, 1982). This may have clinical implications when cimetidine is co-administered with intravenously administered drugs of high hepatic extraction, and would be particularly important if the co-administered drug such as propranolol itself reduced liver blood flow.

Barber HE, et al (1982). Br. J. Pharmac., In press
Gillespie JS, et al (1970). Br. J. PHarmac., 40, 257-267
Smith KJ, et al (1982). Clin. Sci., 63, 5P
Somogyi A and Gugler R. (1982). Clin. Pharmacokin., 7, 23-41
Wilkinson GR and Shand DG. (1975). Clin. Pharmac. Ther., 18, 377-390

THE PHARMACOKINETICS AND PHARMACODYNAMICS OF PROPRANOLOL IN RATS WITH RAISED ERYTHROCYTE SEDIMENTATION RATES

Barber, H.E., Hawksworth, G.M. and Walker, K.A.* Department of Therapeutics and Clinical Pharmacology and Department of Pharmacology, University Medical Buildings, Foresterhill, Aberdeen. AB9 2ZD.

Schneider and Bishop (1979) reported that after a single oral dose of propranolol patients in the active state of an inflammatory disease, with a raised erythrocyte sedimentation rate (ESR), had increased plasma drug concentrations compared with control subjects. This effect can also be observed in rats with adjuvant-induced arthritis. The increase in the area under the blood concentration time curve (AUC) could be due to a number of factors. These include decreased intrinsic clearance and/or increased protein binding. In order to elucidate the mechanism responsible for the increase in the AUC, the pithed rat model was used. This model enables the pharmacokinetics and pharmacological effect of propranolol to be studied simultaneously.

Male Sprague-Dawley rats (180g) were injected with 0.1ml. M. tuberculosis (heat killed strains PN, DT and C) in paraffin oil (3 $mg.ml^{-1}$) into the right hind foot pad. The rats were used approximately 15 days later when the ESR's were elevated (>2 $mm.h^{-1}$). The rats were pithed under halothane anaesthesia and ventilated with 100% oxygen (Gillespie et al., 1970). The pithing rod which acts as an electrode was positioned to produce optimal continuous stimulation of the sympathetic outflow to the heart (C6-T1, 0.05ms, 1-2Hz, 30-60V). {^{3}H}-Propranolol was infused via the jugular vein (0.1 $mg.kg^{-1}$) or a cannulated side branch of the hepatic portal vein (0.2 $mg.kg^{-1}$). Blood samples were removed from the carotid artery and analysed for propranolol and its metabolites (Barber et al., 1982a). Heart rate was measured from the cannulated contralateral carotid artery.

When propranolol was administered intraportally there was a significant increase ($P < 0.05$) in the AUC of the treated rats (663 ± 230 $ng.ml^{-1}min.$) compared with control animals (63 ± 14 $ng.ml^{-1}min.$). No significant difference was seen in the inhibition of the electrically induced tachycardia, nor was there any alteration in the proportion of basic or acidic metabolites or conjugates formed in the two groups of rats.

When propranolol was administered via the jugular vein there was a significant increase ($P < 0.05$) in the AUC of the treated rats (1125 ± 83 $ng.ml^{-1}min.$) compared with the controls (597 ± 21 $ng.ml^{-1}min.$). In contrast to the intraportal results there was a significant decrease ($P < 0.01$) in the treated rats of the inhibition of electrically induced tachycardia (63% ± 3%) compared with controls (82% ± 1%). There was no difference in the metabolic profile between the two groups.

These results show that irrespective of the route of administration, total propranolol blood concentrations are increased in rats with a raised ESR. The pharmacological effect is diminished after jugular vein infusion which may indicate that free drug concentration is decreased and that the increased AUC is due to an increase in binding, possibly to α_1-acid glycoprotein. This agrees with our previous results from *in vitro* studies which suggested that the mechanism responsible is due to increased protein binding and not to a decrease in intrinsic clearance (Barber et al., 1982b).

Barber, H.E. et al (1982a) Br. J. Pharmac. In press
Barber, H.E. et al (1982b) Br. J. Pharmac. 76, 224P
Gillespie, J.S. and Muir, T.C. (1967) Br. J. Pharmac. 30, 78-87
Schneider, R.E. and Bishop, H. (1979) Br. J. Clin. Pharmac. 8, 43-47
Wilkinson, G.R. and Shand, D.G. (1975) Clin. Pharmac. Ther. 18, 377-390

THE METABOLIC ACTIVATION OF AFLATOXIN B_1 IN RAT AND MAN

A R Boobis, G C Kahn, D S Davies, S Plummer* Department of Clinical Pharmacology, Royal Postgraduate Medical School, Ducane Road, London W12 OHS

Recent epidemiological evidence has suggested an association between susceptibility to aflatoxin B_1 (AFB_1) induced cancer and capacity to oxidize the anti-hypertensive drug debrisoquine (Smith et al, 1980). It is possible that a single form of cytochrome P-450 catalyses the oxidation of debrisoquine and the "activation" of AFB_1 to a mutagen. This hypothesis has been investigated in two strains of rat - Fischer and DA, which show a marked difference in their metabolism of debrisoquine(Al-Dabbagh et al, 1981 & Kahn et al, 1981), and their susceptability to AFB_1 induced hepatic tumours.

The cytochrome P-450 mediated "activation" of AFB_1 was tested *in vitro* and *in vivo* in two strains of rat and in a human liver sample. Liver microsomes were incorporated with *Salmonella typhimurium* TA98 and AFB_1 in the Ames Test. The covalent binding of ^{3}H-AFB_1 metabolites to DNA and microsomal protein was also measured. In the Ames Test, with rat liver microsomes, revertants/plate (R/p) increased linearly with increasing microsomal protein concentration to 0.75 mg/plate. R/p rose with increasing AFB_1 concentration to 8.0 μM but began to fall at higher concentrations probably because of cytotoxicity. Liver microsomes from the two strains of rat were equipotent in the activation of AFB_1 and 10 times more active than human liver microsomes. In the Ames Test debrisoquine(2.5 mM) produced only a 25% inhibition of AFB_1 activation by liver microsomes from both rat strains compared with 75% inhibition with metyrapone (3.0 mM). Debrisoquine(2.5 mM) did not inhibit AFB_1 activation by human liver microsomes. There was no difference in the covalent binding of ^{3}H-AFB_1 (20 μM) to microsomal protein from DA (0.71 ± 0.08 nmoles/mg) or Fischer (0.53 ± 0.09 nmoles/mg) rats. Debrisoquine (2.5 mM) produced only a 10% inhibition. At 20 μM AFB_1 binding to human microsomal protein (0.98 nmoles/mg) was greater than to rat microsomal protein (0.63 nmoles/mg), and debrisoquine (2.5mM) caused only a small (25%) non-competitive inhibition.

There was no strain difference in the binding of AFB_1 to DNA *in vitro* (Fischer 183 ± 8.8 pmoles/mg DNA, DA 180 ± 41.6 pmoles/mg DNA). However, the binding of ^{3}H-AFB_1 to rat liver DNA *in vivo* was two fold higher in Fischer (37.5 ± 5.9 pmoles/mg DNA) than DA (18.0 ± 7.9 pmoles/mg DNA) rats.

Microsomal enzymes from Fischer rats oxidize debrisoquine at 8 times the rate of those from DA rats and the former strain is more susceptible to AFB_1 induced liver tumours. However, metabolism of AFB_1 to its supposed reactive epoxide, as measured by covalent binding to DNA or microsomal protein and mutagenicity in the Ames Test, did not show significant differences between the two strains of rat. These data suggest that the same form of cytochrome P-450 does not catalyse the oxidation of debrisoquine and AFB_1. The failure of debrisoquine to inhibit the conversion of AFB_1 to a mutagen by human microsomes and the non-competitive inhibition of AFB_1 binding to human microsomal protein support this conclusion.

Al-Dabbagh et al (1981) J. Pharm. Pharmac. 33, 161-164 (1981)
Kahn, et al Proceedings of the British Pharmacological Society, Dec. 1981
Smith, et al (1980) Environmental chemicals, enzyme function and human disease (Ciba Foundation Symposium, 1979) 219-244, Excepta Medica.

THE METABOLISM OF ETHYLENEDIAMINE IN THE RAT

J. Caldwell & I.A. Cotgreave*, Department of Pharmacology, St. Mary's Hospital Medical School, London W2 1PG

Ethylenediamine (1,2-diaminoethane; EDA) is a small highly basic molecule which finds application in the chemical industry and is used in various pharmaceuticals, notably aminophylline (theophylline ethylenediamine). It has a number of toxic effects, notably allergic reactions, and human exposure in industrial environments is controlled. Despite its use and toxicity, little is known of the disposition of EDA in the body. We now present a preliminary account of the metabolism of EDA in the rat.

^{14}C-EDA was given i.p. to male Lewis rats (16mg/kg) housed in Metabowls for the collection of urine, faeces and CO_2 in the expired air. Excretion of radioactivity was monitored by scintillation counting and urinary metabolites separated and characterized by TLC, HPLC, chemical derivatization and mass spectrometry, by comparison with authentic standards. In some experiments, benzoic acid (500mg/kg p.o.) was given immediately prior to the EDA dose.

In 24h following EDA administration, some 65% of the dose was eliminated, 46% in the urine and 19% as $^{14}CO_2$. The urinary metabolites were unchanged EDA and its mono- and di-acetyl conjugates. In addition, a trace (<1%) of ^{14}C-hippuric acid was present. Coadministration of benzoic acid did not change the acetylation of EDA but increased greatly the formation of ^{14}C-hippuric acid and reduced the excretion of $^{14}CO_2$. These data are summarized in the Table.

Table 1 Metabolism of EDA in the rat

	%dose in 24h after administration of		
	EDA	EDA+benzoic acid	P
Urine	7	64	<0.0002
EDA	9	7	n.s.
MonoacetylEDA	31	35	n.s.
DiacetylEDA	<2	<2	n.s.
Hippuric acid	<1	16	<0.0001
$^{14}CO_2$	17	6	<0.0001
Faeces	trace	trace	

All figures are the means of 4 rats.

The metabolism of EDA is proposed to proceed by two main pathways (a) acetylation at one or both amino groups, and (b) deamination, giving the intermediate aminoacetaldehyde, which is rapidly converted to glycine. This glycine is presumably the source of both $^{14}CO_2$ and ^{14}C-hippuric acid. Upon co-administration of benzoic acid, glycine is diverted from decarboxylation into the glycine conjugation mechanism leading to a great increase in the excretion of ^{14}C-hippuric acid.

We are grateful to Napp Laboratories Ltd for financial support.

BILIARY AND URINARY METABOLITES OF 17α-ETHINYLOESTRADIOL IN WOMEN

A.M. Breckenridge, I.T. Gilmore+, S.F.M. Grimmer, J.L. Maggs, M.L'E. Orme & B.K. Park*. Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool L69 3BX, and +Department of Gastroenterology, Broadgreen Hospital, Liverpool L14 3LB

17α-Ethinylestradiol (EE_2) given orally to women is excreted in urine and faeces. The urinary metabolites of EE_2 have been extensively studied (Williams & Goldzieher, 1980) but little attention has been paid to biliary metabolites, which are, quantitatively, the more important and may contribute to the enterohepatic circulation of EE_2.

We have investigated the metabolism of [6,7-^{3}H]EE_2 in six female patients (aged 46-72 years) who received 50μg of ^{3}H-EE_2 (100 μCi) in aqueous-ethanol p.o. at 06.00 h after an overnight fast. Bile (7-28ml) was collected over 10 min., 4h after dosing, during diagnostic upper gastrointestinal endoscopy. Synthetic cholecystokinin octapeptide (Sincalide) was given i.v. during endoscopy to contract the gall bladder. Urine was collected for 72h. The bile samples obtained contained 1.8 ± 1.4% of the dose, whereas the total recovery of ^{3}H in urine after 72h was 16.6 ± 7.8% (mean ± S.D.).

Aliquots of bile and urine were concentrated using Sep-Pak C_{18} cartridges and the radiolabelled material eluted with methanol. The eluate was concentrated under nitrogen and incubated with H. pomatia extract (arylsulphatase and β-glucuronidase activity) for 16h; incubations contained 10mM ascorbate to prevent decomposition of catecholestrogens. Unconjugated and deconjugated metabolites were extracted into ether and analysed by diol-bonded-phase chromatography (Williams & Goldzieher, 1980). Peaks of ^{3}H were ascribed by co-elution with authentic standards and the ^{3}H associated with each component expressed as a % of total ^{3}H recovered from the column (70-80%).

The metabolites excreted in bile and urine were largely polar conjugates: 1-12% and 1-10% of ^{3}H, respectively, was ether extractable, whereas after enzymic hydrolysis 78 ± 9% and 81 ± 9% of biliary and urinary ^{3}H was extractable. After hydrolysis, unchanged EE_2 was invariably the principal labelled component in bile (43 ± 16%), and a major component in urine (25 - 65%) for the first 48h. The proportion of conjugated EE_2 in urine declined with time, e.g. for one patient from 50% (0-12h) to 16% (48-72h). Both 2-hydroxyethinyloestradiol (4-10%) and 2-methoxyethinyloestradiol (8-22%) conjugates were observed in bile; these are the major biliary metabolites of EE_2 in male and female rats (Grabowski et al., 1982; Maggs et al., 1982). Oestrone and D-homoestrone, were not detected in bile.

In conclusion, it has been found that the major biliary metabolites of EE_2 in women are conjugates of EE_2 and 2-hydroxyethinyloestradiol. There was no evidence for metabolites formed via oxidation of the 17α-ethinyl group of EE_2.

This work was supported by the Wellcome Trust.

Grabowski, P.S. et al., (1982) B.P.S. Meeting Sunderland.
Maggs, J.L. et al., (1982) Xenobiotica, in press.
Williams, M.C. and Goldzieher, J.W. (1980) Steroids 36, 255.

OESTRONE SULPHATE ABSORPTION IN CONVENTIONAL AND GERMFREE RATS

D.J. Back, H.J. Eyssen[1], Suzanne Huijghebaert[1], M.L'E. Orme & Si Mui Sim*, Department of Pharmacology & Therapeutics, University of Liverpool, Liverpool L69 3BX and [1]Rega Institute for Medical Research, Universteit Leuven, B-3000 Leuven, Belgium.

Although estrone sulphate is widely used for the treatment of postmenopausal oestrogen deficiency symptoms, there are still unanswered questions relating to the intestinal absorption of the compound. Studies on estrone metabolism in the human gastrointestinal tract have shown that the intestinal microflora can hydrolyse conjugates (Adlercreutz et al.,1976) liberating free steroid which is then absorbed; this is the basis of enterohepatic circulation. However, there is evidence of intact absorption of sulphate conjugates (Eriksson, 1971; Schwenk et al., 1981). In a previous study (Back et al., 1981) involving the pretreatment of rats with antibiotics, we suggested that estrone sulphate was partly absorbed intact in the small intestine but completely hydrolysed by the gut microflora in the caecum. There are certain drawbacks to studies involving antibiotic pretreatment and these problems can be largely overcome by using germfree (GF) rats. We have therefore compared the absorption of estrone sulphate in conventional (CVL) and GF rats.

Inbred CVL and GF female Fisher rats (140-170g) were divided each into three groups to study absorption from i) the proximal small intestine (PSI), ii) the distal small intestine (DSI) and iii) the caecum. After starving overnight, rats were anaesthetized with urethane, the bile duct cannulated and appropriate part of the intestinal tract isolated by ligatures. [^{3}H]-Estrone or [^{3}H]-estrone sulphate (10μCi/kg) was then injected into the section of the tract. All experimental procedures in GF rats took place in Trexler isolators. Bile samples were collected at regular intervals to 5h and radioactive content determined.

Steroids are extensively excreted in the bile of rats and therefore biliary excretion is a good reflection of absorption. There was no significant difference in bile flow rate between CVL and GF rats. There was no significant difference in the 5h excretion of steroid following administration of [^{3}H]-estrone sulphate in the PSI of CVL (17.8 ± 6.2%; mean ± S.D.) or GF (28.2 ± 5.3%) rats. A similar finding resulted from administration into the DSI (CVL, 22.3 ± 11.8; GF, 11.4 ± 3.7%). However, when the drug was given into the caecum, excretion in the bile of CVL rats was 59.1 ± 10.7% whereas in GF rats it was only 1.7 ± 0.9%. [^{3}H]-Estrone absorption was rapid in the small intestine of both CVL and GF rats. After injection into the PSI of CVL and GF rats, biliary excretion was 88.2% and 81.7% respectively and after injection into the DSI was 84.7% and 83.6% respectively. Absorption of estrone was reduced from the caeca of GF rats (49% and 25.3% excreted in the bile of CVL and GF rats respectively).

These results give clear evidence of intact absorption of estrone sulphate from the small intestine of the rat; although at a slower rate than the non-sulphated steroid. Estrone sulphate is not absorbed intact in the caecum but is hydrolysed by the gut microflora prior to absorption.

S.M.S. is supported by the Mersey Regional Health Authority and is in receipt of an ORS award. S.H. is supported by the Belgian National Fonds voor Wetenscheppelijk Onderzoek. The investigation was supported by Abbott Laboratories Ltd.

Adlercreutz, H. et al. (1976) J. clin. Endocr. Metab. 43, 497-505.
Back, D.J. et al. (1981) J. Steroid Biochem. 14, 347-356.
Eriksson, H. (1971) Eur. J. Biochem. 19, 416-423.
Schwenk, M. et al. (1981) Arzneim-Forsch/Drug Res. 31, 1254-1257.

MALTASE AND SUCRASE INHIBITION BY ACARBOSE: A PARADOXICAL EFFECT IN VIVO

Helen M Barker & Rodney H Taylor* (introduced by J J Misiewicz), Dept of Gastroenterology & Nutrition, Central Middlesex Hospital, London NW10 7NS.

The α-glycoside hydrolase inhibitor acarbose is a potent sucrase inhibitor which also inhibits α-amylase in vitro (Puls et al, 1980). Studies of 50% inhibition of intestinal enzyme activity in vitro showed that maltase needed only one fifth of the dose of acarbose that sucrase required (Truscheit et al, 1981). However both sucrase and maltase activity measured in normal human jejunal biopsies in vitro were reduced by a similar percentage by acarbose at two dose levels (Caspary & Graf, 1979). By contrast in human oral tolerance studies the area under the blood glucose response curve to sucrose 50g was reduced by 89% by acarbose 200mg, but by only 19% for maltose 50g (Jenkins & Taylor, 1982). Thus in vitro acarbose inhibits maltase five times more effectively than it does sucrase but in vivo maltose absorption is reduced by one fifth that of sucrose. These studies were designed to measure the effects of acarbose on maltase and sucrase activity in an in vivo perfusion model.

Young adult female Sprague-Dawley rats (160-210g) were anaesthetised with pentobarbitone 60μg g^{-1} body weight i.p. At laparotomy a 20cm segment of proximal jejunum was cannulated and perfused in vivo at 0.27ml min^{-1}. The perfusion solution contained either maltose or sucrose 11.7mmol l^{-1}, NaCl 147mmol l^{-1}, PEG 4000 3g l^{-1} labelled with 1μCi ^{14}C as a non-absorbable marker and had an osmolality of 290mosmol. After ½h stabilisation, aspirates were collected at 10 min intervals for 3h. In the second ½h of the 3h period the perfusion solution also contained acarbose 30mg l^{-1}. Sugars were measured by high pressure liquid chromatography, electrolytes by flame photometry and absorption rates were calculated in the standard way.

After the first ½h the luminal sucrose disappearance rate fell from 71±7 to 7±1μmol h^{-1} 20cm $segment^{-1}$ when acarbose was in the perfusion solution ($p<0.001$; $n=7$). The reduction in luminal maltose disappearance rate due to acarbose was much less, falling from 138±12 to 56±4μmol h^{-1} 20cm $segment^{-1}$ ($p<0.001$; $n=7$). There was an associated reduction in the rate of sodium and water absorption. In the presence of acarbose luminal glucose levels fell to 20% in the maltose experiments and luminal glucose and fructose levels fell almost to zero in the sucrose perfusions. There was a gradual recovery of both maltase and sucrase activity over the next 2h with a 50% recovery of the pre-acarbose level of both after 1h. Luminal glucose and fructose levels showed a similar recovery and there was some increase in sodium and water absorption.

These experiments show that in the rat in vivo using the same dose of acarbose there was a reduction in luminal maltose disappearance rate of less than 60% whereas the sucrose disappearance rate fell by more than 90%. This suggests that though acarbose may be a more potent inhibitor of maltase than sucrase in vitro, the reverse is true in vivo. This is compatible with the data from human test meal studies. Other experiments suggest that acarbose does not affect monosaccharide transport, therefore the explanation for this paradox must lie in the mechanism of function and inhibition of the enzymes in the intestinal brush border.

RHT is a Wellcome senior research fellow in clinical science. The assistance of Bayer (UK) Ltd is gratefully acknowledged.

Caspary, W.F. & Graf, S. (1979) Res.Exp.Med. 175, 1-6
Jenkins, D.J.A. & Taylor, R.H. (1982) Proc.1st.Int.Symp.Acarbose, 86-96
Puls, W. et al (1980) Frontiers Horm.Res. 7, 235-47
Truscheit, E. et al (1981) Angew.Chem.(Int.Ed.) 20, 744

DIVALENT CATIONS REGULATE THE RECEPTOR-SPECIFIC BINDING OF MUSCARINIC AGONISTS

C.P. Berrie, N.J.M.Birdsall, A.S.V. Burgen, E.C. Hulme* & M. Jameson, Division of Molecular Pharmacology, National Institute for Medical Research, Mill Hill, London NW7 1AA.

Addition of Mg^{2+} (10 mM) to unwashed membrane preparations from rat brain induces a modest increase in the affinities of muscarinic agonists such as carbachol (Table 1) and oxotremorine-M for muscarinic binding sites in all regions except the hypothalamus, whilst muscarinic agonist binding to heart membranes is unaffected. Conversely, washing of membranes with EDTA (10 mM) perceptibly decreases agonist affinity in the medulla-pons, cerebellum and heart, but has little or no effect elsewhere (Table 1).

This inhibiting effect of EDTA is fully reversed by Mg^{2+} (10 mM), yielding agonist affinities which are, in some cases, rather higher than those resulting from the addition of Mg^{2+} to unwashed membranes (Table 1). Higher (> 10 mM) concentrations of Mg^{2+} cause progressive inhibition. Quantitative analysis of the binding curves (Birdsall et al., 1980) shows that 10 mM Mg induces an apparent conversion of low to high and high to superhigh affinity sites.

Table 1. The effect of EDTA, Mg, and EDTA + Mg on carbachol binding to muscarinic receptors in rat brain and heart*

Region	IC_{50} carbachol (M)			
	unwashed membranes	EDTA	Mg	EDTA + Mg
Cerebellum	5.5×10^{-7}	1.9×10^{-6}	1.1×10^{-7}	1.3×10^{-7}
Medulla-pons	1.2×10^{-6}	3.0×10^{-6}	3.3×10^{-7}	1.4×10^{-7}
Hypothalamus	3.7×10^{-6}	3.7×10^{-6}	3.1×10^{-6}	3.7×10^{-6}
Hippocampus	5.8×10^{-5}	1.3×10^{-4}	8.2×10^{-6}	5.0×10^{-6}
Cerebral cortex	2.5×10^{-5}	3.6×10^{-5}	9.5×10^{-6}	4.3×10^{-6}
Striatum	4.9×10^{-5}	4.0×10^{-5}	2.4×10^{-6}	1.1×10^{-5}
Heart	1.2×10^{-6}	5.2×10^{-6}	1.2×10^{-6}	7.6×10^{-7}

* Membranes (10 mg protein/ml) were pretreated with 10 mM EDTA in 20 mM Na-HEPES, 100 mM NaCl, pH 7.5 at 30°C for 30 min, and diluted to 1 mg/ml for assay. The free [Mg] was 10 mM. Carbachol IC_{50} was determined by inhibition of specific binding of ^{3}H-propylbenzilylcholine (10^{-9} M brain) or ^{3}H-N-methylscopolamine (2×10^{-10} M, heart) and corrected for antagonist occupancy.

A number of divalent and trivalent cations influence muscarinic agonist binding. The pattern of effect is always stimulation at low concentrations followed by inhibition at higher concentrations. The results are complicated, but it appears that La^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+} and Ni^{2+} interact particularly strongly, that Ca^{2+} has considerable effects, particularly in the heart, but that Ba^{2+} is rather ineffective. For a given cation, the order of potency is heart >> medulla pons > hippocampus (e.g. for Mg^{2+}, the EC_{50} values for stimulation are 2×10^{-5} M, 2.0×10^{-4} M, 3.5×10^{-4} M in 20 mM Na-HEPES buffer). The ionic specificity accords with that reported for divalent cation binding to membranes (cf. Bers & Langer, 1979) and with the ability of cations to antagonise or maintain Ca^{2+}-mediated physiological responses in several different systems (Hagiwara & Byerly, 1981).

Birdsall, N.J.M., Hulme, E.C. & Burgen, A.S.V.(1980) Proc.R.Soc.Lond.B. 1-12.
Bers, D.M. & Langer, G.A. (1979) Am. J. Physiol., 237, 332-341.
Hagiwara, S. & Byerly, L. (1981) Ann. Rev. Neurosci. 4, 69-125.

THE BINDING PROPERTIES OF MUSCARINIC RECEPTORS IN THE RAT LACRIMAL GLAND: COMPARISON WITH THE CEREBRAL CORTEX AND MYOCARDIUM

C.P. Berrie*, N.J.M. Birdsall, A.S.V. Burgen & E.C. Hulme, Division of Molecular Pharmacology, National Institute for Medical Research, Mill Hill, London NW7 1AA.

The binding properties of muscarinic receptors in a typical exocrine gland, the lacrimal gland of the rat, have been characterised and compared with those of the cerebral cortex and myocardium. Receptor-specific binding of ^{3}H-N-methylscopolamine (83.6 Ci/mmole, NEN) was determined as described previously (Hulme et al., 1978), and the binding of unlabelled ligands studied by equilibrium competition in a medium containing 100 mM NaCl, 20 mM HEPES, pH 7.5, 30°C.

The affinity constants for well known antagonists such as NMS and atropine were extremely close to those found in forebrain regions (Table 1), and distinctly higher than in the heart. This difference was greatest (26-fold) in the case of diphenacetyl-N-methylpiperidine methiodide (Di-4) whose selective action on the ileum as opposed to the heart has been demonstrated by Barlow et al., 1976. The only drug to distinguish the lacrimal from the forebrain receptor was the selective antagonist pirenzepine which binds less strongly in the gland than in the cortex, but more strongly than in the heart.

Table 1. Binding of antagonists to muscarinic receptors in the rat lacrimal gland: comparison with cerebral cortex and heart

Antagonist	Affinity constant (M^{-1})		
	Lacrimal	Cerebral cortex	Myocardium
(-)-N-Methylscopolamine	6.3×10^{9}	6.2×10^{9}	1.4×10^{9}
(-)-Atropine	1.9×10^{9}	1.8×10^{9}	4.5×10^{8}
(-)-N-Methylatropine	2.7×10^{9}	4.5×10^{9}	-
Di-4	1.6×10^{9}	1.0×10^{9}	6.1×10^{7}
Pirenzepine	7.2×10^{6} *	5.0×10^{7} *	1.2×10^{6}

* These binding curves are not simple mass action curves. The affinity constants refer to the major component (77% in lacrimal, 66% in cerebral cortex).

The binding of agonists to the lacrimal receptor was predominantly of the low affinity type, as in the forebrain, particularly the hippocampus (Birdsall et al., 1980); a further similarity exists in that the GTP (10^{-3} M)-induced reduction in agonist affinity was small (ca 3-fold). Again there is a contrast with the myocardium, where agonist affinity is high, and the GTP effect large (Berrie et al.,1979).

The differences in binding properties between the lacrimal and forebrain muscarinic receptors are small but there are large differences between both tissues and the myocardium.

Hulme, E.C., Birdsall, N.J.M., Burgen, A.S.V. & Mehta, P. (1978) Molec. Pharmac. 14, 737-750.
Barlow, R.B., Berry, K.J., Glenton, P.A.M. Nicolaou, N.M. & Soh, K.S. (1976) Brit. J. Pharmac., 56, 613-620.
Birdsall, N.J.M., Hulme, E.C. & Burgen, A.S.V. (1980) Proc.R.Soc.Lond.B. 207, 1-12.
Berrie, C.P., Birdsall, N.J.M., Burgen, A.S.V. & Hulme, E.C. (1979) B.B.R.C. 87, 1000-1005.

INFLUENCE OF 5HT UPTAKE ON THE 5HT ANTAGONIST ACTIVITY OF METOCLOPRAMIDE ON THE RAT SUPERIOR CERVICAL GANGLION

S.J. Ireland*, D.H. Fortune & M.B. Tyers, Neuropharmacology Department, Glaxo Group Research Ltd., Ware, Hertfordshire.

Metoclopramide selectively inhibits 5-hydroxytryptamine (5HT) - induced depolarisations of the rat isolated superior cervical ganglion (SCG), and behaves as a competitive antagonist of 5HT-induced depolarisations of the rat isolated vagus nerve (VN) (Ireland et al, 1982). Both these effects of 5HT may be mediated via the so-called "M" receptor (Gaddum & Picarelli, 1957). In the present study, the 5HT antagonist action of metoclopramide has been examined in detail in the SCG, and compared to that in the VN.

Male Lister Hooded rats (270-330g) were anaesthetised with chloral hydrate (300mg/kg i.p.), and the SCG or VN were excised and desheathed. 5HT-induced depolarisations were recorded extracellularly from VN or SCG preparations mounted in 2-compartment perspex baths, and superfused with Krebs-Henseleit solution at 27°C (Ireland et al, 1982).

While metoclopramide, $1x10^{-6}$-$1x10^{-4}$M, produced parallel rightwards shifts of the 5HT concentration - depolarisation curves in both the SCG and VN, the apparent pA_2 values differed. For the SCG, a plot of log (dose ratio -1) against log concentration of antagonist (Arunlakshana & Schild, 1959) had a slope of 0.82 and yielded a pA_2 of 5.74, the corresponding values for the VN were 0.98 and 6.60 respectively. Neither slope was significantly different from unity ($p>0.05$).

Since a saturable uptake system for an agonist may distort apparent antagonist kinetics (Langer & Trendelenburg, 1969), the present study was extended to investigate the presence and possible influence of a 5-HT uptake mechanism on the 5HT antagonist properties of metoclopramide in the SCG and VN.

For uptake studies, whole SCGs or VN segments were incubated individually at 27°C in 2ml of Krebs-Henseleit medium containing nialamide, $1x10^{-5}$M.

Uptake of ^{3}H-5HT by the SCG was linear over 60 minutes; it was saturable, temperature and sodium dependent, sensitive to ouabain, $1x10^{-3}$M, and 5HT uptake inhibitors. Using ^{3}H-5-HT, $1x10^{-8}$M, the IC_{50} values for desipramine, chlomipramine, zimelidine and paroxetine were $1.4x10^{-6}$, $8.7x10^{-9}$, $1.5x10^{-7}$ and $4.3x10^{-8}$ M respectively. These results compare well with published IC_{50} values for inhibition of 5HT uptake into rat brain synaptosomes (Ross, 1982). In contrast significant saturable uptake of ^{3}H-5HT by the VN could not be demonstrated.

The 5HT antagonist effects of metoclopramide were measured in the presence of the 5HT uptake inhibitor paroxetine, $1x10^{-6}$M. A plot of data obtained on the SCG in the presence of paroxetine had a slope of 0.93 and gave a pA_2 value of 6.25. Paroxetine did not alter the effects of metoclopramide on the VN.

The results suggest that the apparent differences in the antagonist kinetics of metoclopramide against 5HT-induced depolarisations of the rat SCG and VN are due to the influence of a 5HT uptake mechanism in the SCG.

Arunlakshana, O. & Schild, H.O. (1959) Br.J.Pharmac. Chemother. 14, 48-58
Gaddum, J.H. & Picarelli, Z.P. (1957) Br.J.Pharmac. Chemother. 12, 323-328
Ireland, S.J. et al (1982) Br.J.Pharmac. 72, Proc. Suppl. 16P
Langer, S.Z. & Trendelenburg, U. (1969) J.Pharmac.Exp.Ther. 169, 117-142
Ross, S.B. (1982) In "Biology of Serotonergic Transmission" ed. N.N. Osbourne. pp 159-195

α-ADRENOCEPTOR-MEDIATED VASOCONSTRICTION IN THE PITHED RAT INDUCED BY STIMULATION OF SUBCLASSES OF GANGLIONIC MUSCARINIC RECEPTORS

D. Davidesko, P.B.M.W.M. Timmermans, B. Wilffert* & P.A. van Zwieten, Department of Pharmacy, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands

The innervation of vascular α_1-adrenoceptors has been the subject of several investigations (Langer et al, 1981; Yamaguchi & Kopin, 1980; Wilffert et al, 1982a). However, until recently it has been uncertain whether vascular α_2-adrenoceptors are innervated or not. Although it has been suggested that α_2-adrenoceptors are not under sympathetic nervous system control (Langer et al, 1981), Wilffert et al (1982b) reported that in the pithed normotensive rat there are indications for an innervation of vascular α_2-adrenoceptors via the muscarinic pathway. Muscarinic receptors can be divided into two subtypes with high and low affinity for pirenzepine, respectively. Binding studies have identified both receptor types in sympathetic ganglia. Pirenzepine has a high potency in antagonizing the pressor effect of the selective agonist McN-A-343 (Hammer & Giachetti, 1982). The involvement of α_1- and α_2-adrenoceptors in the pressor effects of the selective muscarinic receptor agonist McN-A-343 and the non-selective muscarinic receptor agonist 1,1-dimethyl--4-carboxypiperidine methylester (DMCPM), was assessed by means of the selective α_1-adrenoceptor antagonist prazosin (0.24×10^{-6} mol/kg) and the selective α_2-adrenoceptor antagonist rauwolscine (2.56×10^{-6} mol/kg) in the pithed normotensive rat. Pithed, artificially ventilated, male normotensive Wistar rats (190-240 g) were used throughout. Catheters were introduced into a jugular vein and into a common carotid artery for drug administration and measurement of arterial pressure, respectively. Drugs dissolved in saline were injected in a volume of 0.5 ml/kg. Antagonists were administered 15 min before the injection of the agonist. The pressor effects due to i.v. bolus injections of McN-A-343 were antagonized by prazosin but not by rauwolscine. The combination of prazosin and rauwolscine, however, attenuated the increase in diastolic pressure evoked by McN-A-343 more effectively than prazosin alone, indicating a modest participation of α_2-adrenoceptors. The hypertensive action of the non-selective muscarinic receptor agonist, DMCPM, was antagonized much more effectively by rauwolscine, pointing to a greater involvement of α_2-adrenoceptors in this pressor response. The results indicate that in the sympathetic ganglion of the normotensive rat both subclasses of muscarinic receptors may possess a functional role. Stimulation of ganglionic receptors with low affinity for pirenzepine gives rise to α_2-adrenoceptor-induced increases in arterial pressure. The receptors with high affinity for pirenzepine predominantly cause α_1--adrenoceptor-mediated pressor effects.

Hammer, R. & Giachetti, A. (1982) Life Sci. 31, 2991
Langer, S.Z. et al (1981) Hypertension 3, supp. I, I-112
Wilffert, B. et al (1982a) J.Pharmac.Exp.Ther. 221, 762
Wilffert, B. et al (1982b) J.Pharmac.Exp.Ther. 223, 219
Yamaguchi, I. & Kopin, I.J. (1980) J.Pharmac.Exp.Ther. 214, 275

DOPAMINE RECEPTOR MODULATION OF CHOLINERGIC TRANSMISSION IN THE RABBIT RECTOCOCCYGEUS MUSCLE

G.M. Drew & A. Hilditch*, Department of Pharmacology, Glaxo Group Research Limited, Ware, Hertfordshire, SG12 0DJ.

Many dopamine-receptor agonists and antagonists exert effects at α- and β-adrenoceptors as well as at dopamine receptors. These effects may impede measurement of the activities of these compounds at presynaptic dopamine receptors in isolated tissues. We have tried to find a preparation in which the effects of drugs at presynaptic dopamine receptors can be examined without these complicating factors.

Field stimulation of the rabbit isolated rectococcygeus muscle preparation elicits contractile responses mediated by acetylcholine acting at muscarinic receptors (Ambache et al., 1974). Stimulation of preparations at 0.1 Hz (0.4 ms, supramaximal voltage) in Krebs solution at 32°C containing cocaine (3 x 10^{-5} M), corticosterone (4 x 10^{-5} M), propranolol (1 x 10^{-6} M) and ascorbic acid (1 x 10^{-4} M), and gassed with 95% O_2 and 5% CO_2, produced individual twitch responses. Dopamine (1 x 10^{-8} - 1 x 10^{-5} M) and noradrenaline (1 x 10^{-9} - 1 x 10^{-7} M) caused concentration-dependent inhibition of the twitch response but did not relax preparations contracted with carbachol (3 x 10^{-6}M). However, higher concentrations of noradrenaline (3 x 10^{-7} - 1 x 10^{-5} M) relaxed carbachol-induced tone. Sulpiride (1 x 10^{-8} - 1 x 10^{-5} M) reversed the effect of dopamine, but not noradrenaline, on the twitch responses to field stimulation. Phentolamine (1 x 10^{-8} - 1 x 10^{-5} M) was inactive against either agonist.

The effects of dopamine were mimicked by 6,7-ADTN, N,N-di-n-propyl-5,6-ADTN, Sandoz 27-403, epinine, 5,6-ADTN, apomorphine, N,N-diethyl dopamine and N,N-di-n-propyldopamine (equipotent concentration ratios, dopamine = 1, were 0.04, 0.09, 0.25, 0.46, 2.9, 3.1, 11.8 and 26.4 respectively). The inhibitory effects of all compounds were reversed by sulpiride (1x10^{-5} M). N,N-di-n-propyl-6,7-ADTN inhibited the twitch response but was not reversed by sulpiride. SKF 38393 (1 x 10^{-5} M) was inactive.

After 30 min incubation, haloperidol and sulpiride had no effect on the twitch responses, but both competitively antagonised the twitch-inhibitory effect of dopamine; their pA_2 values were 8.39 and 7.75, respectively. Neither neuroleptic blocked the effects of noradrenaline. In contrast, the twitch-inhibitory effects of dopamine were not consistently antagonised by cis α-flupenthixol or fluphenazine in concentrations up to 1 x 10^{-5} M.

These results suggest that dopamine and noradrenaline inhibit cholinergic transmission in the rabbit rectococcygeus muscle through different mechanisms. The effects of dopamine appear to be mediated via dopamine receptors, probably located on cholinergic nerve terminals. Although these receptors show some similarities to the presynaptic dopamine receptors in the cat heart (Drew & Hilditch, 1982) they are clearly different from the vascular dopamine receptors in the rabbit splenic artery (Hilditch & Drew, 1981).

Ambache, N. et al. (1974) Br. J. Pharmac., 52, 175.
Drew, G.M. & Hilditch, A. (1982) Br. J. Pharmac. 75, 145P.
Hilditch, A. & Drew, G.M. (1981) Eur. J. Pharmac., 72, 287.

BIPHASIC RELAXATION OF THE MOUSE ANOCOCCYGEUS MUSCLE DURING FIELD STIMULATION

A. Gibson, Department of Pharmacology, Chelsea College, University of London, Manresa Road, London SW3 6LX.

Biphasic relaxations of the mouse anococcygeus muscle have been observed during trains of field stimulation (Gibson & Tucker, 1982) and, in this paper, some further details of the phenomenon are presented.

Male mice anococcygeus muscles were set up for recording isometric contractions as described previously (Gibson & Wedmore, 1981). Field stimulation was applied by two parallel platinum electrodes running down either side of the tissue. These were attached to a square wave pulse generator (1 msec pulse width ; supramaximal voltage). Sympathetic motor responses to field stimulation were prevented by preincubation with guanethidine (30 μM ; 15 min) and by inclusion of phentolamine (1 μM) in the Krebs bathing medium. Muscle tone was raised by carbachol (50 μM).

60 sec trains of field stimulation (5, 10, and 25 Hz) caused a biphasic relaxation of carbachol-induced tone. Separation of the two phases was greatest at 10 Hz. At this frequency the time to peak (sec) and the magnitude (% reduction in carbachol-induced tone) of the first phase relaxation were 6.3 ± 0.3 and 50.5 ± 2.4 respectively ; those of the second phase were 46.5 ± 1.6 and 61.5 ± 2.8 respectively. Neither phase was affected by propranolol (1 μM), (+)-tubocurarine (100 μM), or neostigmine (1 μM). The magnitude of both phases was reduced by tetrodotoxin (TTX ; 124 nM ; first phase reduced by 50.2 ± 4.5% ; second phase reduced by 54.0 ± 3.3% ; $P > 0.05$). Haemolysed blood (40 μl/ml of a 1:1 dilution of mouse blood with distilled water) produced a greater reduction of the magnitude of the first phase (49.7 ± 3.2%) than of the second phase (29.8 ± 4.9% ; $0.01 > P > 0.001$). Following a prolonged train of inhibitory field stimulation (10 Hz ; 10 min) the magnitude of the first phase was reduced by 8.5 ± 2.5% while that of the second phase was reduced by 41.4 ± 2.9% ($P < 0.001$).

The results confirm that field stimulation of the mouse anococcygeus muscle causes a biphasic relaxation. The initiation of both phases appears to be neuronal since they are equally sensitive to block by TTX. The second phase is relatively resistant to block by haemolysed blood, and is reduced to a greater extent by a preceding burst of inhibitory field stimulation. In these respects the second phase inhibition resembles the relaxations induced by vasoactive intestinal polypeptide (VIP) in the mouse anococcygeus (Gibson & Tucker, 1982), suggesting that if VIP is involved in non-adrenergic, non-cholinergic inhibitory transmission in this tissue then it is more likely to be associated with the second, rather than the first, phase of nerve-induced relaxation.

The support of the Central Research Fund of London University is appreciated.

Gibson, A. & Tucker, J.F. (1982) Br. J. Pharmac. in press.
Gibson, A. & Wedmore, C.V. (1981) J. Auton. Pharmac. 1, 225-233

COEXISTENCE OF β_1- AND β_2-ADRENOCEPTORS IN HUMAN HEART AND LUNG

O.-E. Brodde*, K. Karad, H.-R. Zerkowski+, N. Rohm+ and J. Chr. Reidemeister+ (Introduced by S.R. Nahorski). Div. Renal & Hypertensive Diseases, Med. Klinik & Poliklinik and +Div. Thoracic and Cardiovasc. Surgery, University of Essen, D-4300 Essen, Fed. Rep. Germany.

Radioligand binding studies have led to rapid progress in quantitative analysis of distribution of β_1- and β_2-adrenoceptors in various organs (Barnett et al., 1978; Minneman et al., 1981). In the present study we have used the highly specific β-receptor radioligand (±)-^{125}iodocyanopindolol (ICYP, Engel et al., 1981; Brodde et al., 1981) to subclassify β-adrenoceptors in human heart and lung.

Strips of human right atria and lungs, obtained during surgery, were homogenized in 10 vol ice-cold 10 mM Tris-HCl pH 7.4; the homogenate was centrifuged with 700g x 15 min and the supernatant was centrifuged with 20,000g x 15 min. The resulting pellets were three times washed with ice-cold incubation buffer (50 mM Tris-HCl, 10 mM $MgCl_2$ pH 7.4) by resuspension and recentrifugation. ICYP binding assay was performed as recently described (Brodde et al., 1981).

Binding of ICYP to both tissues was saturable (B_{max} = 40-60 fmoles/mg protein in heart; 100-130 fmoles/mg protein in lung), of high affinity (K_D = 40-65 pM), rapid, reversible and stereospecific. The relative potencies of isoprenaline, adrenaline and noradrenaline for inhibition of ICYP binding and activation of adenylate cyclase were with cardiac membranes 1:10:10 indicating a population of mainly β_1-adrenoceptors. With lung membranes the relative potencies were 1:10:100 indicating a population of mainly β_2-adrenoceptors.

Inhibition of ICYP binding by β_1-(practolol, betaxolol) and β_2-(IPS 339; ICI 118.551) selective drugs, however, resulted in both tissues in biphasic displacement curves with pseudo Hill-coefficients (n_H) of 0.6. Non-linear regression analysis (Engel et al., 1981) of these curves revealed a β_1:β_2-ratio of 80:20 in heart and of 30:70 in lung membranes. Unselective β-adrenergic drugs like propranolol or isoprenaline (in the presence of 10^{-4} M GTP), on the contrary, inhibited binding with monophasic displacement curves and n_H of 1.0.

Interaction of agonists with β-adrenoceptors has been shown to induce the high and low affinity state of the receptor. GTP converts this heterogeneous binding into a homogeneous one of low affinity (Kent et al., 1980). The same holds true for β-adrenoceptors in human heart and lung. In the absence of GTP (-)-isoprenaline displacement curve was shallow with n_H of about 0.6 in both tissues. In the presence of GTP (10^{-4} M) the curves were shifted to the right, became steeper with n_H of about 1.0. It is concluded that in human heart and lung β_1- and β_2-adrenoceptors coexist, whereby in heart β_1-, in lung β_2-adrenoceptors predominate.

Barnett, D.B. et al. (1978) Nature 273, 166-168.
Brodde, O.-E. et al. (1981) Life Sciences 29, 2189-2198.
Engel, G. et al. (1981) Arch. Pharmacol. 317, 277-285.
Kent, R.S. et al. (1980) Molec. Pharmacol. 17, 14-23.
Minneman, K.P. et al (1981) Annu. Rev. Neurosci. 4, 419-461.

A STUDY OF RAT JEJUNAL α-ADRENOCEPTORS

D.J. Cotterell*, B.J. Parsons, Judith A. Poat and Penny A. Roberts. (Introduced by G.N. Woodruff), Department of Physiology and Pharmacology, University of Southampton, Hampshire.

It has been shown that noradrenaline stimulates sodium and fluid transport across the small intestine in vivo (Levens et al 1979). This response is blocked by α- but not β-adrenoceptor antagonists. Furthermore, specific binding sites for α-adrenoceptor ligands, (^{3}H)-prazosin and (^{3}H)-clonidine have been located on membranes prepared from isolated rat jejunal cells (Cotterell et al 1982). The present study compares the effects of α-adrenoceptor agonists and antagonists upon fluid transport with their effects upon (^{3}H)-prazosin binding.

Fluid transport was measured using everted rat jejunal sacs according to the method of Wilson and Wiseman (1954). Membranes were prepared from isolated jejunal epithelial cells and specific (^{3}H)-prazosin binding assessed using 1-2 nM (^{3}H)-prazosin and 1 μM prazosin to define specific binding. The details of the method are described previously (Cotterell et al 1982).

The results obtained with a number of different α-adrenoceptor agonists and antagonists are shown in Table 1.

Table 1

	Fluid transport	(^{3}H)-prazosin binding
Agonists	EC_{50}	Ki
Noradrenaline	4 μM	1.8 μM
Adrenaline	36 μM	22.2 μM
Clonidine	5 mM	9.0 μM
Phenylephrine	> 10 mM	84.8 μM
Methoxamine	> 10 mM	162.0 μM
Antagonists	IC_{50}	Ki
Prazosin	450.0 nM	3.5 nM
Phentolamine	6.8 μM	304.4 nM
WB4101	17.0 μM	475.6 nM
Yohimbine	100.0 μM	6.2 μM
Rauwolscine	309.0 μM	9.0 μM

IC_{50} is expressed as the amount of antagonist required to cause 50% inhibition of the maximum response to noradrenaline; EC_{50} is the amount of drug required to produce 50% of the noradrnenaline response. Ki values for binding studies were calculated from the Chen Prusoff equation (1973).

The results show that there is good agreement between the effects of α-adrenoceptor agonists and antagonists on binding studies and the physiological response. They suggest that α-adrenoceptors are important in the control of intestinal transport and suggest that the effects in rat jejunum are mediated by α_1 adrenoceptors.

Chen, Y-C and Prusoff, W.H. (1973). Biochem. Pharmac. 22, 3099.
Cotterell, D.J., Munday, K.A. and Poat, J.A. (1982). Brit. J. Pharmac. 76, 36P.
Levens, N.R., Munday, K.A., Parsons, B.J., Poat, J.A. and Stewart, C. (1979) J. Physiol. 286, 351.
Wilson, T.H. and Wiseman, G. (1954). J. Physiol. 123, 116.

α-ADRENOCEPTORS AND THE INHIBITION BY CLONIDINE OF INTESTINAL SECRETION IN THE ANAESTHETIZED RAT

K.T. Bunce & C.F. Spraggs*, Department of Pharmacology, Glaxo Group Research Ltd., Ware, Hertfordshire, SG12 ODJ.

Clonidine has been reported to inhibit fluid secretion induced by various secretagogues in rat jejunum (Nakaki et al, 1982). We have investigated the effects of the predominantly α_2-adrenoceptor agonist, clonidine, and the α_1-adrenoceptor agonist, phenylephrine, on secretion induced by prostaglandin E_1 (PGE_1) plus theophylline in segments of jejunum, ileum and colon in the anaesthetized rat. The effects of the α-adrenoceptor antagonists yohimbine and prazosin on intestinal secretion have also been determined.

Male rats were anaesthetized with an allobarbital/urethane mixture and predosed with indomethacin, 10 mg kg^{-1} s.c. Segments of jejunum, ileum and colon were ligated and the animals injected intravenously with the test drugs. After 30 min, the segments were filled with 1.5 ml of Tyrode solution which contained [^{14}C]-polyethylene glycol 4000 (5g/l; 20 μCi/l) as a volume marker. Intestinal secretion was stimulated by the addition of theophylline (25 mM) to the intraluminal solution and infusion of PGE_1 (10 nmol kg^{-1} min^{-1}) retrogradely into the left carotid artery. After 60 min the intraluminal contents were collected and the net water flux determined.

Under control conditions the segments of jejunum, ileum and colon absorbed water at rates of -56 ± 4, -40 ± 6 and -94 ± 6 μl $cm^{-1}h^{-1}$ respectively (negative sign indicates net absorption, positive sign indicates net secretion of water). Clonidine (1 μmol kg^{-1} i.v.) had no significant effect on these basal rates of absorption. Administration of PGE_1 plus theophylline reduced the net absorption of water in the jejunum and ileum (-5 ± 6 and +3 ± 5 μl $cm^{-1}h^{-1}$ respectively) and induced a net secretion in the colon (+37 ± 8 μl $cm^{-1}h^{-1}$). In the jejunum, clonidine (0.1 to 3.0 μmol kg^{-1} i.v.) caused a dose related inhibition of the PGE_1/theophylline-induced response, returning water absorption to control levels with doses of 1 and 3 μmol kg^{-1} i.v.. The same doses of clonidine had no significant effect on the secretagogue-induced changes in the ileum and colon. Phenylephrine (1 μmol kg^{-1} i.v.) did not significantly inhibit the effect of PGE_1/theophylline in any segment of intestine. Yohimbine (0.03 to 3 μmol kg^{-1} i.v.) caused a dose related reversal of the inhibitory effect of clonidine (1 μmol kg^{-1} i.v.) in the jejunum, while prazosin (3 μmol kg^{-1} i.v.) had no significant effect.

Clonidine is a potent antidiarrhoeal agent in the rat (Lal & Shearman, 1981) and the inhibition of intestinal fluid accumulation may be an important aspect of such an effect. The present results suggest that this antisecretory action may be exerted only in the upper small intestine in this species. In addition the lack of effect of phenylephrine and the reversal of clonidine's antisecretory effect by yohimbine, but not by prazosin, are consistent with the view that this effect is mediated by α_2-adrenoceptors.

Lal, H. & Shearman, G.T. (1981) Drug Dev. Res. 1, 37-41
Nakaki, T. et al (1982) J. Pharmac. exp. Ther. 220, 637-641.

STIMULATION OF DOPAMINE-β-HYDROXYLASE IN RAT ADRENALS BY REPEATED CS_2 EXPOSURES

S. Caroldi*, J.A.E. Jarvis & L. Magos, MRC Toxicology Unit, MRC Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF.

The increased incidence of hypertension (Szobor, 1962) and coronary death (Tiller et al, 1968) in viscose rayon workers exposed to CS_2 initiated research on the effects of this toxic agent on catecholamine metabolism (Magos and Jarvis, 1970). The present study was undertaken to investigate catecholamine contents and dopamine-β-hydroxylase (D-β-H) activity in the adrenals of rats exposed repeatedly to CS_2. After a single 4 h exposure to 2.0 mg/l CS_2 the adrenal dopamine (DA) content increased by 135% and the noradrenaline (NA) and adrenaline (A) contents decreased by about 10%. Multiple exposure had no additional effect on DA but the NA and A contents declined by a further 10% after 9 daily exposures (table 1). These changes were in agreement with the known inhibitory effect of CS_2 on D-β-H. However the in vitro conversion of tyramine to octopamine indicated the gradual induction of this enzyme. D-β-H activity showed no noticable change after a single exposure, it increased by 24% after 5 and 53% after 9 exposures. The activity of D-β-H was estimated also in vivo 24 h after the 9th exposure (table 1).

Table 1 Total DA, NA and A contents and D-β-H activity in pairs of rat adrenals after 9 daily 4 h exposures to 2.0 mg CS_2/1. (The number of animals are shown in brackets).

	Mean ± s.e. mean		
	controls	h after CS_2 exposure	
		1	24
DA in nmol	0.7 ± 0.1 (10)	1.7 ± 0.1 (4)**	1.2 ± 0.1 (5)**
NA in nmol	42.4 ± 2.1 (10)	31.3 ± 1.1 (4)**	35.3 ± 2.5 (5)*
A in nmol	114.4 ± 2.6 (10)	90.5 ± 2.6 (4)**	103.0 ± 6.3 (5)
D-β-H activity in nmol octop/45 min	24.1 ± 0.9 (18)	36.7 ± 2.2 (5)**	29.9 ± 1.5 (13)**
Conversion of DA to NA in % of DA pool min^{-1}	4.5 ± 0.2 (6)		3.1 ± 0.1 (5)**
in Da pmol.min^{-1}	31.4 ± 1.3 (6)		36.9 ± 1.9 (5)*

*P<0.05 **P<0.01 by the two tailed Student's t-test.

The conversion of (^{14}C) DA into (^{14}C) NA was significantly lower in exposed than in control rats. This suggested a reduced DA turnover. Nevertheless the larger DA pool resulted in that in absolute amount more DA was converted to NA in the exposed rats. However the increase in D-β-H activity was not sufficient to restore at 24 h the catecholamine contents of the adrenals to the normal level.

The results show that exposure to CS_2 has a cumulative effect on the catecholamine metabolism of adrenals, and many of the features of this abnormal catecholamine metabolism are similar to the effects of repeated stress.

Szobor, A. (1962) Psychiat.Neurol.Basel, 143, 178-196
Tiller, J.R. et al (1968) Brit.Med.J. 4, 407-411
Magos, L. & Jarvis, J.A.E. (1970) Br.J.Pharmacol. 39, 26-33

BEHAVIOURAL EFFECTS OF Ro 5-4864, A LIGAND FOR THE "MICROMOLAR BENZODIAZEPINE RECEPTOR"

Sandra E. File* & P.S.Mabbutt, Department of Pharmacology, The School of Pharmacy, University of London, Brunswick Square, London WC1N 1AX.

A micromolar benzodiazepine receptor has been characterised in the rat CNS (Bowling & deLorenzo,1982), which can be distinguished from both the CNS nanomolar and from the peripheral benzodiazepine receptors, on the basis of binding,kinetics and pharmacological characteristics. Phenytoin also binds to these micromolar receptors, as does RO 5-4864 which has little affinity for the nanomolar receptors. In general, there are insignificant correlations between the potencies with which benzodiazepines bind to the micromolar receptor and their behavioural effects; however, a high correlation has been reported with protection against electroshock -induced convulsions (deLorenzo et al, 1981).

The present study explored further the possible behavioural significance of the micromolar receptor, by investigating the effects of RO 5-4864. Possible sedative effects have not yet been reported and these were therefore investigated in a holeboard test. This allows independent assessment of directed exploration (measured by head-dipping), locomotor activity and rearing. Male hooded rats were tested for 7.5 min, 20 min after i.p. injection with vehicle (water) or RO 5-4864 (20 mg/kg). Exploratory head-dipping was unchanged, but locomotor activity and rearing were significantly reduced ($P < .001$ &.01, respectively). Picrotoxin and the specific benzodiazepine antagonists RO 15-1788 & CGS 8216 counteract chlordiazepoxide-induced sedation (File,1982; File et al,1982; File & Lister,1983), and therefore the effects of these drugs on RO 5-4864-induced sedation were investigated. The sedation was potentiated by picrotoxin (1 & 2 mg/kg) and by CGS (3 mg/kg), but reversed by RO 15-1788 (4 & 10 mg/kg).

During the holeboard test some of the rats treated with both RO 5-4864 and picrotoxin (2 mg/kg) convulsed. These effects were further investigated in male mice. A subconvulsant dose of picrotoxin (4 mg/kg) significantly potentiated seizures in mice treated with 10 & 20 mg/kg RO 5-4864. Seizures induced by 30 mg/kg RO 5-4864 were reduced in a dose-related manner by pretreatment 1 hr beforehand with phenytoin (20 & 40 mg/kg) or with the quinoline derivative PK 11195 (30 & 60 mg/kg). The high incidence of frank seizures induced by 30 & 40 mg/kg RO 5-4864 are difficult to reconcile with its reported protection against electroshock (MED 36 mg/kg), but the antagonism by phenytoin suggests that the two drugs may be acting as agonist and antagonist at the same receptor.

However, these convulsant effects may not necessarily reflect actions at the CNS micromolar receptor, since RO 5-4864 and PK11195 are ligands for the peripheral-type benzodiazepine receptor and these behavioural effects could be mediated through such a receptor. The antagonism of the sedative effects by RO 15-1788 and its potentiation by CGS 8216 (ligands that do not bind to the peripheral receptor) suggest that these effects are centrally mediated.

Bowling,A.C. & deLorenzo,R.J. (1982) Science 216, 1247-1249
deLorenzo,R.J. et al (1981) Science 213, 546-548
File,S.E. (1982) Pharmac. Biochem. Behav. 17, in press
File,S.E. et al (1982) Neuroscience Letters, in press
File,S.E. & Lister R.G. (1983) This meeting

THE EFFECTS OF METHYL β-CARBOLINE-3-CARBOXYLATE ON THE RESPONSES TO GABA OF RAT HIPPOCAMPAL NEURONES

I.A. Paterson* & M.H.T. Roberts, Department of Physiology, University College, P.O. Box 78, Cathays Park, Cardiff.

An attempt has been made to determine if Methyl β-carboline-3-carboxylate (β-CCM) blocked GABA responses. This would be expected as β-CCM is a convulsant (Jones & Oakley, 1981) which binds with high affinity to benzodiazepine receptors which are functionally linked to GABA receptors (Braestrup & Nielsen, 1981). Extracellular recordings were made with 5 barrelled glass microelectrodes (tip diameter of 8 to 12 μm) of spontaneously firing CA1 hippocampal pyramidal cells in male Wistar rats under halothane anaesthesia. GABA (0.2M, pH 3.5) and Glutamate (0.2M, pH 8.0) were applied iontophoretically. β-CCM (250 μg/ml of 0.16M NaCl, pH 3.5) was applied by pressure-ejection as the electrical conductivity of β-CCM solutions was insufficient for iontophoresis. NaCl (0.165M, pH 3.5) was applied by pressure-ejection as a control. The GABA responses of one cell only were reduced by β-CCM and the responses of 5 others were apparently unaffected by β-CCM.

Binding studies have shown that β-CCM has a very high affinity for benzodiazepine receptors (Braestrup & Nielsen, 1981) and the pressure-ejection system has no means of preventing diffusion of drug from the electrode. This continual release of β-CCM may have been affecting the cells as 86% (n = 22) were consistently firing in bursts (trains of 3 or more spikes). When electrodes containing no β-CCM were used, only 34% (n = 38) of the cells observed were firing bursts. To reduce the diffusion of β-CCM from the electrode the concentration of β-CCM was reduced ten fold to 25 μg/ml of 0.165M NaCl (pH 7.0). Only 46% (n = 26) of cells recorded with these electrodes fired in bursts. Studies of GABA responses with this concentration of β-CCM were more successful. β-CCM reduced GABA responses in 7 of 11 cells without affecting responses to Glutamate. β-CCM also elevated the spontaneous firing rate of these cells.

It is thought that GABA is the neurotransmitter mediating recurrent inhibition of hippocampal pyramidal neurones (Anderson *et. al.*, 1964; Ben-Ari *et. al.*, 1979). In view of the reduction of GABA responses reported here it is most likely that β-CCM reduces the efficacy of endogenous GABA in the hippocampus resulting in an elevated firing rate and an increased tendency to fire in bursts. The effect of β-CCM upon GABA responses is similar to the effect of systemically administered Ethyl β-carboline-3-carboxylate upon hippocampal neurones reported by Polc *et. al.* (1981). Further studies will make a quantitative comparison of the actions of these drugs.

We thank Glaxo for supplying β-CCM.

I.A.P. is an SRC (CASE) student.

Anderson, P. *et. al.* (1964) J. Neurophysiol. 27, 608-619.
Ben-Ari, Y. *et. al.* (1979) Can. J. Physiol. Pharmacol. 57, 1462-1466.
Braestrup, C. and Nielsen, M. (1981) Nature (Lond.) 294, 472-474.
Jones, B.J. and Oakley, N.R. (1981) Br. J. Pharmacol. 74, 884P.
Polc, P. *et. al.* (1981) Brain Research 217, 216-220.

EFFECT OF ANTI-INFLAMMATORY DRUGS ON cAMP-STIMULATED RAT GASTRIC ACID SECRETION, IN VIVO AND IN VITRO

K.M. Donaldson* and I.H.M. Main, Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX

Indomethacin potentiates dibutyryl cyclicAMP (dbcAMP) stimulated rat gastric acid secretion, (Main & Whittle, 1975; Donaldson & Main, 1981). In the present study the effects of a number of anti-inflammatory drugs has been compared in vivo and in vitro.

For in vivo experiments (Main & Whittle, 1975), anti-inflammatory agents were given subcutaneously 6 hours before 20 mg/kg dbcAMP was administered intravenously to anaesthetised rats. Samples of gastric perfusate were collected and the acid content estimated. Secretory responses, (μmol/75 min), were larger after treatment with 15 mg/kg indomethacin, (23.5±7.2, n=6, $p < 0.05$) 100 mg/kg phenylbutazone (24.5±6.1, n=7, $p < 0.05$) or 30 mg/kg sulindac (15.3±2.0, n=5, $p < 0.005$) when compared with controls (7.4±1.4, n=6). Sodium salicylate (100 mg/kg) had no effect (5.8±1.2, n=5). All drugs except sodium salicylate, caused gastric erosions.

In vitro (Main & Pearce, 1978) paired test and control mucosae were used. The test was treated with an anti-inflammatory drug given serosally 1 hour before adding dbcAMP. EC_{50} values for potentiation of 0.1 mM dbcAMP were calculated for indomethacin (0.1 μM) and sulindac (17 μM). Potentiation was caused by 10 and 100 μM BW755C ($p < 0.05$) but not by sodium salicylate (0.01-10 mM) or phenylbutazone (0.1-1 mM). However, when rats used for in vitro experiments were given an anti-inflammatory drug subcutaneously 6 hours before the addition of dbcAMP the responses ($\mu mol/cm^2/h$) were significantly ($p < 0.01$) larger for both 15 mg/kg indomethacin (7.1±1.0, n=11) and 100 mg/kg phenylbutazone (6.9±1.0, n=10) than in controls (3.7±0.8, n=12). When mucosae from phenylbutazone treated rats were bathed in a 0.1 mM solution of this drug the potentiation was masked (2.2±0.5, n=10) by a local inhibitory effect.

These results show that drugs which inhibit cyclo-oxygenase and cause gastric erosions can potentiate dbcAMP stimulated acid secretion in vivo and in vitro, whereas sodium salicylate, which does not inhibit rat mucosal cyclo-oxygenase or cause erosions (Whittle et al, 1980) has no effect. Potentiation of dbcAMP-induced secretion by anti-inflammatory drugs may therefore, reflect their ability to inhibit gastric mucosal cyclo-oxygenase and their ulcerogenic potential.

K.M.D. is an MRC Scholar

Donaldson, K.M. & Main, I.H.M. (1981). Br. J. Pharmac. 74, 970P
Main, I.H.M. & Pearce, J.P. (1978). J. Pharmac. Methods 1, 27-38
Main, I.H.M. & Whittle, B.J.R. (1975). Br. J. Pharmac. 53, 217-224
Whittle, B.J.R. et al, (1980). Nature 284, 271-273.

EVIDENCE THAT (D-PRO^2, D-TRP^{7,9})-SP, A PUTATIVE SUBSTANCE P ANTAGONIST, IS AN ANTAGONIST AT 'E'-TYPE RECEPTORS

J.W. Growcott* and A.V. Tarpey, Bioscience Dept. II, ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG

A series of Substance P analogues with D-amino acid substituents have recently been demonstrated to possess specific Substance P-antagonist activity in various assays (Folkers et al., 1981; Engberg et al., 1981). Amongst this series is tri-substituted analogue [D-Pro^2, D-Trp^{7,9}]-SP which has been reported to be a specific and competitive antagonist of Substance P in the guinea-pig ileum (Rosell and Folkers, 1982). Moreover, in the guinea-pig taenia coli, this compound has been shown to block the actions of the tachykinins physalaemin and eledoisin (Leander et al., 1981).

The recent suggestions of Lee et al. (1982) and Hawcock et al. (1982), that there may be multiple receptors for Substance P, led us to determine if [D-Pro^2,D-Trp^{7,9}]-SP was specific for a particular receptor sub-population in vitro. Thus, in the current study, we have determined the antagonist profile of the compound in guinea-pig ileum, urinary bladder and field-stimulated vasa deferentia against three tachykinins. The putative antagonist was allowed 15 min for equilibration at 10^{-5}M.

Our results (Table 1) support the hypothesis for multiple Substance P receptors, but indicate that the putative Substance P antagonist [D-Pro^2,D-Trp^{7,9}]-SP acts predominantly at Substance P 'E'-type receptors.

Table 1 Potency of [D-Pro^2,D-Trp^{7,9}]-SP against tachykinins on isolated tissues

	Substance P	Physalaemin	Eledoisin
Guinea-pig ileum	6.6 ± 0.5 (4)	9.9 ± 0.7 (3)	< 2.0 (3)
Guinea-pig ileum + atropine (1μM)	4.3 ± 0.9 (3)	12.4 ± 1.8 (3)	31.9 ± 2.2 (3)
Guinea-pig urinary bladder	< 2.0 (3)	< 2.0 (3)	4.0 ± 0.4 (3)
Guinea-pig field-stimulated vas deferens	< 2.0 (3)	8.0 ± 1.6 (3)	26.6 ± 4.3 (3)

Figures denote ratios (mean ± s.e.m. (n))

Engberg, G. et al. (1981) Nature (London) 293, 222-223
Hawcock, A.B. et al. (1982) Eur. J. Pharmacol. 80, 135-138
Leander, S. et al. (1981) Nature (London) 294, 267-269
Lee, B.E.B. et al. (1982) N.S. Arch. Pharmacol. 318, 281
Rosell, S. and Folkers, K. (1982) T.I.P.S., 3, 21-212

IDENTIFICATION AND PHARMACOLOGY OF VOLTAGE SENSITIVE CALCIUM CHANNELS IN CLONAL CELL LINES

S.B. Freedman*, G. Dawson[1], R.J. Miller, M.L. Villereal & R.E. West, Jr., Dept. of Pharmacological and Physiological Sciences and [1]Depts. of Pediatrics and Biochemistry, University of Chicago, Chicago, Illinois 60637, USA

Organic calcium antagonists are known to block voltage sensitive channels in a variety of tissues. However, it has been suggested that these drugs exhibit a different sensitivity towards cardiac and smooth muscle compared with nerve terminals (Nachshen and Blaustein, 1979). We have examined a variety of neuronal cell lines for the presence of voltage sensitive calcium channels. This study has included neuroblastoma N4TG1, neuroblastoma x glioma hybrid NG108-15 and neuroblastoma x brain hybrid NCB-20.

Cultured cells were grown and assayed for $^{45}Ca^{2+}$ uptake as previously described (West et al., 1982). Depolarisation of cells with 50 mM KCl produced a large increase in the uptake of $^{45}Ca^{2+}$ into NCB-20 cells (0.97 $\pm$ 0.03 nmol/mg protein/10 min, normal K; 3.02 $\pm$ 0.06 nmol/mg protein/10 min, high K; n=4). Calcium uptake was also stimulated by 10 μM veratridine (72% of high K uptake). Results of 5 hour incubations suggested that the increase was due to a change in uptake rate rather than compartment size. In contrast to NCB-20 cells, only a small increase was seen with N4TG1 cells (1.43 $\pm$ 0.03 nmol/mg protein/10 min, normal K; 2.02 $\pm$ 0.16 nmol/mg protein/10 min, high K; n=4) whilst no increase was seen with NG108-15 cells (1.96 $\pm$ 0.1 nmol/mg protein/10 min, normal K; 2.32 $\pm$ 0.26 nmol/mg protein/10 min, high K; n=4).

Table 1 Effect of Calcium Channel Antagonists on $^{45}Ca^{2+}$ Uptake into NCB-20 Cells

Drug	IC_{50}(nM)
Nisoldipine	0.58
Felodipine	2.10
Nimodipine	5.60
Nitrendipine	6.40
Nifedipine	9.20
D-600	350.00
Flunarizine	750.00
Cinnarizine	750.00
Verapamil	1,800.00
Diltiazem	4,500.00

The depolarisation sensitive uptake in NCB-20 cells was inhibited by a wide variety of organic calcium antagonists (Table 1), whereas these drugs had little effect on basal calcium uptake. Calcium uptake was also antagonised by a variety of divalent cations. $CdCl_2$, $NiCl_2$, $MnCl_2$, $CoCl_2$ and $SrCl_2$ (all at 1 mM) produced an inhibition of depolarisation sensitive uptake by 100,72,69,59 and 26% respectively. Interestingly, 1 mM $LaCl_3$ produced an identical increase in both normal and high K uptake (114% above high K uptake), whereas 1 mM $BaCl_2$ selectively stimulated depolarisation sensitive uptake (665% above high K uptake).

These results suggest that clonal cell lines may differ in the number of calcium channels or in their sensitivity to depolarising stimuli. In particular, NCB-20 cells appear to be a useful model to study voltage sensitive calcium channels and to examine the mechanisms of action of organic calcium antagonists.

We thank Miles, Janssen, Pfizer, Knoll and Marion for gifts of drugs.

Supported by USPHS grant DA-02121 and a SERC/NATO U.K. Fellowship to S.B.F.

Nachshen, D.A. & Blaustein, M.P. (1979) Mol. Pharmacol. 16, 579-586
West, Jr., R.E. et al (1982) Life Sci. In press

TISSUE-SPECIFIC REGULATION OF (^{3}H)-NIMODIPINE BINDING TO PUTATIVE CALCIUM-CHANNELS BY THE BIOLOGICALLY ACTIVE ISOMER OF DILTIAZEM

D.R. Ferry & H. Glossmann*, Rudolf-Buchheim Institut für Pharmakologie, Frankfurterstr. 107, 6300 Giessen, F.R.G.

Nimodipine is a 1,4-dihydropyridine Ca^{2+} -antagonist with an ED_{50} of 0.5-1.0 nM (Towart and Kazda 1979). In guinea-pig brain membranes stereospecific, saturable and fully reversible high affinity [^{3}H]-nimodipine ([^{3}H]-NIM), (160-200 Ci/mmol) binding with a Hill slope of 1.0 has been reported (Ferry and Glossmann 1982). In these membranes (at 37° C in 50 mM TRIS-HCl, pH=7.4) d-cis-diltiazem (a potent Ca^{2+} -antagonist chemically unrelated to 1,4-dihydropyridines, Nagao et al 1972) enhances [^{3}H]-NIM binding with an ED_{50} of 383 ± 72 nM (n=3). This enhancement is due to a decrease in the dissociation constant (K_D); the maximum number of binding sites (B_{max}) remains constant. This regulation is stereospecific as l-cis-diltiazem inhibits saturable [^{3}H]-NIM binding with an IC_{50} of 221± 60 μM (n=3).

Guinea-pig skeletal muscle, duodenum and heart homogenates were freed of cellular debris by a low speed spin (2000xg/15min). High speed centrifugation yielded a plasma membrane fraction. In the case of skeletal muscle this membrane fraction was enriched (with respect to the homogenate) 4.98± 0.18 (n=3) in [^{3}H]-ouabain binding sites and 5.20 ±0.39 (n=3) fold in [^{3}H]-NIM binding sites. In skeletal muscle membranes d-cis-diltiazem enhanced [^{3}H]-NIM binding with an ED_{50} of 1017± 134 nM (n=3). The enhancement was due to a significant increase in B_{max} from 2.0± 0.25 to 10.6 ±2.1 pmol/mg protein. ($P < 0.001$). The K_D of [^{3}H]-NIM was also significantly reduced from 1.5 ±0.03 (n=3) to 0.99 ±0.15 nM (n=3) ($P < 0.05$), although the Hill slope of the saturation was unity under both experimental conditions. l-cis-Diltiazem inhibited [^{3}H]-NIM binding in skeletal muscle membranes with an IC_{50} of 42 ± 18 µM (n=3), and was a significantly more potent inhibitor in the skeletal muscle than in the brain.

In heart membranes the K_D of [^{3}H]-NIM was 0.26 ±0.003 nM (n=3). d-cis-Diltiazem caused no change in K_D but increased the B_{max} from 333 ± 13 to 423 ± 22 fmol/mg protein ($P < 0.05$). l-cis-Diltiazem inhibited [^{3}H]-NIM binding to the heart membranes with an IC_{50} of 241 ±42 µM (n=3).

Guinea-pig duodenum, in contrast to the other tissues described above, is a poor source of [^{3}H]-NIM binding sites and the Scatchard plots of saturation isotherms are curvilinear. The low affinity site had a K_D of 2-5 nM and B_{max} of 170-230 fmol/mg protein, whilst the high affinity site had a K_D of 0.2-0.5 nM with a B_{max} of 50-80 fmol/mg protein. High protein concentrations are required to get a analysable signal (300 µg/ml gives 1000 cpm signal over 900 cpm blank at 2.5 nM [^{3}H]-NIM). The duodenum is a problematic tissue to study Ca^{2+} -antagonist binding.

In conclusion, [^{3}H]-NIM binding sites co-purify with [^{3}H]-ouabain binding sites and may be located in the plasma membrane. d-cis-Diltiazem acts at a distinct site from 1,4-dihydropyridines in the putative Ca^{2+} -channel. Ca^{2+} -channels in isolated guinea-pig membranes from different tissues respond differentially to d-cis-diltiazem regulation of 1,4-dihydropyridine binding. The greater potency of d-cis-diltiazem (the biologically active isomer) over l-cis-diltiazem (the biologically inactive isomer) suggests that radiolabelled Ca^{2+}-antagonist binding is to phyiologically relevant Ca^{2+}-channels.

Ferry, D.R. and Glossmann, H. (1982) Naunyn-Schmied. Arch. Pharmac. (in press).
Nagao, T. et al (1972) Japan. J. Pharmac. 22, 467.
Towart, R. and Kazda, S. (1979) Br. J. Pharmac. 67, P409.

SPECIFIC BINDING OF (^{3}H)-LYSERGIC ACID DIETHYLAMIDE (LSD) TO HUMAN PLATELET MEMBRANES

D.P. Geaney*, M. Schachter, J.M. Elliott and D.G. Grahame-Smith, MRC Unit and University Department of Clinical Pharmacology, The Radcliffe Infirmary, Oxford.

5-Hydroxytryptamine (5-HT) interacts with a specific receptor on intact human platelets to induce shape change and aggregation. A separate recognition site mediates the active uptake of 5-HT by intact cells. (^{3}H)-5-HT labels both these binding sites (Peters and Grahame-Smith 1980), complicating the analysis of binding to each separate site. LSD is a potent inhibitor of 5-HT induced platelet aggregation, but has no significant effect on active 5-HT uptake. We have therefore investigated the binding of (^{3}H)-LSD to human platelet membranes.

Platelet-rich plasma was prepared by low-speed centrifugation of venous blood, anti-coagulated with 1% EDTA. This was centrifuged at 1200 x g for 7.5 min at 10°C to produce a platelet pellet which was resuspended in hypotonic Tris-EDTA buffer, pH 7.5. The suspension was twice homogenised and centrifuged at 30 000 x g for 15 min. The membrane pellet was suspended in 50 mM Tris-HCl buffer, pH 7.3, containing 120 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$ and 0.05% ascorbic acid. The centrifugation was repeated, and the final pellet was resuspended in the same medium. In binding assays, aliquots of the membrane suspension were incubated with 0.25 - 2.5 nM (^{3}H)-LSD for 4 h at 37°C in a total volume of 0.5 ml. Specific binding was defined as the difference in (^{3}H)-LSD binding occurring in the presence and absence of 300 nM spiperone. Incubations were terminated by filtration under reduced pressure through Whatman GF/F filters. Aliquots of membrane suspension were taken for protein assay.

Specific binding of (^{3}H)-LSD is saturable, reversible and linearly related to membrane protein concentration. Scatchard analysis of binding data showed an affinity (mean K_D ± SD, n = 19) of 0.53 ± 0.1 nM and a capacity (B_{max} ± SD) of 57.1 ± 24.4 fmol/mg protein. By kinetic analysis, the K_D was 0.33 nM with an association rate constant of 0.012 $nM^{-1}min^{-1}$ and a dissociation rate constant of 0.004 min^{-1}. Binding studies on a single subject on 4 separate occasions showed a coefficient of variation of 16% for the K_D and 11% for the B_{max}. The rank order of potency for inhibitors of specific (^{3}H)-LSD binding is: d-LSD > metergoline > pirenperone > spiperone > ketanserin >> mepyramine > l-propranolol > phentolamine, and showed stereo specificity (d-butaclamol >> l-butaclamol). Among the biogenic amines tested, 5-HT was by far the most potent inhibitor of(^{3}H)-LSD binding.

The characteristics of the (^{3}H)-LSD binding site resemble those of the 5-HT receptor involved in platelet aggregation. This technique may prove useful in studying pathological and drug-induced changes in 5-HT receptors in man.

Peters, J.R. and Grahame-Smith, D.G. (1980) Eur J Pharmacol **68**, 243.

(^{125}I)LSD, A NEW LIGAND WITH SELECTIVITY FOR 5-HT$_2$ RECEPTORS

G. Engel*, E. Müller-Schweinitzer, P. Stadler (introduced by B. Berde). Preclinical Research, Sandoz Ltd., CH 4002 Basle, Switzerland

Two distinct types of serotonin (5-HT) receptors have been postulated by ligand binding studies. 5-HT$_1$ receptors are labelled by ^{3}H-5-HT (Peroutka & Snyder, 1979) and 5-HT$_2$ receptors by ^{3}H-spiperone or ^{3}H-ketanserin (Leysen et al., 1982) to mention only two examples. ^{3}H-LSD was reported as a non discriminating ligand for both receptor types (Peroutka & Snyder, 1979). We iodinated LSD with ^{125}I Na and chloramine T (Hunter & Greenwood, 1962) to get the radioligand ^{125}I-LSD (^{125}IOL) and with N-I-succinimide to get the non-radioactive compound 2-I-LSD (IOL) for comparative pharmacological studies. IOL and ^{125}IOL showed the same chromatographic pattern in HPLC and HPTLC analysis. The radioligand, with a specific radioactivity of 2175 Ci/mmol, was very stable under our assay (1h incubation, 37°C) or storage conditions (-20°C, 3 months).

In comparison with non-iodinated LSD, the introduction of iodine in position 2 of the ergot ring system leads to selectivity for 5-HT$_2$ receptors. Analysis of the binding characteristics of ^{125}IOL to rat brain cortex membranes yields a dissociation constant K_D = 0.9 ± 0.1 nM and a maximum of binding sites B_{max} = 240 ± 20 fmoles/mg protein and indicated a uniform class of binding sites. Non-specific binding is routinely determined in presence of 0.1 µM ketanserin or 3 µM cinanserin and does not exceed 40% of total binding at the K_D concentration. In kinetic experiments, the rate constants for association and dissociation are 1.6 ± 0.5x10^8 M^{-1} min^{-1} and 0.042 min^{-1}, respectively. In competition experiments, 5-HT antagonists show monophasic and 5-HT agonists biphasic displacement curves. The K_I-values of the antagonists correlate well with pD_2'-values for inhibition of 5-HT-induced contraction of canine basilar artery.

In a routine binding screen with various ligands, the IC_{50} constants of IOL for α_1, β, histamine, muscarinic and 5-HT$_1$ receptors are higher than 100 nM. A moderate affinity of IOL to dopamine receptors (IC_{50} = 26 nM) was observed.

The low dissociation constant of ^{125}IOL together with its high specific radioactivity permits detection of even small 5-HT$_2$ receptor densities such as are found in the guinea pig ileum.

We thank Dr. A. Closse and Dr. W. Frick for investigating IOL in their binding screen.

Hunter, W.M. & Greenwood, H. (1962). Nature 194, 495-496.
Leysen, J.E. et al. (1982). Mol.Pharmacol. 21, 301-314.
Peroutka & Snyder, S.N. (1979). Mol.Pharmacol. 16, 687-699.
Troxler, F. & Hofmann, A. (1957). Helv.chim.Acta 40, 2160.

REFLEX CHANGES IN BREATHING TO 5-HT IN THE ANAESTHETIZED RAT

H.W. Mitchell, Department of Physiology, Sheffield University, Sheffield s10 2TN.

Activation of the J-receptors (Paintal, 1955) in cats, dogs and rabbits causes transient apnoea and possible bronchoconstriction (reviewed by Widdicombe, 1974). These reflex responses are evoked by activation of nonmyelinated nerve fibres which run in the vagi. Pharmacological agents capable of stimulating the reflex include 5-HT, capsaicin and phenyldiguanide. In this paper a reflex apnoea to 5-HT is described. Further, the effect of sodium cromoglycate was measured on the evoked reflex since this drug has been shown to reduce lung 'C' fibre discharge in the dog (Dixon et al., 1980).

Male rats (250-300 g) were anaesthetised with urethane (1.5 g/kg ip). The trachea was cannulated for recording airflow ($\dot{V}$) and tidal volume (VT, obtained by integration of $\dot{V}$). Drugs (made up in normal saline) were administered via a cannula in the jugular vein. The cannula was advanced to within 1 cm of the heart. In some experiments blood pressure was also recorded from a carotid artery, and in other experiments the cervical vagi were cooled with brass thermodes. The signals were displayed on a chart recorder.

Injection of 5-HT (10-25 µg) caused inspiratory apnoea, bradycardia and hypotension. There was also a reduction in $\dot{V}$ and VT. In 9 rats the duration of apnoea was 3.3 $\pm$ 0.6 s (mean $\pm$ s.e. mean $P < 0.001$). Also $\dot{V}$ and VT were reduced to 80.3 $\pm$ 3.6% ($P < 0.01$) and 70.7 $\pm$ 3.6% ($P < 0.001$) respectively of control levels. The latency of the apnoea was very short (< 1.5 s). Apnoea, bradycardia and hypotension, of short latency, were also observed to capsaicin (10 µg) and phenyldiguanide (5 µg). Saline and acetylcholine controls were without effect on the breathing rate. Section of the cervical vagi abolished the 5-HT induced apnoea, bradycardia and hypotension but not the changes in $\dot{V}$ and VT. In 4 rats when the cervical vagi were cooled, the apnoea in response to 5-HT was attenuated. The apnoea was abolished in 2 cases at $<$ 1-4 ^{o}C and at 7-10^{o}C in the remaining 2 animals.

Sodium cromoglycate (0.1-1 mg/kg iv) did not alter the apnoea following 5-HT, or the changes in $\dot{V}$ and VT.

The vagal reflex described in the anaesthetised rat is characteristic of the pulmonary J-receptor reflex found in other species. However, the cooling results are equivocal and may implicate both myelinated and nonmyelinated nerves. The reflex does not appear to be attenuated by sodium cromoglycate.

A gift of sodium cromoglycate from Fisons Pharmaceuticals is acknowledged. Supported by the Sheffield University Medical Research Fund.

Dixon, M. et al., (1980) Br. J. Pharmac. 70, 11-13
Paintal, A.S. (1955) Q. J. exp. Physiol. 40, 89-111
Widdicombe, J.G. (1974) Rec. Adv. Physiol. 9, 239-278

EFFECTS OF 5-HYDROXYTRYPTAMINE ON A NEW PHARMACOLOGICAL PREPARATION, THE MOUSE BLADDER STRIP

J.H. Wyllie,* Marie Cooper & N.M. Please, Surgical Unit, and the Department of Statistics, University College, London.

The urinary bladder of a mouse was cut into a strip and mounted between two pieces of 36 SWG stainless steel wire so as to allow stimulation of the tissue by electrical pulses (8 volts, 2 msec, 10 Hz), recording of contractions using a pendulum lever, (resting tension about 0.3 g) and superfusion by Krebs' solution containing hyoscine 10^{-7}M (37°C 5 ml/min).

The tissue developed a steady resting tone and responded to trains of pulses with a twitch. The increase in tension was proportional to the logarithm of the number of pulses applied, up to 32 or 64 pulses. The optimum frequency was 10 Hz. Eight volts was about twice threshold for 2 msec pulses. The tissue proved robust, responding reproducibly for many hours.

When 5-hydroxytryptamine (5HT, 10 - 1000 nM) was added to the superfusion fluid it usually increased basal tone, but the effect was slight and was abolished by 10^{-6}M methysergide. Another effect of 5HT was potentiation of the response to electrical stimulation; this was usually marked (e.g. doubling the effect of each electrical pulse), sensitive (EC50 = 50 nM) consistent, sustained, and reproducible. In contrast to the effect on basal tone, potentiation was not abolished by 10^{-6}M methysergide or any or all of the other six 5HT antagonists we have tested - cyproheptadine, trazodone, ketanserin, chlorpromazine, quipazine and morphine, all 1 uM (see Table).

EFFECTS OF SOME 5-HT ANTAGONISTS ON RESPONSES OF SUPERFUSED MOUSE BLADDER

	% change K1	F	P	% change K2	F	P	K3 (nM) control	test	F	P
Chlorpromazine	0.2	0.0	NS	-14.1	3.0	NS	42	28	4.7	NS
Cyproheptadine	5.7	9.1	*	-31.0	14.3	**	68	95	2.6	*
Ketanserin	-16.3	40.18	**	18.3	4.7	NS	51	54	0.0	NS
Methysergide	-2.1	2.3	NS	7.4	1.6	NS	28	51	7.8	*
Morphine	-5.7	3.3	NS	9.6	1.3	NS	40	41	0.0	NS
Quipazine	15.4	4.8	NS	-30.3	39.6	**	51	67	1.4	NS
Trazodone	-0.8	0.4	NS	-7.0	1.0	NS	85	77	0.7	NS

K1 is the twitch height in the absence of 5-HT. K2 is the maximum increase in twitch height produced by 5-HT. K3 is the nanomolar concentration of 5-HT required to produce half the maximum possible potentiation. All three parameters were estimated by computer and values shown are means from 3 experiments with each drug in which K1, K2, and K3 were estimated 3 times before and 3 times after addition of the drug. Analysis of variance was used to indicate the significance of changes in parameters: * = <0.05, ** = <0.01.

PRE- AND POSTJUNCTIONAL EFFECTS OF TUBOCURARINE AND TRIMETAPHAN INVOLVED IN TETANIC FADE AT THE RAT NEUROMUSCULAR JUNCTION

A.J. Gibb* & I.G. Marshall, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW.

Tetanic-fade during tubocurarine-induced neuromuscular block has been explained in terms of ion channel block and/or prejunctional block (see Bowman, 1980 for review). Trimetaphan was shown to be a powerful channel-blocking drug (Gibb & Marshall, 1982) and we have now compared the effects of tubocurarine with trimetaphan in isolated rat phrenic nerve-hemidiaphragm preparations.

In intact nerve-muscle preparations tubocurarine (2.5×10^{-7} - 10^{-6}M) produced a concentration-dependent decrease in single twitch tension (0.1Hz), and a concentration-dependent fade of trains of four twitches (2Hz for 2 sec) and of tetanic contractions (50Hz for 1.9 sec) (Table 1). In voltage-clamped hemidiaphragms cut to prevent muscle contractions, tubocurarine produced a concentration-dependent reduction of the peak amplitude (I_p) and time constant (τ_{epc}) of single (0.5Hz) neurally evoked endplate currents (epcs) (Table 1). The reduction of τ_{epc} by the concentrations of tubocurarine studied showed no marked dependence on membrane potential over the range -20mV to -90mV. Tubocurarine produced a concentration-dependent increase in the rundown of trains of neurally evoked epcs (50Hz for 0.4 sec) (Table 1). In agreement with the results of Magleby et al (1981) this increase in rundown was not dependent on membrane potential. In contrast to the rundown of trains of neurally-evoked epcs, tubocurarine (2.5×10^{-7}M) produced no rundown of trains of iontophoretically evoked epcs (10, 20 and 50Hz for 0.4 sec) in uncut tetrodotoxin-treated (10^{-7}M) preparations voltage clamped at -80mV. Tubocurarine simply reduced the amplitude of each epc relative to the corresponding current in control trains by 51 ± 11% (n=3).

In contrast to the results observed with tubocurarine, trimetaphan (2.5×10^{-5}M), which has previously been shown to cause tetanic fade and a voltage-dependent rundown of neurally evoked trains of epcs (Gibb & Marshall, 1982), produced a voltage- and frequency- dependent rundown of trains of iontophoretically evoked epcs. For example, trimetaphan produced 41 ± 7% (n=4) rundown of 50Hz trains at -90mV.

We conclude that postjunctional channel block contributes to tetanic fade produced by trimetaphan, but cannot account for the fade produced by tubocurarine.

Table 1 Percentage change in muscle tension and endplate currents produced by tubocurarine ± S.E. mean

Tubocurarine concn (M)	2.5×10^{-7}	5×10^{-7}	7.5×10^{-7}	10^{-6}	n
Single twitch block	1 ± 2	2 ± 1	8 ± 3	20 ± 5	6
Train of four fade	5 ± 1	10 ± 2	30 ± 2	58 ± 9	6
Tetanic fade	14 ± 3	63 ± 8	98 ± 1	100 ± 0	6
Reduction in I_p (-60mV)	67 ± 8	87 ± 2	94 ± 2	-	3
Reduction in τ_{epc}(-60mV)	38 ± 6	35 ± 5	45 ± 1	-	3
Increased epc rundown (-60mV)	9 ± 4	17 ± 1	36(n=1)	-	3

Supported by grants from the Medical Research Council and the Smith Kline & French Foundation. AJG holds a Science and Engineering Research Council CASE award.

Bowman, W.C. (1980) Anesth. Analg. 59, 935-943.
Gibb, A.J. & Marshall, I.G. (1982) Br. J. Pharmac. 76, 187P.
Magleby, K.L. et al (1981) J. Physiol. 312, 97-113.

DOES METHOHEXITONE FACILITATE NEUROMUSCULAR TRANSMISSION AT THE CHICK NEUROMUSCULAR JUNCTION?

R.C. Elliott and F.A. Wali**Department of Applied Biology, Brunel University, Uxbridge, Middlesex, UB8 3PH, England.

Bell & Wali (1981), using the moving fluid electrode technique (Fatt, 1950) and working on the isolated chick biventer cervicis (BVC) nerve-muscle preparation, found that methohexitone (88μM) increased the amplitude of the depolarizations and contractions produced by repetitive nerve stimulation, at 0.2 Hz with 5V and 0.5 ms pulse duration. In contrast, methohexitone reduced the depolarizations and contractures produced by bath application of acetylcholine (ACh).
The present study is an extension of the results previously obtained by Bell & Wali (1981). The effects of methohexitone (88μM) on the depolarizations & contractions produced by repetitive nerve stimulation at various frequencies (1-30 Hz) were studies in the same preparation.

Repetitive electrical stimulation of the motor nerve produced frequency-dependent depolarizations and contractions in the chick BVC muscle. These responses were increased by methohexitone (88μM). The mean (±SEM) frequency of stimulation which produced 50% maxium contraction (Freq. 50% max.) in the control Krebs solution and in methohexitone were 9.2±0.16 Hz and 8.5±0.23 Hz, n=6, $P < 0.05$, respectively. The mean (±SEM) Freq. 50s for the depolarizations were 7.2±0.16 Hz and 5.8±0.1 Hz, n=6, $P < 0.001$, respectively. Maximum contraction of 2.17±0.04 g tension was produced by repetitive nerve stimulation at 20 Hz in the control Krebs solution, and this was increased in methohexitone to 2.6±0.05 g tension (an increase of 17%±1.63, n=6, $P < 0.05$). Maximum depolarization of 0.78±0.02 mV was produced by 20 Hz in the control Krebs solution, and this was increased by 19%±1.38 (n=6, $P < 0.001$) in methohexitone.

Methohexitone did not alter the relationship between the depolarization and contraction responses produced by repetitive nerve stimulation at 1-30 Hz, with 5V and 0.5 ms pulse duration. The calculated slopes for the depolarization and contraction responses in the control Krebs solution and in methohexitone were 3.2 $g.mV^{-1}$ and 3.0 $g.mV^{-1}$ respectively. The intercepts on the Y axis were -0.56 g and -0.59 g respectively, and the corresponding correlation coefficients were 0.96 and 0.96.

In conclusion, the present results confirm those previously obtained by Bell & Wali (1981) and Elliott (1979), and show that methohexitone, in low concentrations may facilitate neuromuscular transmission (Westmoreland, Ward & John, 1971). However, methohexitone may have other actions at the chick BVC muscle (Wali, 1982). Experiments are in progress, using intracellular recording, to further investigate the precise mode(s) of action of methohexitone at the chick neuromuscular junction.

Bell, C. & Wali, F.A. (1981). Br. J. Pharmacol. 73, 311 P
Elliott, R.C. (1979). Br. J. Pharmacol. 66, 391-395
Fatt, P. (1950). J. Physiol. 111, 408-422
Wali, F.A. (1982). Br. J. Pharmacol. 76, 186 P
Westmoreland, B.F., Ward, D. & John, T.R. (1971). Brain Res. 26, 465-468

**Present address: Anaesthetics Unit, The London Hospital Medical College, Whitechapel, London E1 1BB.