# CARDIOVASCULAR EFFECTS OF RX781094 IN THE RABBIT: POSSIBLE PARTIAL AGONIST EFFECT IN ADDITION TO $\alpha_2$-ADRENOCEPTOR ANTAGONISM

H.W. Dalrymple, C.A. Hamilton, J.A.M. Hannah, J.L. Reid
University Department of Materia Medica, Stobhill General Hospital, Glasgow.

RX781094 (2-(2(1,4 benzodioxanyl))2-imidazoline hydrochloride) has been shown to be a potent and selective $\alpha_2$ adrenoceptor antagonist in rats and mice both centrally (Dettmar et al 1981) and peripherally (Chapleo et al 1981). In rabbits we have confirmed that RX781094 antagonises the cardiovascular effects of clonidine. However, in addition, in conscious rabbits RX781094 causes an acute transient dose related pressor response after IV dosing and a concentration related contractile response ($10^{-8}$-$10^{-5}$M) in rabbit isolated aortic strips.

Table 1 Pressor Responses (mmHg mean ± SD) to I.V. RX781094

| Time (min) | Saline | RX781094 (mg/kg) 0.1 | 0.5 | 1.0 | 5.0 |
|---|---|---|---|---|---|
| 0.5 | +2 ± 1 | +9 ± 5 | +17 ± 14 | +25 ± 12 | +26 ± 17 |
| 5 | -1 ± 4 | +2 ± 4 | + 1 ± 4 | + 4 ± 2 | - 6 ± 3 |
| 15 | +2 ± 3 | -2 ± 6 | + 4 ± 2 | 0 ± 6 | - 2 ± 3 |

Pretreatment of rabbits in vivo with prazosin (0.1 and 0.5 mg/kg) or α yohimbine (1 mg/kg) significantly reduced ($p < 0.01$) the pressor response to RX781094 (1.0 mg/kg IV) from 25 ± 12 mmHg to 8 ± 4, 9 ± 5 and 6 ± 3 mmHg respectively. In vitro contractile response to $10^{-6}$M RX781094 was reduced by 80% by $10^{-8}$M prazosin.

The rapid onset of the pressor response (30 sec to maximum) and the in vitro contractile response make a central mechanism unlikely. In order to differentiate between a prejunction site of action either via $\alpha_2$ adrenoceptor antagonism or an indirect sympathomimetric effect and a post junctional, direct, effect on alpha adrenoceptors, 6 hydroxydopamine (50 mg/kg IV) was administered to rabbits 24-48 hours prior to study. In conscious rabbits the immediate pressor response to RX781094 (0.1 and 1.0 mg/kg) did not differ significantly at 14 ± 5 and 22 ± 5 mmHg respectively to the response in control animals and the contractile response to $10^{-6}$M RX781094 of 0.29 ± 0.10g was also similar to controls (0.30 ± 0.13g).

There is evidence that the post junctional adrenoceptors in the rabbit aorta are only $\alpha_1$ in character (Docherty & Starke 1981). Thus the in vitro findings suggest that, while RX781094 is a relatively selective $\alpha_2$ adrenoceptor antagonist it may possess some partial agonist activity at post junctional $\alpha_1$ adrenoceptors. The in vivo data from intact and 6 hydroxydopamine treated animals also supports the hypothesis that RX781094 may act as a partial agonist. As the pressor response is attenuated by both α yohimbine and prazosin it is possible that both $\alpha_1$ and $\alpha_2$ adrenoceptors at postsynaptic sites contribute to the response.

Chapleo et al 1981 Br. J. Pharmac. 74, 842P.
Dettmar, Lynn & Tulloch 1981 Br. J. Pharmac. 74, 843P.
Docherty & Starke 1981 J. Cardiovasc. Res. 3, 854.

# SPECIES DIFFERENCES IN THE EFFECTS OF RX781094 ON HAEMOGLOBIN CONCENTRATION

J.M.Clifford[1], Leonie W.Frank and J.E.Mackenzie, Pharmacology and Clinical[1] Departments, Reckitt and Colman, Pharmaceutical Division, Hull HU8 7DS.

RX 781094 has been shown to be a selective $\alpha_2$-adrenoceptor antagonist (Chapleo et al., 1981). When RX 781094 was infused into the chloralose/ urethane (60mg/kg : 600mg/kg i.v.) anaesthetized greyhound (n=9) at the $\alpha_2$-selective dose of 0.01 mg/kg/min for 20 min it produced a significant elevation of blood haemoglobin concentration (from 15.5 ± 0.4 to 18.0 ± 0.7 g/dL; as measured by an OSM2 haemoximeter). Control infusions of saline produced no changes in haemoglobin concentration (15.3 ± 0.5 to 15.5±0.5 g/dL; n=5). The same degree of increase in haemoglobin concentration was produced by RX 781094 when infused into greyhounds anaesthetized with pentobarbitone (35 mg/kg i.v.). As a previous study has shown that the spleen of the guinea-pig contains $\alpha_2$-adrenoceptors (McPherson and Summers, 1982) it was thought possible that the effects of RX 781094 were secondary to an action on the spleen. After ligation of the spleen the same infusion of RX 781094 produced no change in haemoglobin concentration (16.6 ± 0.8 of 16.4 ± 0.6 g/dL; n=6). Ahmed et al., (1982) have recently shown that the $\alpha_2$-adrenoceptors of the rat spleen are unaffected by the less selective $\alpha_2$-adrenoceptor antagonist yohimbine. These observations were supported by the present study since yohimbine infused as above at infusion rates of 0.02 and 0.2 mg/kg/min had no effect on the blood haemoglobin concentration of the greyhound.

The spleen of the greyhound is disproportionately large in comparison with other species (weighing ≈1.5kg in a 30kg greyhound) and may account for the fact that RX 781094 (1mg/kg i.v.) failed to produce any change in haemoglobin concentration when given to the pentobarbitone anaesthetized beagle dog (n=3), the rat (n=7), guinea pig (n=5), and rabbit (n=5). Similarly no change in haemoglobin concentration was produced by RX 781094 when infused in conscious man at doses up to 0.3mg/kg i.v.(n=3).

In conclusion it would appear that splenic contraction in the greyhound produced after infusion of the $\alpha_2$-adrenoceptor antagonist RX 781094 can lead to a significant elevation of blood haemoglobin concentration and that the influence of the spleen on haemodynamics in the greyhound require special consideration when evaluating pharmacological studies.

Ahmed M. I. and Naylor I. L. (1982) Br. J. Pharmac. 77 538P.

Chapleo et al., (1981) Br. J. Pharmac. 74 842P.

McPherson G. A. and Summers R. J. (1982) Clin. Exp. Pharmac. and Physiol 9 77-87.

# THE CARDIOVASCULAR EFFECTS OF RX781094 IN THE ANAESTHETIZED GREYHOUND

A.S.Hersom and J.E.Mackenzie, Pharmacology Department, Pharmaceutical Division, Reckitt and Colman, Dansom Lane, Hull, HU8 7DS.

RX 781094 is a potent α-adrenoceptor antagonist which is highly selective for $\alpha_2$-adrenoceptors (Chapleo et al., 1981). In this study the cardiovascular effects of RX 781094 have been examined in greyhounds (20-30kg) anaesthetized with chloralose 60 mg/kg i.v. - urethane 600 mg/kg i.v. Arterial and venous femoral catheters were introduced to measure systemic blood pressure and infuse RX 781094 respectively. Pulmonary artery pressure and pulmonary artery wedge pressure were measured by a balloon tip catheter introduced via a jugular vein. Left ventricular pressure (LV) and LVdP/dt were monitored by a catheter introduced via the carotid artery. Cardiac output was measured by the thermodilution method. An electromagnetic flow probe was used to determine femoral blood flow.

RX 781094 was infused for 20 min at 0.01, 0.1 and 1.0 mg/kg/min with 30 min between each infusion (n=8). A control group of dogs (n=5) received saline. The results obtained are summarised in Table 1.

Table 1.

| | Cumulative dose (mg/kg,i.v.) | | | |
|---|---|---|---|---|
| Parameter | basal | 0.2 | 2.0 | 20 |
| Systolic pressure (mmHg) | 191±7 | 188±6 | 183±15 | 153±14 |
| Diastolic pressure (mmHg) | 150±6 | 143±9 | 126±9 | 98±11* |
| Heart rate (beat/min) | 177±16 | 173±15 | 206±17 | 211±16* |
| Cardiac output (L/min) | 3.3±0.3 | 3.4±0.3 | 4.2±0.7 | 4.9±0.6* |
| Total peripheral resistance (units) | 52±4 | 48±3 | 40±5 | 26±4* |
| Pulmonary artery pressure(mmHg) | 21±3 | 24±4 | 28±5 | 24±4 |
| Right ventricular stroke work index ($g/m/m^2$) | 5.6±1.0 | 6.5±1.0* | 8.0±2.2 | 8.7±2.4 |

* P<0.05 relative to basal controls and compensating for saline control values

There were no arrhythmias at any dose, however at 1.0 mg/kg/min the ECG (lead II) had a reduced QRS amplitude and QT interval (P<0.05). With the exception of an elevation in the haemoglobin concentration (Clifford et al., 1983) all other parameters including blood gases remained unaltered after RX 781094.

In conclusion these results show that although the existence of $\alpha_2$-adrenoceptors have been demonstrated at pre- and post synaptic sites in the cardiovascular system (Constantine et al, 1980), infusion of the selective antagonist RX 781094 (0.2 mg/kg cumulative dose) resulted in negligible net haemodynamic changes.

Chapleo C.B. et al. (1981). Br.J.Pharmac. 74, 842P.
Constantine J.W. et al. (1980) Eur.J.Pharmac. 66, 281-286.
Clifford, J.M. et al. (1983). Br.J.Pharmac. This meeting.

# ELECTRICAL STIMULATION-INDUCED RELEASE OF ($^3$H)-5-HYDROXYTRYPTAMINE IN CANINE ISOLATED BLOOD VESSELS

T.J. Verbeuren*, F.H. Jordaens & A.G. Herman, Division of Pharmacology, Universitaire Instelling Antwerpen, B-2610 Wilrijk, Belgium

Experiments were designed to test whether $^3$H-5-hydroxytryptamine ($^3$H-5HT) can be released by electrical stimulation from isolated dog blood vessels previously incubated in the tritiated indoleamine. Four types of canine blood vessels were studied : the saphenous vein (n = 10) and the splenic artery (n = 3) which are both densely innervated by adrenergic nerve endings (Vanhoutte et al, 1981), and the basilar artery (n = 8) and the arteries of the circle of Willis (n = 5) for which the existence of a direct serotoninergic innervation has been suggested (Chan Palay, 1976; Napoleone et al, 1982).

Helical strips of the blood vessels were incubated for 2 hours at 37°C in aerated Krebs-Ringer solution containing 3 x $10^{-7}$M of $^3$H-5HT. After incubation the tissues were mounted in superfusion set-ups for determination of the efflux of radioactivity. After a washout period of 2 hours, sampling of the superfusate was started. Some samples were afterwards analyzed chromatographically to separate $^3$H-5HT and its main metabolite 5-hydroxyindole acetic acid (5-HIAA).

In all blood vessels a basal efflux of total tritium was detected. Column chromatographic analysis of superfusate samples obtained from saphenous veins and basilar arteries indicated that intact $^3$H-5HT represented less than 10% of this basal efflux. Electrical stimulation (2 Hz; 6 min.) of the preparations caused an increased efflux of tritium in all blood vessels; chromatographic analysis of samples obtained from saphenous veins and basilar arteries showed that this stimulation induced overflow of radioactivity was due to an overflow of intact $^3$H-5HT and of $^3$H-5HIAA. After the first stimulation period (S1) the efflux returned to prestimulation levels. At 30 minutes interval a second (S2) and third (S3) stimulation period were applied which again resulted in an increased efflux of tritium in all blood vessels tested. In saphenous veins and basilar arteries, tetrodotoxin ($10^{-7}$M) infused 20 minutes before S3 nearly abolished the stimulation induced overflow of tritium. The release of $^3$H was frequency-dependent since electrical stimulation at 5 Hz in basilar arteries evoked a larger overflow of tritium than stimulation at 2 Hz; furthermore, this 5 Hz-induced overflow of $^3$H was also sensitive to pretreatment with tetrodotoxin ($10^{-7}$M).

The present experiments indicate that $^3$H-5HT accumulates in different isolated canine blood vessels and that the $^3$H-amine can subsequently be released from the tissues by electrical impulses via a tetrodotoxin sensitive mechanism.

Chan Palay, V. (1976) Brain Res. 102, 103-133
Napoleone, P. et al (1982) Neurosci. Letters 28, 57-60
Vanhoutte, P.M. et al (1981) Physiol. Rev. 61, 151-247

# CENTRAL CARDIOVASCULAR EFFECTS OF 5-HYDROXYTRYPTAMINE IN THE CONSCIOUS RAT

D.W. Dalton, D.H. Fortune & M.B. Tyers, Neuropharmacology Department, Glaxo Group Research Limited, Ware, Hertfordshire.

There is evidence to suggest that central 5-hydroxytryptamine (5-HT) neurones may participate in blood pressure regulation, although its precise role remains unclear (Kuhn et al., 1980). In the anaesthetised rat, central administration of 5-HT generally produces increases in blood pressure. However, because cardiovascular reflexes are altered under anaesthesia (Vatner et al., 1971), the present study was undertaken to examine the cardiovascular effects of centrally administered 5-HT to conscious normotensive and DOCA-salt hypertensive rats.

Normotensive and hypertensive rats (male, C. River CD, 300-350g) were anaesthetised with Althesin, 10mg/kg i.v., and cannulae were implanted in the left carotid artery and into the lateral cerebral ventricle. Animals were allowed at least 2 days to recover from the operation before use. Drugs or artificial CSF, 5ul, were injected directly into the lateral ventricle, whilst blood pressure and heart rate were continuously recorded. Cannula placement was confirmed histologically.

In DOCA-salt hypertensive rats, 5-HT, 1-30ug i.c.v. (n=8), produced pressor responses (15-24mm Hg. after 2 mins) and dose-related decreases in heart rate (10-67 beats/min after 5-15 mins). After 30ug i.c.v. 5-HT, small delayed decreases in blood pressure (8mm Hg.) were also observed. In normotensive rats, 5-HT, 1-30ug i.c.v. (n=8), produced smaller initial pressor responses (5-7mm Hg. after 2 mins) followed by decreases in blood pressure, (5-14mm Hg. after 10-15 mins) and heart rate (24-82 beats/min). The cardiovascular effects of 5-HT, 3-30ug i.c.v, were abolished by pretreatment of methysergide, 30ug i.c.v. (n=8).

In normotensive rats, pretreatment with atenolol, 0.5mg/kg i.a. (n=8), or N-methylatropine, 0.5mg/kg i.a. (n=8), attenuated the 5-HT, 3-30ug i.c.v., induced bradycardia. In combination, both antagonists (n=6) virtually abolished the 5-HT induced bradycardia, suggesting that this may result from increased parasympathetic and decreased sympathetic tone. The secondary vasodepressor responses were attenuated also indicating that they were mainly a result of the bradycardia induced by 5-HT. Pressor responses were unaffected.

In summary, in the conscious rat, centrally injected 5-HT caused an initial pressor response and marked bradycardia. In normotensive rats, as the bradycardia became more profound at higher doses of 5-HT, secondary decreases in blood pressure were more pronounced. Therefore, 5-HT administered centrally, appears to exert an initial vasopressor response, but alterations in autonomic tone to the heart produce a marked bradycardia resulting in a later decrease in blood pressure.

We wish to thank Mr. M. Avkiran for his excellent technical assistance.

Kuhn, D.M., Wolf, W.A. & Lovenberg, W. (1980) Hypertension 2, 243-255.
Vatner, S.F., Franklin, D. & Braunwauld, E. (1971) Am.J.Physiol., 220, 1249-1255.

# CALCIUM ENTRY BLOCKADE DOES NOT INHIBIT VASOCONSTRICTION BY SEROTONIN IN VIVO

H.O. Kalkman, P.B.M.W.M. Timmermans & P.A. van Zwieten, Department of Pharmacy, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands

Vasoconstriction induced by serotonin in vitro is reported to be inhibited by calcium entry blockers (van Nueten & Vanhoutte, 1980; Towart, 1981). Under in vivo circumstances, however, nifedipine (in doses up to 1 mg/kg, i.a.) did not affect the vasoconstriction by serotonin (Kalkman et al, 1982). In order to investigate this discrepancy in more detail, a number of calcium entry blockers was tested with respect to their ability to inhibit serotonin-induced vasoconstriction in the pithed normotensive rat.

Male Wistar, normotensive rats (weight 190-240 g) were pithed and subjected to artificial ventilation. Body temperature was kept at 37°C. The right jugular vein and common carotid artery were cannulated to allow administration of drugs and continuous measurement of arterial pressure, respectively.

I.v. bolus injections with serotonin dose-dependently increased diastolic pressure to a maximum of approximately 110 mm Hg. The dihydropyridines nimodipine (0.3 mg/kg) and PY 108-068 (0.3 mg/kg) applied i.a. 15 min previously were without effect on the serotonin-induced pressor responses. Flunarizine (3 mg/kg, i.a., -15 min) was also ineffective. Both the (+)- (1-10 mg/kg) and the (-)-isomer (0.3-3 mg/kg) of verapamil shifted the log dose-pressor response curve of serotonin to the right in a dose-related and parallel manner. The (-)-isomer was found about 10 times more potent than the (+)-enantiomer. The magnitude of the vasoconstriction by serotonin after an i.a. infusion (15 min) of $Na_2$-EDTA (120 mg/kg) was not significantly ($p > 0.05$) different from that evaluated in control experiments (Ca-EDTA, 120 mg/kg). It may be added that the doses of the calcium entry blockers and $Na_2$-EDTA used in the present experiments reduced the vasoconstriction to the $\alpha_2$-adrenoceptor agonist B-HT 920 by at least 70%.

In a receptor binding assay, using ($^3$H)mianserin to label $5HT_2$-receptors in membranes from rat frontal cortex (Peroutka & Snyder, 1981), the following affinities (-log $IC_{50}$ values) were determined: nimodipine: 4.8; PY 108-068: 4.8; flunarizine: 5.3; (+)-verapamil: 5.8; (-)-verapamil: 6.4.

It is concluded that the vasoconstriction evoked by i.v. bolus injections with serotonin in vivo is not dependent on a transmembrane influx of calcium ions, in view of the inability of $Na_2$-EDTA and calcium entry blockers to interfere with this process. Based upon the marked affinity of (-)-verapamil for $5HT_2$-receptors, it is likely that this compound inhibits serotonin-induced vasoconstriction by a genuine antagonism and not via a blockade of calcium influx.

Nueten, J.M. van & Vanhoutte, P.M. (1980) Eur.J.Pharmac. 64, 173
Kalkman, H.O. et al (1982) Eur.J.Pharmac. 78, 107
Peroutka, S.J. & Snyder, S.H. (1981) J.Pharmac.Exp.Ther. 216, 142
Towart, R. (1981) Circ.Res. 48, 650

# POSTJUNCTIONAL $\alpha_2$-ADRENOCEPTORS AND CALCIUM ENTRY BLOCKADE IN THE ISOLATED PERFUSED HINDQUARTERS OF THE RAT

J.C.A. van Meel, P.B.M.W.M. Timmermans & P.A. van Zwieten, Department of Pharmacy, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands

The existence of postjunctional $\alpha_2$-adrenoceptors in vascular smooth muscle has been established convincingly in vivo. However, the only two in vitro blood vessel preparations where responses towards selective $\alpha_2$-agonists mediated by postsynaptic $\alpha_2$-adrenoceptors have been demonstrated unequivocally, are the dog saphenous vein and the isolated perfused hindquarters of the rat after reserpine treatment (for reviews see Timmermans & van Zwieten, 1982; McGrath, 1982). The latter model, introduced by Kobinger & Pichler (1981) was used in this study to further characterize the $\alpha$-adrenoceptors involved in the vasoconstrictor processes in vitro. In addition, we examined the calcium dependency of the process of vasoconstriction via $\alpha_2$-adrenoceptors.

Male normotensive Wistar rats (240-260 g) were treated with reserpine (5 mg/kg, i.p.) and killed by a blow on the neck 16-22 h later. The ileolumbar vessels were exposed and ligated bilaterally. The hindquaters were perfused with modified Tyrode (for details see Kobinger & Pichler, 1981) plus 5% albumin at constant pressure (55-60 cm $H_2O$) through the abdominal aorta. The outflow from the venous side was measured continuously. After 3-5 min perfusion time the venous effluent was constant and amounted to 4.68 $\pm$ 0.10 ml/min (n=12). The $\alpha$-agonists B-HT 920 and methoxamine were injected into the cannula proximal to the aorta in a volume of 0.1 ml.

In the perfused hindquarters of the rat treated with reserpine, the $\alpha_1$-agonist methoxamine caused a complete inhibition of the flow (= 100%) reflecting intense vasoconstriction. This effect was competitively antagonized by prazosin ($pA_2$ = 8.83). The apparent $pA_2$ of yohimbine towards methoxamine-induced vasoconstriction was 6.14. The $\alpha_2$-agonist B-HT 920 reduced the flow to about 40% of the initial value. Yohimbine ($pA_2$ = 8.95) competitively antagonized the $\alpha_2$-agonist-induced constrictor effect, whereas an apparent $pA_2$ = 7.58 was obtained for prazosin. Nisoldipine ($3.10^{-9}$ - $10^{-7}$ M), $Co^{2+}$($10^{-4}$ M) and $La^{3+}$($10^{-4}$ and $3.10^{-4}$ M) produced a non-competitive type of interaction with the B-HT 920-induced vasoconstrictor effects, whereas the vasoconstriction induced by methoxamine was virtually unaffected by the calcium entry blockers. Omission of calcium in the perfusion fluid abolished the responses to B-HT 920, but the vasoconstrictor responses to methoxamine were attenuated to a minor degree without depression of the maximal response. On the other hand, increasing the calcium concentration (from 1.8 to 11.8 mM) in the perfusion fluid markedly potentiated the vasoconstrictor effects via activation of postjunctional $\alpha_2$-adrenoceptors without influencing the vasoconstriction induced by methoxamine.

These findings strengthen the hypothesis (van Meel et al, 1981) that extracellular calcium ions are essential for the process of vasoconstriction initiated by postjunctional $\alpha_2$-adrenoceptor activation.

Kobinger, W. & Pichler, L. (1981) Eur.J.Pharmac. 76, 101
McGrath, J.C. (1982) Biochem.Pharmac. 31, 467
van Meel, J.C.A. et al (1981) Naunyn-Schmiedeb.Arch.Pharmac. 316, 288
Timmermans, P.B.M.W.M. & van Zwieten, P.A. (1982) J.Med.Chem. in press

# CENTRAL AND PERIPHERAL MECHANISMS IN THE CLONIDINE WITHDRAWAL PHENOMENON IN THE SH RAT

M.J.M.C. Thoolen, P.B.M.W.M. Timmermans, D.H.G. Versteeg[1] & P.A. van Zwieten, Department of Pharmacy, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam and [1]Rudolf Magnus Institute for Pharmacology, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands

In rats, the clonidine rebound phenomenon is characterized by an overshoot of heart rate and the appearance of blood pressure upswings (Thoolen et al, 1981). Recent investigations have indicated that the Locus Coeruleus (LC) could play a role in this phenomenon (Engberg et al, 1982). The present study was undertaken to distinguish between central and peripheral factors in the clonidine rebound phenomenon by suppressing the symptoms with i.p. and i.c.v. applications of morphine and oxymetazoline. Moreover, the effects of clonidine withdrawal in SH rats were studied in which the LC had been stereotactically destroyed.

Clonidine (500 µg/kg/day) was infused for twelve days via ALZET miniosmopumps in male SH rats (300-330 g). Blood pressure and heart rate were measured via permanently indwelling abdominal aortic catheters. Between 8 and 14 h after clonidine withdrawal, the effects of morphine (3 and 10 µg/kg, i.c.v. and 0.01, 0.1, 1.0 and 3.0 mg/kg, i.p.) and oxymetazoline (30 µg/kg, i.c.v. as well as i.p.) were studied on heart rate and the appearance of blood pressure upswings. Morphine dose-dependently abolished the appearance of b.p. upswings, but had little influence on the rebound tachycardia (about 460 b.p.m.). I.p. injection of oxymetazoline (30 µg/kg) lowered heart rate from 456 $\pm$ 12 to 289 $\pm$ 10 b.p.m. (n=6), thus completely abolishing the frequency overshoot. This drug had no effect on the appearance of b.p. upswings. I.c.v. injection of oxymetazoline abolished the upswings as well as the heart rate overshoot (7.9/h to 0/h and 450 $\pm$ 16 b.p.m. to 290 $\pm$ 12 b.p.m., respectively, n=6).

In the SH rats in which the LC had been destroyed, the continuous infusion of clonidine (500 µg/kg/day) reduced the mean arterial pressure and the heart rate to the same extent as in Sham-lesioned SH rats. Following withdrawal, a discontinuation syndrome appeared, which was equal in severity, duration and appearance in both groups.

The results indicate, that the blood pressure upswings after clonidine withdrawal are due to a central mechanism, whereas the tachycardia is, at least in part, caused by a peripheral action. It is unlikely, that the locus coeruleus is the only central nucleus, responsible for the appearance of blood pressure upswings.

Thoolen, M.J.M.C. et al (1981) Gen.Pharmac. 12, 303
Engberg, G. et al (1982) Life Sci. 30, 235

# EXAMINATION OF CARDIAC β-ADRENOCEPTOR SUBTYPES BY PHARMACOLOGICAL AND RADIOLIGAND BINDING TECHNIQUES

K.J. Broadley & M.H. Hawthorn, Department of Applied Pharmacology, Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cardiff CF1 3NU

The responses to catecholamines of certain tissues, such as the isolated trachea of the guinea-pig, have been shown by pharmacological analysis to be mediated via a mixed population of $\beta_1$ and $\beta_2$-adrenoceptors (Zaagsma et al, 1979). In contrast, the responses of left and right atria and ventricular strips have been shown pharmacologically to be mediated solely via $\beta_1$-adrenoceptors, whereas in the lung strip they are solely via $\beta_2$-adrenoceptors (Zaagsma et al, 1979). Recently, however, although only a single $\beta_2$-adrenoceptors population was found in guinea-pig lung strips pharmacologically, both $\beta_1$ and $\beta_2$-adrenoceptors were identified in the same tissue by radioligand binding techniques (Carswell & Nahorski, 1982). The present study was undertaken to compare the β-adrenoceptors in cardiac tissue by using both radioligand binding and pharmacological techniques.

β-adrenoceptor binding of $^3$H-dihydroalprenolol to membrane fractions obtained from homogenates of guinea-pig ventricles was examined as described previously (Hawthorn & Broadley, 1982). The dissociation constant ($K_D$) and $^B$max of $^3$H-DHA binding were $1.6\pm0.25\times10^{-9}$M and $93.1\pm16.5$fmol mg$^{-1}$ protein respectively (n=6). Displacement of $^3$H-DHA binding by the antagonists practolol ($\beta_1$-selective) and ICI 118,551 ($\beta_2$-selective) was examined. Hofstee analysis of the displacement curves revealed them to be biphasic indicative of a dual β-adrenoceptor population, the proportion of $\beta_1$ and $\beta_2$-adrenoceptors being $52.0\pm5.4$ and $48.0\pm4.5$% from practolol displacement (n=4) and $64.1\pm2.4$ and $35.9\pm2.4$% with ICI 118,551 (n=4).

For pharmacological analysis of cardiac β-adrenoceptors, left atria, right atria and papillary muscles were suspended in Krebs-bicarbonate solution at 38°C containing cocaine ($10^{-5}$M), metanephrine ($10^{-5}$M) and phentolamine ($5\times10^{-6}$M) to inhibit neuronal and extraneuronal uptake and α-adrenoceptors. Left atria and papillary muscles were paced at 2 Hz with threshold voltage (+50%) and isometric tension recorded. Right atrial rate responses were recorded. $pA_2$ values were determined for the antagonism by practolol and ICI 118,551 of the responses to fenoterol and noradrenaline as $\beta_2$ and $\beta_1$-selective agonists respectively (Table 1).

Table 1 Mean (n=12) $pA_2$ values and Schild plot slopes for antagonism by practolol and ICI 118,551 of fenoterol (FEN) and noradrenaline (NA)

| | | Practolol | | ICI 118,551 | |
|---|---|---|---|---|---|
| | | $pA_2$ | slope | $pA_2$ | slope |
| Right atria | FEN | 6.36±0.13 | 1.07 | 6.43±0.13 | 0.92 |
| | NA | 6.25±0.06 | 0.86 | 6.51±0.08 | 1.11 |
| Left atria | FEN | 6.59±0.05 | 1.12 | 6.64±0.12 | 1.23 |
| | NA | 6.34±0.05 | 0.86 | 6.30±0.07 | 1.10 |
| Papillary muscle | FEN | 6.43±0.11 | 1.14 | 6.56±0.13 | 1.05 |
| | NA | 6.10±0.05 | 1.17 | 6.64±0.10 | 1.50 |

In each tissue, the $pA_2$ values were the same whether FEN or NA was the agonist indicating a single receptor mediating these responses, and confirming Zaagsma et al (1979). This is at variance with the radioligand binding data which indicates the additional presence of $\beta_2$-adrenoceptors, the functional role of which, if any, remains to be established.

Carswell, H. & Nahorski, S.R. (1982) Proc. Br. Pharmac. Soc. (Sunderland) C.65
Hawthorn, M.H. & Broadley, K.J. (1982) Naunyn-Schmiedebergs Arch. Pharmac. (in press)
Zaagsma, J. et al (1979) Catecholamines.Basic and Clinical Frontiers, Vol.1, 435, Pergamon Press

# THE EFFECT OF CADMIUM AND ZINC ON THE DEVELOPMENT OF DOCA/SALT HYPERTENSION IN RATS

Ann F. Johnston & H.G. Dean, Department of Pharmacology, The University, Leeds LS2 9JT,

The induction of hypertension in rats by the administration of deoxycorticosterone acetate (DOCA) and saline as drinking fluid has been used experimentally for many years. This model, however, shows a variation in the time course of the development of the hypertension which does not appear to be related to any obvious factor. We therefore decided to investigate the effect of the cadmium ion, a possible environmental contaminant which is known to affect blood pressure in its own right (Perry 1976), and zinc, which has been reported to reverse some of the hypertensive effects of cadmium (Perry et al, 1980), on the development of DOCA/salt hypertension in rats.

Cadmium (25 µg/ml and 5 µg/ml) was administered in the drinking fluid to 90 g, female, Wistar rats over a period of 19 days. The rats had been either implanted with DOCA and given 1% saline to drink or had undergone a sham operation and were receiving distilled water to drink.

Cadmium at either level (actual dose 300 and 600 µg/rat/day) reduced both the rise in blood pressure and elevated saline intake in the DOCA-treated rats in a dose-related manner.

Zinc (200 µg/ml actual dose 7 mg/rat/day) given concurrently in the drinking fluid with cadmium (25 µg/ml, actual dose 300 µg/rat/day) did not antagonise the effects of the latter in DOCA/salt animals and had a similar effect on blood pressure and fluid intake.

Blood and plasma levels of both metal ions were determined as appropriate by flameless atomic absorption spectroscopy. Rats receiving cadmium had elevated levels of this metal irrespective of whether zinc was administered at the same time.

The blood and plasma levels of zinc in animals receiving this metal were unrelated to the administration of zinc or cadmium.

Thus both zinc and cadmium appear to reduce blood pressure and fluid intake in DOCA/salt rats. However, we consider that it cannot be concluded whether these metals are acting directly on the control of blood pressure, or indirectly through reducing saline intake until the relationship between salt and/or water intake and blood pressure in DOCA-treated rats has been further investigated.

Perry, H.M. (1976) Review of hypertension induced in animals by chronic ingestion of cadmium. Trace elements in human health and disease vol II, ch 43. Academic Press, London.
Perry, H.M., Erlanger, M. & Perry, E.F. (1980) Inhibition of cadmium induced hypertension in rats. Sci. Total Environ. 14, 153-166.

# STEREOSELECTIVE AGONIST ACTIVITY OF 5,6-DIHYDROXY-2-DIPROPYLAMINO-TETRALIN AT PERIPHERAL DOPAMINE RECEPTORS

R.A.Brown, Janet C.Hall, F.Ince, S.E.O'Connor and G.W.Smith, Departments of Pharmacology and Medicinal Chemistry, Fisons plc, Pharmaceutical Division, Science & Technology Laboratories, Bakewell Road, Loughborough, Leicestershire, LE11 ORH

(±)-5,6-dihydroxy-2-dipropylaminotetralin (DP-5,6-ADTN) a conformationally restricted analogue of dopamine (DA), is a potent agonist at both prejunctional and postjunctional (vascular) DA receptors (Brown et al, 1982). We have examined the potency of its two enantiomers in peripheral DA receptor models.

Prejunctional effects were assessed in the rabbit isolated ear artery preparation (REA; Brown & O'Connor, 1981), and postjunctional effects were measured as falls in renal vascular resistance in the pentobarbitone - anaesthetised dog (DRV; modified from Goldberg et al, 1978). Dogs were pretreated with phenoxybenzamine (10 mg/kg i.v.), haloperidol (50 µg/kg i.v. each hour) and ICI 118,551 (200 µg/kg $h^{-1}$ i.r.a. infusion) to block α-adrenoceptors, prejunctional DA receptors and $\beta_2$-adrenoceptors respectively. Results are summarised below.

| | Relative equipotent molar doses | |
|---|---|---|
| | REA (n=6) | DRV (n=4) |
| Dopamine | 1.0 | 1.0 |
| RS(±)-DP-5,6-ADTN | 0.028 | 0.5 |
| S(-)-DP-5,6-ADTN | 0.005 | 0.2 |
| R(+)-DP-5,6-ADTN | 4.6 | >30 |

S(-)-DP-5,6-ADTN is approximately 900 x more potent than R(+)-DP-5,6-ADTN at peripheral prejunctional DA receptors and >150 x more potent at postjunctional sites. Thus, functionally and pharmacologically distinct peripheral DA receptors share common stereochemical features with respect to agonists. Pure enantiomers of potent asymmetric non-selective DA receptor agonists are therefore unlikely to differentiate between pre- and postjunctional sites. Interestingly, the DA receptor antagonist sulpiride shows marked steric dependence (S(-)) at prejunctional receptors, but this requirement is absent (Shepperson et al, 1982) or opposite (Goldberg et al, 1979) at postjunctional sites.

McDermed (1982) has resolved a number of DA receptor agonists including DP-5,6-ADTN. In contrast to DP-5,6-ADTN, the R(+)-enantiomer of 6,7-dihydroxy-2-aminotetralin (6,7-ADTN) is the more potent both at CNS DA receptors (Woodruff, 1982) and at peripheral postjunctional sites (Goldberg et al, 1979). The different preferred arrangements of DP-5,6-ADTN and 6,7-ADTN can be explained by their interactions with the complementary area of the DA receptor as proposed by McDermed (1982).

Brown, R.A.& O'Connor S.E. (1981) Br.J.Pharmac 73, 189P
Brown, R.A. et al (1982) Proc.Int.Symp. on Dopamine Agonists, Stockholm. In press
Goldberg, L.I. et al (1978) Ann.Rev.Pharmac.Toxicol. 18, 57-79
Goldberg, L.I. et al (1979) In:Catecholamines, Basic & Clinical Frontiers. Eds. Usdin E., Kopin I.J. & Barchas J pp. 447-449. Pergamon Press
McDermed, J.D. (1982) Proc.Int.Symp. on Dopamine Agonists, Stockholm. In press
Shepperson, N.B. et al (1982) J. Pharmacol. exp. Therap. 221, 735-761
Woodruff, G.N. (1982) TIPS February pp. 59-61

# CARDIOVASCULAR EFFECTS OF LY141865, A NEW DOPAMINE RECEPTOR AGONIST, IN RATS

I. Cavero, L.E.R.S., 58 rue de la Glacière, 75013 Paris, France.

LY141865, trans-(±)-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-2H-pyrazolo-[3,4-g]-quinoline dihydrochloride, is a "partial ergoline" derivative which stimulates dopamine receptors (Goldstein & Kinugasa, 1983). The aim of this work was to verify whether, in the rat, LY141865 exerts cardiovascular effects similar to those of pergolide (Cavero & Lefèvre-Borg, 1981) or N,N-di-n-propyl-dopamine (Cavero et al, 1981a, b), two agents possessing agonist activity at prejunctional neuronal $DA_2$ dopamine receptors.

Male rats (Sprague Dawley) weighing 220-250g were anaesthetized with pentobarbitone and prepared for arterial pressure and heart rate measurement as previously described (Cavero et al, 1981a). LY141865 (150.0 µg/kg, i.v., given during a 15 min period) was studied in intact rats after the i.v. administration of either saline (0.3 ml/kg) or sulpiride (0.3 mg/kg). Furthermore, LY141865 was given to saline, sulpiride and/or phentolamine (0.3 mg/kg, i.v.) pretreated pithed rat in which either pressor (0.5-1.0 Hz, 1.0 msec, 50.0 V for 15 sec) or tachycardic (0.2 Hz, 1.0 msec, 50.0 V for 30 min) responses were evoked.

In anaesthetized rats, mean carotid arterial pressure was decreased, at the end of the infusion of LY141865, by 37.2 ± 4.5 mmHg from a control value of 127.2 ± 2.4 mmHg (n=6). At the same time, heart rate was maximally decreased by 74.0 ± 6.0 beats/min (control value 441.7 ± 9.2). The hypotensive effect was 30% less 30 min later and the bradycardia was almost of the same magnitude. Pretreatment of the rats with sulpiride prevented by 80% the bradycardia and entirely antagonized the hypotension. In pithed rats, LY141865 did not modify baseline heart rate but reduced by 65% a neurally evoked sustained tachycardia (84.0 ± 3.3 beats/min, n=5). This effect was more inhibited by phentolamine (40-60%) than sulpiride (10-30%). However, in pithed rats pretreated with the combination of these two antagonists, LY141865 did not modify the sympathetic tachycardia. Furthermore, the pressor responses evoked by short periods of electrical stimulation of the spinal cord were decreased (approximatively 40%) by LY141865 and the latter effect was antagonized by sulpiride.

In conclusion, these results indicate that, in rats, LY141865 produces cardiovascular effects similar to those already described for other dopamine receptor agonists, like pergolide and N,N-di-n-propyl-dopamine (Cavero & Lefèvre-Borg, 1981; Cavero et al, 1981a, b).

Cavero, I. & Lefèvre-Borg, F. (1981) Br.J.Pharmac. 74, 927P
Cavero, I. et al (1981a) J.Pharmacol.Exp.Ther. 218, 515-524
Cavero, I. et al (1981b) J.Pharmacol.Exp.Ther. 219, 510-519
Goldstein, M. & Kinugasa, K. (1982) Pharmacologist 24, 137 (Abs. 240)

# EFFECTS OF TL-99 ON CARDIOVASCULAR α- AND β-ADRENOCEPTORS IN PITHED RATS

Françoise Lefèvre-Borg, L.E.R.S., 58 rue de la Glacière, 75013 Paris, France.

TL-99 (6,7-dihydroxy-2-dimethylaminotetralin) and M-7 (5,6-dihydroxy-2-dimethylaminotetralin) are chemically related to dopamine with which they share the property of stimulating dopamine receptors (Long, et al, 1975 ; Kitzen et al., 1978). Recently, Cavero & Lefèvre (1982) reported that M-7 also stimulates vascular $\beta_2$- in addition to $\alpha_2$-adrenoceptors. The effects mediated by the former receptors were shown to affect the determination of the potency of yohimbine as an $\alpha_2$-adrenoceptor antagonist. Since TL-99 is also a relatively selective agonist of $\alpha_2$-adrenoceptors (Hicks & Cannon, 1980), a study was carried out to assess whether it activates $\beta_1$- and $\beta_2$-adrenoceptors in pithed rats.

Male rats (Sprague Dawley) were anaesthetized with pentobarbitone (55.0 mg/kg, i.p.) and then pithed. Mean carotid arterial pressure and heart rate were measured. Dose-response curves to TL-99 were determined in rats pretreated with either i.v. saline (0.3 ml/kg), prazosin (0.1 mg/kg) or yohimbine (0.3 and 1.0 mg/kg), alone or plus propanolol.

TL-99 produced dose-pressor response curves which were not affected by pretreating the rats with either prazosin or propranolol alone. However, yohimbine shifted the control dose-response curve to the right without changing neither the slope nor the maximum response. The i.v. doses of TL-99 producing a 50 mmHg increase in arterial pressure were 0.97 $\pm$ 0.04 µg/kg (n=9) in the control group, and 6.2 $\pm$ 0.6 (n=7) and 14.7 $\pm$ 1.5 (n=4) µg/kg in rats pretreated with 0.3 and 1.0 mg/kg, i.v., yohimbine, respectively. These values were not modified by propranolol. However, this antagonist blocked the increases in heart rate produced by TL-99 in the pithed rat.

TL-99, exhibits a relatively high selectivity as agonist of $\alpha_2$-adrenoceptors, like its close analogue M-7 (Cavero & Lefèvre-Borg, 1982). However, in contrast to M-7 (Cavero & Lefèvre-Borg, 1982), TL-99 does not stimulate $\beta_2$-adrenoceptors but activates cardiac $\beta_1$-adrenoceptors. The blockade of the latter receptors with propranolol does not appear to modify the dose-pressor response curves to TL-99 in rats pretreated with yohimbine, a relatively selective $\alpha_2$-adrenoceptor antagonist.

Cavero, I. & Lefèvre-Borg, F. (1982) Br.J.Pharmac. (in press)
Hicks, P.E. & Cannon, J.G. (1980) J.Pharm.Pharmacol. 32, 786-788
Kitzen, J.M. et al (1978) J.Pharmac.Exp.Ther. 206, 239-247
Long, J.P. et al (1975) J.Pharmac.Exp.Ther. 192, 336-342

# ANTIHYPERTENSIVE ACTIVITY OF BETAXOLOL IN CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS

I. Cavero and Françoise Lefèvre-Borg, L.E.R.S., 58 rue de la Glacière, 75013 Paris, France.

Betaxolol is a new potent β-adrenoceptor antagonist possessing a high selectivity toward cardiac β-adrenoceptors (Boudot et al, 1979). Since most of β-adrenoceptor antagonists exhibit antihypertensive properties in man (Prichard, 1978) but not in animals with an experimental hypertension (Buckingham, 1979), we studied whether betaxolol reduced the established hypertension or affected the natural development of high arterial pressure in spontaneously hypertensive rats (SHR).

In a first series of experiments, betaxolol (2.5 or 5.0 mg/kg, p.o., twice a day) was given for two days to freely moving male SHR (about 1 year of age) with established hypertension. These animals were prepared for continuous blood pressure and heart rate recording through a catheter implanted in the abdominal aorta at least one day before the experiment (Lefèvre-Borg & Cavero, 1980). In a second series of experiments, 4-6 weeks old SHR were dosed with either placebo (water), betaxolol, metoprolol or propranolol, at 2.0 or 5.0 mg/kg, p.o., (n=10-15/group) for 13 weeks. Systolic tail arterial pressure and heart rate were indirectly measured before, at the 4th, 9th, 13th week of treatment and then, every 3 weeks during the 3 months following the end of these treatments.

Betaxolol, given for 2 days, markedly decreased the mean aortic pressure (MAP) and the heart rate (HR) of the SHR with established hypertension (initial values: MAP = 143±4 mmHg, HR = 429±10 beats/min; n=18). This effect was maximal 2 hours after the first dose on day 1 (-30±6 and -40±6 mmHg after 2.5 and 5.0 mg/kg, respectively) and lasted over 5 hours. Heart rate was maximally decreased (-63±19 and -141±18 beats/min for 2.5 and 5.0 mg/kg, respectively) by betaxolol within 30 min following the administration, and this lasted over 5 hours. Similar changes in the recorded parameters were measured after the morning dose of day 2. The progressive age related natural rise in arterial blood pressure was significantly attenuated (approximatively 25 mmHg at the end of the treatment) by small oral doses of betaxolol but not metoprolol or propranolol. This beneficial effect persisted for the 13 week follow-up period after the end of the treatment.

These results indicate that small doses of betaxolol reduced the established high arterial pressure in SHR. Furthermore, young SHR given betaxolol during the period of natural rise of arterial pressure exhibited a lower level of established hypertension. In a separate communication, a study on possible mechanisms of the antihypertensive activity of betaxolol will be presented (Cavero, 1983).

Boudot, J.P. et al (1979) Br.J.Pharmac. 63, 445P
Buckingham, R.E. & Hamilton, T.C. (1979) Gen.Pharmac. 10, 1-13
Cavero, I. (1983) This meeting
Lefèvre-Borg, F. & Cavero, I. (1980) Clin.Sci. 59, 291s-294s
Prichard, B.N.C. (1978) Br.J.Clin.Pharmacol. 5, 379-399.

# A COMPARISON OF THE CARDIOVASCULAR EFFECTS OF NALOXONE AND ICI 154129 FOLLOWING HAEMORRHAGIC SHOCK IN ANAESTHETIZED RATS

J. Froggatt, J. Parkes and M. H. Todd. Bioscience II Department, ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire.

Naloxone, an opiate receptor antagonist, reverses haemorrhagic shock in rats (Faden and Holaday, 1979). The mechanism involved in this effect is not clear and may involve one or more opiate receptor sub-types since naloxone blocks both $\mu$- and $\delta$-receptors at high doses ( Wheeler 1982). ICI 154129 is a selective $\delta$-receptor antagonist ( Shaw et al 1982) and we have therefore compared the cardiovascular actions of this compound with naloxone in haemorrhagic shock.

Female normotensive rats weighing between 200 and 250gm were anaesthetised with a mixture of urethane ( 800mg.Kg$^{-1}$) and chloralose (60mg.Kg$^{-1}$). A tracheal cannula was inserted to facilitate spontaneous respiration and blood pressure and heart rate were monitored from a cannula inserted in the left common carotid artery. Drugs or vehicle were administered via the left jugular vein. After an equilibration period of 30 min haemorrhagic shock was induced by the removal of blood (17ml/Kg) from the carotid cannula over a period of 5 min. Following this manoevre diastolic blood pressure ( DBP) fell from 124 $\pm$ 5 to 37 $\pm$ 3 mmHg. Subsequently, spontaneous recovery of blood pressure occurred so that immediately before drug administration DBP was 54 $\pm$ 4 mmHg. Naloxone (10mg.Kg$^{-1}$) ICI 154129 (50mg.Kg$^{-1}$) or vehicle were administered 30 min after the induction of shock. Comparisons of drugs or vehicle effects were carried out in groups of six animals between pre-dose values and the highest value of DBP within the period 15 min after drug administration.

Saline vehicle produced no effect on DBP and naloxone induced a small but not significant increase in blood pressure ( Table 1). ICI 154129 increased DBP and heart rate significantly by 21 $\pm$ 4 mmHg and 44 $\pm$ 16 bts-min$^{-1}$ respectively.

Table 1

| | Diastolic Blood Pressure (mmHg) | | |
|---|---|---|---|
| | Vehicle | Naloxone | M 154129 |
| Pre-drug | 42.5 (4.2) | 54.2 (10.5) | 65.8 (10.1) |
| Post-drug | 42.5 (3.4) | 63.3 (13.9) | 86.7 ( 9.8) * |

*$p < 0.01$

ICI 154129 attenuated the blood pressure changes induced by haemorrhage, results which extend observations that this compound reverses blood pressure changes in endotoxic shock without affecting morphine analgesia (Holaday et al, 1982). The relative magnitude of these effects and of the effect of naloxone in this model may reflect the severity of the shock induced by 40% haemorrhage. These results provide further evidence that opioid antagonists may be beneficial in shock states characterised by low blood pressure.

We are grateful to Endo Laboratories Inc. for supplying naloxone hydrochloride.

References

Faden, A.I. and Holaday, J.W. (1979) Science 205 317-318.
Holaday, J.W., Ruvio, B.A., Robles, L.E. and Johnson, C.E. (1982). Life Science 31 ( in press).
Shaw, J.S., Miller, L., Turnbull, M.J., Gormley, J.J. and Morley, J.S. Life Science 31 ( in press).
Wheeler, H. (1982) Neuropharmacology ( in press ).

# THE IN VITRO PROFILE OF ICI 154129: A SELECTIVE ANTAGONIST AT THE OPIATE DELTA-RECEPTOR

Lynne Miller and J.S. Shaw, Bioscience Dept. II, ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG

The existence of multiple opiate receptor sub-types is now well documented (Martin et al., 1976; Lord et al., 1977). However, despite the existence of agonists with high specificity for the different receptors, the existing antagonists such as naloxone and naltrexone are relatively non-specific, possessing affinity for both μ and δ-receptors, although all have some degree of selectivity for the μ-receptor (Shaw et al., 1982).

We have recently reported that ICI 154129 (N,N-diallyl Tyr-Gly-Gly-$\psi$-($CH_2$S)-Phe-Leu) is a selective antagonist at the opiate δ-receptor both *in vitro* (Shaw et al. 1982) and *in vivo* (Gormley et al., 1982). In the present study we have determined the antagonist potencies of ICI 154129 and naloxone against a range of opiate agonists in the field-stimulated mouse vas deferens preparation (mvd). We have also compared the relative potencies of the agonists in two *in vitro* models predictive of δ- (mvd) and μ- (field-stimulated guinea-pig ileum - gpi) agonist activity. The methods were as previously described (Shaw & Turnbull, 1978).

The results (Table 1) demonstrate that these techniques are capable of distinguishing between the μ-selective agonists morphine and normorphine and the δ-selective enkephalins. However, the sensitivity of the agonists to ICI 154129 proved to be the most reliable index of receptor selectivity since only this technique was able to reveal the mixed μ/δ activity of both etorphine and β-endorphin. Furthermore, the selectivity ratios obtained with ICI 154129 are in close agreement with the results from receptor binding studies reported by Kosterlitz et al. (1980).

Table 1 Tissue selectivity and antagonism by naloxone and ICI 154129 of opiate agonists

| | Potency ratio gpi/mvd* | Ke value on mvd (nM) ICI 154129 | Naloxone |
|---|---|---|---|
| Leu-enkephalin | 0.26 | 261 ± 19.8 | 28.5 ± 3.8 |
| [D-$Ala^2$,D-$Leu^5$]-enkephalin | 0.18 | 419 ± 167 | 29.0 ± 3.1 |
| Met-enkephalin | 1.0 | 595 ± 118 | 33.6 ± 5.9 |
| β-endorphin | 9.04 | 1002 ± 120 | 28.7 ± 3.9 |
| [D-$Met^2$, $Pro^5$]-enkephalinamide | 30.1 | 2130 ± 460 | 14.7 ± 2.3 |
| Etorphin | 65.4 | 4170 ± 1320 | 14.4 ± 3.3 |
| Morphine | 61.0 | 7780 ± 1230 | 4.23 ± 0.55 |
| Normorphine | 63.0 | 8170 ± 1660 | 4.14 ± 0.46 |

* expressed relative to met-enkephalin = 1

Gormley J.J. et al. (1982) Life Sci. 31, 1263
Kosterlitz, H.W. et al. (1980) Br. J. Pharmac. 68, 333
Lord, J.A.H. et al. (1977) Nature 267, 495
Martin, W.R. et al. (1976) J. Pharmac. Exp. Ther. 197, 517
Shaw, J.S. and Turnbull, M.J. (1978) Eur. J. Pharmac. 49, 313
Shaw, J.S. et al. (1982) Life Sci. 31, 1259

# TRITIUM RELEASE BY DMPP AND FIELD STIMULATION AFTER INCUBATION OF HUMAN COLONIC MUSCLE STRIPS IN ($^3$H)-CHOLINE

D.E. Burleigh and S.J. Trout, Department of Pharmacology and Therapeutics, The London Hospital Medical College, Turner Street, London E1 2AD.

Conventional organ bath studies have shown that ganglion stimulant drugs usually only relax human colonic muscle, even though intrinsic cholinergic nerves are present (Stockley and Bennett, 1974); occasionally biphasic responses occur. In this investigation we have used a radio-chemical method to determine whether 1,1-dimethyl-4-phenylpiperazinium (DMPP) can release tritiated material possibly from cholinergic nerves thereby accounting for the contractile phase, and compared this with release elicited by electrical field stimulation (EFS).

Circular muscle strips of human colon were immersed in Krebs fluid (37$^{o}$C, gassed with 95% $O_2/CO_2$) containing ($^3$H)-choline ($HOCH_2CH_2N^+(C^3H_3)_3.Cl$, Amersham, 2μCi/ml, 15 Ci/mmol) for 60 min. Tissues were then superfused with Krebs fluid for 90 min before injecting DMPP into the superfusion circuit or stimulating the tissue for 30 seconds with platinum ring electrodes connected to a Grass S88 stimulator delivering a current of 77 mA. Superfusion fluid was collected every 2 min using a fraction collector and samples prepared for liquid scintillation counting. Hemicholinium ($1.7 \times 10^{-5}$ M) and physostigmine ($6.0 \times 10^{-7}$ M) were present in the superfusion fluid.

Resting efflux was measured over three 2 min periods immediately before giving DMPP or EFS. Stimulated efflux of tritium was measured over three 2 min periods immediately after challenge with DMPP or EFS. The resting efflux was then subtracted from the stimulated efflux and the difference expressed as a percentage of the resting value (i.e. % increase in tritium release, Table 1).

Table 1

| Challenge | % increase in tritium release | Size of contraction (mm)* | |
|---|---|---|---|
| DMPP $3.14 \times 10^{-8}$ mol | 14.2 ± 7.3 | 14.7 ± 4.7 | (n = 12) |
| DMPP $3.14 \times 10^{-7}$ mol | 93.9 ± 20.3 | 29.6 ± 7.5 | (n = 11) |
| EFS 0.1 Hz 0.5 ms | 59.0 ± 19.2 | 38.0 ± 9.7 | (n = 3) |
| EFS 0.5 Hz 1.0 ms | 359.5 ± 35.1 | 112.3 ± 9.8 | (n = 4) |

* DMPP caused a biphasic response while EFS produced only contractions. For comparative purposes only the contractions to DMPP are recorded.
Results are expressed as mean ± s.e. mean.

Radiochemical methods suggest that DMPP releases acetylcholine. This would probably have been greater if physostigmine had been used to 'optimise' recovery of tritiated material as hemicholinium reduced tissue responses to DMPP.

D E B thanks the University of London for a CRF grant.

S J T holds an SERC-CASE award in conjunction with Janssen Pharmaceutical Ltd.

Stockley, H L and Bennett A (1974) Proc. 4th Int. Symp. on GI Motility. Ed: E E Daniel. Mitchell Press, Vancouver. p 165-176.

# HISTAMINE POTENTIATES RESPONSES OF RABBIT ISOLATED DISTAL COLON TO PELVIC NERVE STIMULATION

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

Histamine can modulate the response of several tissues to sympathetic nerve stimulation (Marshall, 1981) but there are few reports of the effect of histamine on responses to parasympathetic nerve stimulation. In the present study the effect of histamine on the response of the rabbit isolated distal colon to pelvic motor nerve stimulation has been investigated.

About 4 cm of distal colon with the pelvic motor innervation was removed from male New Zealand White rabbits (1.6 - 2.5 kg) (Garry and Gillespie, 1954). About 4 cm of proximal colon was also removed and stripped of mesentery. Tissues were set up in Krebs Henseleit solution (36°C, 95% $O_2$, 5% $CO_2$) under 1 g tension. The pelvic nerves were stimulated using square wave pulses, 0.5 ms duration, supramaximal voltage, and the contraction of the tissues was measured isometrically.

Cumulative response curves were constructed to histamine ($EC_{50}$ 6.7±0.7 μM, mean ± s.e. mean, n = 6) and to carbachol ($EC_{50}$ 0.39 ± 0.05 μM, n = 5) on the proximal colon. Responses to histamine were competitively inhibited by the histamine $H_1$-receptor antagonist mepyramine, 10 nM (log $K_B$ 8.4) but were unaffected by the histamine $H_2$-receptor antagonist cimetidine, 100 μM, or the muscarinic receptor antagonist atropine, 10 nM. Responses to carbachol were competitively inhibited by atropine, 10 nM (log $K_B$ 8.9), but were unaffected by mepyramine, 10 nM, or cimetidine, 100 μM. The selective histamine $H_2$-receptor agonist dimaprit, 100 μM, had no effect on either the resting tone of the tissue or on tone raised by carbachol, 1 μM.

Distal colon contracted in response to carbachol ($EC_{50}$ 0.35 ± 0.09 μM, n = 4) and to histamine ($EC_{50}$ 34 ± 12 μM, n = 4). Stimulation of the pelvic motor innervation to the distal colon caused a frequency related contraction. Maximal responses were seen after stimulation at 5-10 Hz. Responses to 2 Hz stimulation were inhibited (70.6 ± 12.2%, n = 4) by atropine, 100 nM. Responses to stimulation at 1 Hz and 2 Hz were unaffected by mepyramine, 100 nM, or cimetidine, 100 μM, but were potentiated by histamine 0.3 and 1 μM. Higher concentrations of histamine caused a contraction. The potentiation of the response to nerve stimulation was inhibited by mepyramine, 100 nM, but was unaffected by cimetidine, 100 μM. Dimaprit, 100 μM, had no effect on either the tone of the tissue or on responses to nerve stimulation.

Responses to acetylcholine matching those seen after nerve stimulation at 1 Hz and 2 Hz were also potentiated by histamine, 1 μM. This potentiation was antagonized by mepyramine, 100 nM.

These results suggest the effects of histamine on the rabbit proximal and distal colon are mediated via histamine $H_1$-receptors.

PMR is an SERC CASE award student in collaboration with Smith, Kline & French Research Ltd.

Garry, R.C. & Gillespie, J.S. (1954) J. Physiol. 123, 60-61P
Marshall, I. (1981) J. autonomic Pharmac. 1, 235-250

# COMPARISON BETWEEN PRESYNAPTIC $\alpha_2$-ADRENOCEPTORS IN THE VAS DEFERENS AND THOSE IN THE FRONTAL CORTEX OF THE MOUSE

M. Adams & I. Marshall, Department of Pharmacology & Therapeutics, The Middlesex Hospital Medical School, London W1P 7PN.

Presynaptic $\alpha_2$-adrenoceptors involved in the feedback modulation of noradrenaline release have been characterised in a wide variety of peripheral tissues, including the vas deferens. There have been fewer studies of similar receptors on central noradrenergic neurones, especially in the mouse. In the present work the $\alpha_2$-adrenoceptors in the vas deferens have been compared with those in the frontal cortex of the mouse using similar stimulation parameters and the selective $\alpha_2$-adrenoceptor antagonist RX 781094 (Chapleo et al, 1981)

Mouse vasa deferentia or slices of frontal cortex (0.3 mm thick) were loaded with 1-7,8-($^3$H)-noradrenaline (0.59 μM, Sp. Act. 34 Ci/mmol). Uptake into brain slices was inhibited by low sodium (29 ± 4% inhibition, mean ± s.e. mean in 40 mM NaCl Krebs solution) and by cocaine (59 ± 3% inhibition, at 30 μM).

In the vas deferens field stimulation (100 pulses, 1 Hz, 2 ms) gave a fractional release per pulse of ($^3$H)-noradrenaline (separated from its ($^3$H)-metabolites) of 2.8±0.5 x $10^{-6}$. Clonidine (1-300 nM) caused a concentration dependent inhibition of both the fractional release of ($^3$H)-noradrenaline and twitch tension ($EC_{50}$, concentration to halve the control value, 4.7±0.6 nM and 12.6±3.9 nM respectively, with more than 90% inhibition of both parameters at 100 nM. The antagonist RX 781094 (0.1, 0.3 and 1.0 μM) was added to the Krebs solution (minimum equilibration 20 min). This produced a shift to the right of 2-point concentration-inhibition curves for clonidine on ($^3$H)-noradrenaline release and twitch tension (dose ratio 88 ± 18 and 68 ± 16 respectively at RX 781094 1.0 μM) with $pA_2$ values for RX 781094 of 7.9 (slope 0.94) and 7.7 (slope 1.04) respectively.

The electrically evoked release of ($^3$H)-noradrenaline from superfused cortical slices was current dependent (5-52 mA) and at 30 mA was prevented by omission of calcium or the addition of guanethidine (4 μM). Stimulation (100 pulses, 1 Hz, 2 ms, 30 mA) gave a fractional release per pulse of ($^3$H)-noradrenaline of 26.0 ± 4.6 x $10^{-6}$. This was reduced by clonidine (0.3 - 100 nM; $EC_{50}$ 6.4 ± 1.3 nM) with about 90% inhibition at the highest concentration used. Two point clonidine concentration-inhibition curves were moved to the right by RX 781094 (0.1, 0.3, and 1.0 μM) with a dose ratio of 139 ± 31 at 1 μM and a $pA_2$ value of 8.0 (slope 1.00).

In peripheral tissues presynaptic $\alpha_2$-adrenoceptors are usually defined by $pA_2$ values obtained using the post-junctional response to stimulation. The present work with RX 781094 shows that the $pA_2$ value is similar whether measured using clonidine inhibition of the twitch response or of ($^3$H)-noradrenaline release. Furthermore the results indicate that the mouse vas deferens and frontal cortex contain a similar $\alpha_2$-adrenoceptor through which the release of ($^3$H)-noradrenaline can be modulated.

We thank the MRC for support and Glaxo (Greenford) for the brain slice chambers. MA holds an SERC CASE Award in collaboration with Reckitt & Colman Ltd.

Chapleo, C.B. et al (1981) Br. J. Pharmac. 74, 842P

# ARE THERE INNERVATED POST-SYNAPTIC $\alpha_2$-ADRENOCEPTORS IN THE VAS DEFERENS?

Susan Baker & I. Marshall, Department of Pharmacology & Therapeutics, The Middlesex Hospital Medical School, London W1P 7PN.

Recent evidence from radio-ligand binding experiments with homogenates of rat vas deferens suggests the presence of post-synaptic $\alpha_2$-adrenoceptors in this tissue (Watanabe et al., 1982). These receptors would be available for drugs used to study the presynaptic $\alpha_2$-adrenoceptors and complicate the interpretation of experiments based on post-junctional twitch responses. Therefore the presence of post-synaptic $\alpha_2$-adrenoceptors in the mouse vas deferens has been investigated.

Three $\alpha_2$-adrenoceptor antagonists have been used of which two, yohimbine and RX781094, are known to be selective in the mouse vas deferens (Marshall et al, 1978; Baker & Marshall, 1982). The third compound was Wy26703 (Lattimer et al, 1982). The selectivity of this drug for $\alpha_2$-adrenoceptors was assessed using cumulative clonidine (1-1000 nM)-inhibition of twitch response curves (0.2 Hz, 2 ms) with prazosin (100 nM) present in the Krebs solution. One concentration of Wy26703 (0.1, 0.3, 1.0 or 3.0 μM) was equilibrated for 30 min and the clonidine curve repeated. At the highest concentration the dose ratio was 98±17 (mean ± s.e. mean). The apparent $pA_2$ for Wy26703 was 7.84 (slope 0.83). To assess the activity of the drug at $\alpha_1$-adrenoceptors isolated vasa (without prazosin in the Krebs solution) were contracted by phenylephrine (1 μM - 3 mM). Wy26703 (10, 30 and 100 μM) shifted the concentration-effect curve to the right (dose ratio 105 ± 26 at 100 μM) with a $pA_2$ value of 5.75 (slope 1.12). The selectivity ratio (antilog ($\alpha_2 pA_2 - \alpha_1 pA_2$)) for Wy26703 was 123.

To assess the presence of a post-synaptic $\alpha_2$-adrenoceptor the effects of the 3 selective antagonists were studied on the response of the isolated vas deferens to a single electrical pulse. With a single pulse there is no detectable feedback by released noradrenaline on to presynaptic $\alpha_2$-adrenoceptors (Baker & Marshall, 1982) to complicate the interpretation of the results.

The response of the vas deferens to a single pulse (2 ms, repeated every 10 min; prazosin 100 nM present in Krebs solution) was recorded on a Grass polygraph (single phase response, peak tension 171 ± 26 mg). Each tissue was exposed to 4 increasing concentrations (30, 100, 300 and 1000 nM) of one antagonist. The equilibration time for yohimbine and RX781094 was 20 min and for Wy26703 it was 30 min. The antagonists did not produce consistent effects on the smooth muscle response to a single electrical pulse. Significant increases in tension over controls were produced by yohimbine 100 and 300 nM, RX781094 30 nM and Wy26703 30 nM and 300 nM ($P < 0.05$, paired t test). The greatest increases (about 50%) were elicited by yohimbine and Wy26703. However, results with all three antagonists failed to produce concentration-effect relationships in line with those of the drugs at pre-synaptic $\alpha_2$-adrenoceptors in the same tissue.

These results do not support the presence of post-synaptic $\alpha_2$-adrenoceptors with similar characteristics to the pre-synaptic $\alpha_2$-adrenoceptors in the vas deferens of the mouse.

We thank the MRC for support.

Baker, S. & Marshall, I. (1982) Br. J. Pharmac. 76, Proc. Suppl., 212P
Lattimer, N. et al (1982) Br. J. Pharmac. 75, Proc. Suppl., 154P
Marshall, I. et al (1978) Br. J. Pharmac. 62, 147-151
Watanabe, Y. et al (1982) Eur. J. Pharmac. 80, 105-108

# EFFECT OF MAZINDOL ON THE RAT VAS DEFERENS AND ANOCOCCYGEUS MUSCLE

M.R. Zarrindast, Department of Pharmacology, Faculty of Medicine, University of Tehran, Tehran 14, Iran

Mazindol is an anorectic agent with different chemical structure from that of phenylethylamines; in addition to anorexia, it increases body temperature, locomotor activity and stereotypic behaviour in rats (Gogerthy et al, 1975). The observed effects might result from inhibition of uptake or increase of the release of monoamines from the brain (Engstrom et al, 1975). We have previously reported an increase of the release of dopamine, induced by mazindol, from rat brain synaptosomes (Kruk & Zarrindast, 1976). In the present work, effect of mazindol on mechanical activity of the rat anococcygeus muscle and the release of tritium from ($^3$H)-noradrenaline labelled vas deferens has been studied.

Mazindol ($3.5 \times 10^{-7}$ M to $1.8 \times 10^{-5}$ M) contracted the anococcygeus of the rat. The contractions appeared in 1-2 min after exposure to mazindol and reached a maximum in 4-8 min. The magnitude of contractions was not dose dependent. This effect could be antagonized by pretreatment of the animals with reserpine (2.5 mg/kg, i.p., 48 and 24 h before experiment) or incubation of the tissue with phentolamine ($4.4 \times 10^{-7}$ M) but not by atropine, cocaine, hexamethonium, methysergide or promethazine.

Mazindol ($7 \times 10^{-7}$ M) potentiated the effect of electrical stimulation (2-50 Hz). In reserpinized tissue, mazindol ($7 \times 10^{-7}$ M) potentiated the effect of noradrenaline. -Log $EC_{50}$ of noradrenaline was 5.57±0.17 in absence and 6.56±0.14 in the presence of mazindol.

In the rat vas deferens, mazindol developed phasic contractions. In vasa deferentia preloaded with ($^3$H)-noradrenaline, mazindol ($7 \times 10^{-5}$ M) increased the outflow of radioactivity from 0.71±0.20 to 1.47± 0.36 (as per cent of radioactivity of the tissue . min$^{-1}$).

It is concluded that mazindol contracts the rat anococcygeus and vas deferens possibly by releasing endogenous noradrenaline.

I wish to thank Miss F. Akhtar-Khavari and Miss E. Rezaei for their technical assistance.

Engstrom, R.G. et al (1975) Arch. int. Pharmacodyn. 214, 308
Gogerthy, J.H. et al (1975) Arch. int. Pharmacodyn. 214, 285
Kruk, Z.L. & Zarrindast, M.R. (1976) Br. J. Pharmac. 58, 272p

# PARADOXICAL AGONIST EFFECT OF PHENTOLAMINE

A. Broadhurst, C. Ennis and N. Lattimer. Department of Pharmacology, Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow, Maidenhead, Berks.

The observation that phentolamine but not phenoxybenzamine is capable of inhibiting the electrically-induced twitch response of the rabbit vas deferens (Adebanjo and Ambache, 1978) is not consistent with the view that the inhibition is due to α-adrenoceptor blockade. In order to further investigate this phenomenon we have studied the effects of phentolamine on the twitch responses of the rat and rabbit vas deferens and on $K^+$-evoked release of NA from rat cortical slices.

The prostatic ends of rat and rabbit vasa deferentia were mounted between platinum ring electrodes in a 5ml organ bath with a resting tension of 0.5g and bathed in Krebs-bicarbonate solution. The vasa were field stimulated at 0.1Hz, 1 msec with supramaximal voltage. Potassium-evoked release of tritium was measured from superfused slices of rat cortex preloaded with {$^3$H}-NA (Dismukes et al, 1977) and the uptake of {$^3$H}-NA into rat cortical synaptosomes was measured by the method of Wood and Wyllie (1981).

Phentolamine completely inhibited the twitch response of the rabbit vas deferens ($IC_{50}$ $1.5 \times 10^{-7}$M) but did not inhibit that of the rat vas deferens where potentiation of the twitch response was observed with concentrations above $10^{-7}$M. Phentolamine ($10^{-9}$ - $10^{-7}$M) produced a concentration - related inhibition of $K^+$-evoked release of {$^3$H}-NA from rat cortical slices with the maximum effect of 50% inhibition at $10^{-7}$M. Concentrations above $10^{-7}$M potentiated the $K^+$-evoked release. Yohimbine antagonised both the inhibition of the rabbit vas deferens twitch ($pA_2$,7.9) and the inhibition of $K^+$-evoked release of {$^3$H}-NA ($pA_2$ 8.1)

Clonidine, an $\alpha_2$-adrenoceptor agonist inhibited both the twitch response of the rabbit vas deferens ($IC_{50}$ $6 \times 10^{-9}$M) and that of the rat vas deferens ($IC_{50}$ $2.7 \times 10^{-9}$M) and inhibited the $K^+$-evoked release of {$^3$H}-NA ($IC_{50}$ $6 \times 10^{-8}$M). The inhibitory effects of clonidine could be antagonised in all three tissues by yohimbine ($pA_2$values 7.7, 7.6 and 7.7 respectively).

The inhibitory effect of phentolamine does not appear to be related to NA $uptake_1$ inhibition since the $IC_{50}$ for phentolamine as an inhibitor of {$^3$H}-NA uptake into rat cortical synaptosomes was $5 \times 10^{-6}$M.

In conclusion, since the responses of all three preparations can be inhibited by classical $\alpha_2$-adrenoceptor agonists eg. clonidine, the receptor at which phentolamine was acting as an agonist, whilst bearing some resemblance to an $\alpha_2$-adrenoceptor does not appear to be identical to it. The rat vas deferens lacks this phentolamine-sensitive receptor.

Adebanjo, A.O. and Ambache, N. (1978) J. Physiol.(Lond)., 281, 28-29P.
Dismukes, R.K. et al: (1977) Naunyn Schmiedeberg's Arch Pharmacol., 299 115-122.
Wood, M.D. and Wyllie, M.G. (1981) J. Neurochem.37 795-797.

# EFFECTS OF ADENOSINE DERIVATIVES ON THE RAT ANOCOCCYGEUS MUSCLE IN VITRO

T.W. Stone, Dept. of Physiology, St. George's Hospital Medical School, University of London, London SW17 ORE.

The effects of a series of purines and derivatives have been studied on the rat anococcygeus muscle in vitro in order to determine the types of purine receptor present. The anococcygeus muscle was removed from male rats as described by Gillespie (1972) and suspended under 1g tension in a 10 ml bath. The tissue was superfused continuously at a rate of 3 ml/min with a bicarbonate solution (NaCl 118 mM, KCl 4.7, $KH_2PO_4$ 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11) bubbled with 95% $O_2$ - 5% $CO_2$ at 37°C. A pair of parallel platinum electrodes were used for stimulation using 1 ms. pulses at 5Hz and supramaximal voltage delivered for 2-3 s every 20 or 50 s. Contractions were recorded isometrically.

Adenosine triphosphate (ATP) induced contraction of the resting anococcygeus muscle. Adenosine and derivatives such as 2-chloroadenosine, (-)$N^6$-phenylisopropyl-adenosine (PIA) and 5'N-ethylcarboxamide adenosine (NECA) did not cause contraction of the resting muscle, but they inhibited contractile responses to field stimulation. The order of potency for this latter action was PIA > NECA > 2-chloroadenosine > adenosine, with PIA having an $IC_{50}$ of about 0.1 μM. The inhibition of evoked contraction could be blocked by theophylline. As responses to noradrenaline were unaffected by any of these purines the inhibition of neurally-mediated contractions probably occurs at a presynaptic site (Stone, 1981). Furthermore, since PIA is the most potent purine of those tested in activating the $A_1/R_i$ receptor (Londos et al., 1980; Van Calker et al., 1979; Stone, 1981) a similar receptor could be involved in the anococcygeus muscle, although it should be noted that the usual order of potency at this site is PIA > adenosine > NECA.

When the tone of the anococcygeus was raised by perfusion with carbachol (50μM) all the purines tested (adenosine, ATP, 2-chloroadenosine, PIA and NECA) caused a contraction. These compounds were approximately equipotent and the responses could not be prevented by theophylline, quinidine, 2, 2'-pyridylisatogen tosylate, phentolamine, methysergide, dipyridamole, hexobendine or indomethacin. The mechanism of these contractile effects therefore is obscure.

When tested on the inhibitory responses to field stimulation seen in muscles with raised tone, none of the purines tested had any inhibitory action. A similar observation has recently been made with GABA which depresses the excitatory but not inhibitory nerves of the anococcygeus muscle (Hughes et al., 1982) suggesting fundamental differences between the two types of innervation.

Gillespie, J. (1972) Br. J. Pharmac. 45, 404 - 416.
Hughes, P.R. et al (1982) Br. J. Pharmac. in press.
Londos, C. et al. (1980) Proc. Nat. Acad. Sci. 77, 2551 - 2554.
Stone, T.W. (1981) Neuroscience 6, 523 - 555.
Van Calker, D. et al., (1979) J. Neurochem. 33, 999 - 1005.

# BDF 6143, A POTENT ANTAGONIST AT $\alpha_2$-ADRENOCEPTOR SITES

I.B. Armah*, D.R. Ferry[1] and H. Glossmann[1]
*Dept. of Pharmacology, BDF, Unnastrasse 48, D-2000 Hamburg 20
[1]Rudolf-Buchheim Institut für Pharmakologie, Frankfurterstr. 107, D-63 Giessen

Yohimbine and Rauwolscine are selective alpha$_2$-adrenoceptor antagonists (Weitzell et al. 1979). We report here the characterization of BDF 6143, (4-chloro-2-(2-imidazoline-2-yl-amino)isoindoline hydrochloride), a potent alpha$_2$-adrenoceptor antagonist in organ bath experiments and radioligand binding studies, and of comparable alpha$_2$-selectivity as other new alpha blockers (Chapelo et al, 1981). With in vivo experiments on pentobarbitone anaesthetized rabbits, the intracisternal (i.ci) application of BDF 6143 abolished dose-dependently the hypotensive effect of i.ci. clonidine (1µg/kg). The $ED_{50}$ value for BDF 6143 was $10^{-8}$M, significantly lower than that of yohimbine at $10^{-7}$M. Prazosin did not inhibit i.ci. clonidine in this test. BDF 6143 was assessed for selectivity of alpha$_2$-adrenoceptor (vs) alpha$_1$-adrenoceptor antagonism. Alpha$_2$-receptor activity was evaluated by using antagonism of clonidine inhibition of 1 Hz field stimulation induced twitch response of the epididymal portion of the isolated rat vas deferens. Alpha$_1$-adrenoceptor blockade was assessed using phenylephrine induced contractions of rat isolated annococcygeal muscle. $pA_2$-values are given in Table 1. Using this paridigm to assess alpha$_2$-selective antagonism BDF 6143 is 26.3 fold alpha$_2$-selective, and a significantly ($< 0.05$) more potent alpha$_2$-antagonist than rauwolscine. At alpha$_1$-adrenoceptors BDF was a partial agonist displaying alpha-mimetic activity in the dose range $10^{-8}$- $10^{-7}$M. Using isolated strips of rabbit pulmonary artery preincubated in $^3$H -noradrenaline, Docherty et al (1982) have found BDF 6143 to be more potent than yohimbine in antagonizing clonidine-induced inhibition of evoked tritium overflow.

Table 1: $pA_2$-values of alpha blockers ± S.D. and selectivities with the number of experiments in parentheses.

| Drug | Alpha$_2$ | Alpha$_1$ | Selectivity Index |
|---|---|---|---|
| BDF 6143 | 9.00 ± 0.25 (12) | 7.58 ± 0.21 ( 9) | 26.3 |
| rauwolscine | 7.93 ± 0.11 ( 9) | 6.72 ± 0.18 ( 9) | 16.2 |
| yohimbine | 7.58 ± 0.11 ( 9) | 6.71 ± 0.16 ( 9) | 7.3 |
| phentolamine | 7.56 ± 0.06 ( 9) | 8.16 ± 0.14 ( 9) | 0.25 |
| BE 2254* | 6.74 ± 0.18 (12) | 9.55 ± 0.09 (12) | 0.002 |
| Prazosin | $\leq$5.0 ( 9) | 8.54 ± 0.11 ( 9) | $\leq$0.0003 |

*BE 2254 = (2-ß-4-hydroxylphenyl)-ethyl-aminomethyl)tetralone.

Using rat cerebral cortex as a source of alpha$_2$ and alpha$_1$-adrenoceptors the selectivity of BDF 6143 was evaluated by displacement of subtype-selective radioliands. Against $^3$H -Rauwolscine (85 Ci/mmol; dissociation constant 2 nM, $B_{max}$ 110 fmol/mg$^{-1}$ protein) BDF 6143 had a $K_i$ value of 1.66nM. Against the alpha$_1$-adrenoceptor ligand $^{125}$I-HEAT (2-ß-3-$^{125}$Iodo,4-hydroxylphenyl)-ethyl-aminomethyl)tetralone (2200 Ci/mmol) (Glossmann et al. 1981) a $K_i$ value of 30 nM was observed. Thus, the in vitro binding alpha$_2$-selectivity of 18.1 fold agrees well with the value obtained from organ bath studies, and confirm BDF 6143 as an alpha$_2$ selective antagonist.

Chapelo, C.B. et al (1981), Br. J. Pharmac., 74, 842P.
Docherty, J.R. et al (1982), Arzneim. Forschung (Drug Res) (in press).
Glossmann, H. et al (1981), Naunyn-Schmied. Arch. Pharmac. 318, 319.
Weitzell, R. et al (1979), Naunyn-Schmied. Arch. Pharmac. 308, 127.

# THE EFFECTS OF Ro 31-1118, A NOVEL $\beta_1$-ADRENOCEPTOR ANTAGONIST, IN THE CAT

L.C. Blaber, D.T. Burden & C. Shivdasani, Pharmacology Department, Roche Products Limited, Welwyn Garden City, Hertfordshire AL7 3AY.

Ro 31-1118 (1-[4-<2-(4-fluorophenethyloxy)ethoxy>phenoxy]-3-isopropy--lamino-2-propanol hydrochloride) is a novel $\beta_1$-adrenoceptor antagonist. The cat has been found to be a useful model for investigating selectivity and potency of β-adrenoceptor agonists and antagonists, and correlates well with activity found in man (Bowman & Raper, 1976; Daly & Levy, 1979; Blaber & Burden, 1982).

Anaesthetised cats, which had been treated with reserpine (0.5 $mg.kg^{-1}$ i.p.) 16 hours before the experiment, were bilaterally vagotomised and a hind limb autoperfused. The dose of Ro 31-1118 reducing isoprenaline-induced tachycardia by 50% was 20 $\mu g.kg^{-1}$ i.v. No reduction in isoprenaline-induced hind limb vasodilator response was observed with doses of Ro 31-1118 up to 3 $mg.kg^{-1}$ i.v. The compound was also a partial agonist producing small increases in heart rate, with a peak effect of 9 bts. $min^{-1}$ at 10 - 30 $\mu g.kg^{-1}$ i.v., and dose related decreases in hind limb perfusion pressure.

In the anaesthetised open-chest cat a positive inotropic effect and increased peak aortic blood flow were observed at doses up to 300 $\mu g.kg^{-1}$ i.v.; above this dose some bradycardia occurred and above 3 $mg.kg^{-1}$ i.v. there was a negative inotropic effect. In the non-reserpinised cat some $\beta_2$-antagonist effects were observed at doses of 1 $mg.kg^{-1}$ and above.

Following oral administration to the conscious cat, Ro 31-1118 (10 $mg.kg^{-1}$) had a duration of action in excess of 12 hours, antagonising the response to i.v. isoprenaline- (0.05 $\mu g.kg^{-1}$ i.v.) or exercise-induced tachycardia. A small antagonism of the isoprenaline-induced vasodepressor response was observed at 3 hrs only.

Thus, in the cat Ro 31-1118 is a $\beta_1$-adrenoceptor antagonist, with specific $\beta_1$-adrenoceptor antagonism in the reserpinised cat. The compound also has some partial agonist activity producing mainly a positive inotropic effect, vasodilator activity and a long duration of action.

We wish to thank Miss Y M Burke for expert technical assistance with the conscious cat studies.

Blaber, L.C. & Burden, D.T. (1982) Brit. J. Pharmacol. 76: 250P.
Bowman, W.C. & Raper, C. (1976) J. Pharm. Pharmacol. 28: 369-374.
Daly, M.J. & Levy, G.P. (1979) Trends in Autonomic Pharmacology 1: 347-419. Ed. S. Kalsner. Urban & Schwarzenberg. Baltimore.

# ENHANCEMENT OF AIRWAY SENSITIVITY TO HISTAMINE IN GUINEA-PIGS BY β-ADRENOCEPTOR BLOCKING AGENTS

U.M. Ney, Preclinical Research, Sandoz Ltd., CH-4002 Basle, Switzerland

It is well known that the β adrenoceptor blocking drugs cause bronchoconstriction in man and can increase airway sensitivity to other constrictor agents e.g. histamine. In guinea-pigs it has been shown that the initial bronchoconstriction is unlikely to be due to β blockade since (+)propranolol, the non-β blocking isomer of propranolol (Howe & Shanks, 1966), produces a similar bronchospasm to the racemate (Maclagan & Ney, 1979). The effects of the two compounds on airway sensitivity to histamine in the guinea-pig have now been studied, together with that of a selective $\beta_1$ antagonist practolol.

Animals were anaesthetised with urethane (1.25 g/kg i.m./i.p.) and allowed to breathe spontaneously. They were prepared as described by Maclagan and Ney (1979) and mean lung resistance ($R_L$) and compliance ($C_{dyn}$) were calculated using a Buxco Pulmonary Mechanics Analyser. The right carotid artery was cannulated for the measurement of BP and HR and the left jugular vein for the injection of drugs. All drugs were made up in 0.9 % saline and injected at a constant volume and rate. After control responses to histamine (1-2 µg/kg) had been obtained the test compound was given followed, 15 min later and at 15 min intervals thereafter, by the same dose of histamine. For comparison, control histamine response =100% $\uparrow R_L$*.

Table 1 Enhancement of histamine-induced bronchoconstriction ($\uparrow R_L$) and its reversal by FPL 55712 (1 mg/kg i.v.)

| Drug | Dose mg/kg | n | Histamine bronchospasm as % of control response* max $R_L$ | 60' | n | after FPL 55712 |
|---|---|---|---|---|---|---|
| practolol | 0.1 | 3 | 201 ± 34 | 151 ± 25 | - | - |
| (±)propranolol | 0.1 | 3 | 233 ± 48 | 153 ± 56 | - | - |
| (+)propranolol | 0.1 | 5 | 221 ± 35 | 196 ± 55 | 2 | 94 (± 6.5) |
| (+)propranolol | 0.5 | 5 | 338 ± 103 | 246 ± 137 | 3 | 106 ± 35 |
| NaCl | 0.2ml | 3 | 98 ± 4 | 108 ± 6 | 2 | 101 (± 25) |

Both (+)propranolol and practolol produced an equivalent enhancement of histamine bronchospasm as (±) propranolol despite the differences in their respective β blocking ability. The increased responses were measured at 15 min and began to decline 1 h after administration of the blocker. After establishment of the increased histamine bronchospasm by (+) propranolol, the SRS antagonist, FPL 55712 (Augstein et al., 1973), was given (1 mg/kg) 1 min before the next histamine challenge. The subsequent bronchospasm was reduced to control levels, indicating the involvement of leukotrienes in the potentiated effect.

It has recently been shown that both (+) and (±) propranolol induce histamine release from mast cells (Terpstra et al., 1981) and thus it is possible that the increased airway sensitivity produced by these drugs may be due to their ability to induce mediator release, in particular leukotrienes, rather than β blockade.

The assistance of F. Telli and S. v. Niederhäusern is gratefully acknowledged.

Augstein, J. et al. (1973). Nature new Biol., 245, 215-217.
Howe, R. & Shanks, R.G. (1966). Nature Lond., 210, 1336-1338.
Maclagan, J. & Ney, U.M. (1979). Eur. J. Pharmac., 66, 404-418.
Terpstra, G.K. et al., (1981). Eur. J. Pharmac., 73, 107-108.

# BRL 16644, AN INHIBITOR OF MONOAMINE UPTAKE, IN VITRO, WITH A LONG DURATION OF ACTION, IN VIVO

A.M. Johnson, Julia M. Loudon, D.R. Nelson, Jane M. Nicholass and D.R. Thomas, Beecham Pharmaceuticals Research Division, Harlow, Essex, CM19 5AD.

In the course of studying the biological activity of a series of 4-aryl chromans (Ainsworth et al, 1982), BRL 16644 (2,2-dimethyl-7-(2-dimethyl-aminoethoxy)-4-[3-trifluoromethyl)phenyl]chroman hydrochloride) was found to inhibit monoamine uptake in vitro. Its biochemical and pharmacological profile was further evaluated and compared to the unsubstituted 4-phenyl analogue (BRL 14831), desipramine, nomifensine and paroxetine.

The effects of the drugs on tritiated dopamine (DA), noradrenaline (NA) and 5-hydroxytryptamine (5-HT) uptake into crude synaptosomes from the corpus striatum and hypothalamic regions of rat brain were investigated. BRL 14831 and BRL 16644 were shown to be active inhibitors in all 3 systems, but BRL 14831 was more active as a catecholamine uptake inhibitor (Table 1).

Table 1 Inhibition of ($^3$H) Monoamine Uptake into Rat Synaptosomes

| | Ki (μM) ± s.e. mean (no. of experiments) | | |
|---|---|---|---|
| Drug | Hypothalamus ($^3$H) 5HT | Hypothalamus ($^3$H) NA | Corpus Striatum ($^3$H) DA |
| BRL 14831 | 0.68 ± 0.12 (3) | 0.29 ± 0.02 (2) | 0.20 ± 0.06 (3) |
| BRL 16644 | 0.83 ± 0.25 (3) | 1.1 ± 0.06 (3) | 2.0 ± 1.18 (3) |
| Desipramine | 1.4 ± 0.2 (3) | 0.29 ± 0.01 (4) | 17.7 ± 3.4 (3) |
| Nomifensine | 5.5 ± 0.8 (3) | 0.55 ± 0.05 (3) | 0.43 ± 0.14 (3) |
| Paroxetine | 0.0083 ± 0.001 (4) | 3.8 ± 1.0 (3) | 2.3 ± 0.6 (2) |
| Kinetic parameters of the uptake, Km(μM): Vmax (pmol/h/mg tissue) | | | |
| Km | 0.043 ± 0.006 (5) | 0.37 ± 0.01 (5) | 0.20 ± 0.01 (4) |
| Vmax | 9.3 ± 2.0 (5) | 7.0 ± 1.5 (5) | 65.1 ± 12.4 (4) |

BRL 14831, BRL 16644, nomifensine and desipramine all prevented reserpine-induced hypothermia in mice (minimal effective doses being 0.3, 1, 3 and 1 mg/kg p.o., respectively). BRL 16644 was most active 24 to 48 hours after administration in contrast to the short time course of the other 3 drugs. The time course of the hypermotility induced in mice by BRL 16644 (40mg/kg p.o.) paralleled that of the prevention of reserpine-induced hypothermia. BRL 16644 (80mg/kg p.o.) induced stereotyped behaviour in the rat which was maintained 4 to 30h after dosing. In contrast, BRL 14831 (20mg/kg p.o.) and nomifensine (60mg/kg p.o.) were most active at 2 to 6h. The behaviours induced by the BRL chromans were inhibited by pimozide (1 mg/kg p.o.). In addition, they induced ipsilateral turning in rats lesioned unilaterally by 6-hydroxydopamine in the nigro-neostriatal pathway indicating that they indirectly activate central dopamine receptors. BRL 16644 and BRL 14831, like paroxetine, potentiated 5-hydroxytryptophan-induced elevation of electroshock threshold in mice, $ED_{50}$'s being 9, 1.9 and 0.6 mg/kg p.o. respectively. The BRL chromans were most active 2 to 24h after administration whereas paroxetine was shorter acting.

In conclusion, BRL 16644 and BRL 14831, inhibitors of NA, DA and 5-HT uptake in vitro, exhibit a pharmacological profile between that of nomifensine and paroxetine. BRL 16644 is characterised by a particularly long duration of action in vivo, in contrast to BRL 14831 and the other drugs investigated. However, toxicological problems have precluded further development of these chromans.

Ainsworth, A. et al (1982) Abstract North Amer. Med. Chem. Symposium, Toronto.

# EFFECTS OF SGD 101/75 AND NORADRENALINE IN THE ANAESTHETIZED RAT

M.A. Chadwick, K. Lawson, & D.F. Weetman, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Sunderland Polytechnic, Tyne and Wear, SR1 3SD.

High sensitivity of preparations to Sgd 101/75 has been used as one criterion in the characterisation of a new subtype of adrenoceptor, $\alpha_{1s}$-adrenoceptor (Coates et al, 1982). Sgd 101/75 also interacts with $\alpha_1$-adrenoceptors where it is a partial agonist, exhibiting a low affinity relative to noradrenaline (NA), tachyphylaxis and antagonism of NA (Ismail et al, 1981).

Female Sprague-Dawley rats (200-250g) were anaesthetised with urethane (1.5 g/kg i.p.). The trachea, right carotid artery and jugular vein were cannulated and the following measurements made: diastolic blood pressure (DBP), heart rate (derived from the BP trace), tension in the left inferior eyelid (IE) and rectal temperature. Responses were recorded on Devices MX2 or Grass 79D polygraphs. After a 30 min stabilisation period, NA was administered at 5 min intervals in an ascending series of doses. Then a single dose of either saline (control) or Sgd 101/75 (0.08-160 μmoles/kg) was administered and 20 min later a second NA dose-response curve was obtained. Mean values and 95% fiducial limits were estimated.

Both NA and Sgd 101/75 increased DBP and contracted the inferior eyelid. NA, but not Sgd 101/75 (up to 80 μmoles/kg), increased heart rate and neither drug affected rectal temperature.

The IE was 10 times less sensitive to Sgd 101/75 than to NA (doses for an equivalent response of 150mg increase in tension with 95% fiducial limits: NA 99 (85-113) nmoles/kg, n=63; Sgd 101/75 1.0(0.25-3.55) μmoles/kg, n=63, $P<0.05$). The dose-response curve for Sgd 101/75 upon DBP showed two distinct regressions, separated by a plateau at an increase of 40mmHg; thus two comparisons between the potency of NA and Sgd 101/75 were made (dose to increase DBP 35mmHg: NA 4.46 (2.06-6.86) nmoles/kg, n=63; Sgd 101/75 0.56 (0.25-1.25) μmoles/kg, n=20, $P<0.05$; relative potency NA:Sgd 101/75 = 1:125; dose to increase DBP 50mmHg: NA 14.0 (11.4-16.6) nmoles/kg, n=63; Sgd 101/75 33.5 (5.0-200) μmoles/kg, n=27, $P<0.05$; relative potency NA:Sgd 101/75 = 1:2400).

The second dose-response curve for noradrenaline after saline revealed no significant difference in sensitivity to the catecholamine on all systems. However, after Sgd 101/75 (20-160 μmoles/kg) a dose-related reduction in the sensitivity to NA was seen on the DBP (DR with 160 μmoles/kg = 73.0 (22.0-124); n=4). Although there was a small reduction in the sensitivity to NA on the IE after Sgd 101/75 (20-160 μmoles/kg), a dose-related effect was not apparent (DR with 160 μmoles/kg = 6.8 (3.6-10.0); n=5). Sgd 101/75 produced no significant reduction in the sensitivity to NA on the heart rate.

A single administration of Sgd 101/75 (50μmoles/kg) produced a rise in DBP 75(49-101)mmHg; n=5); a second administration of Sgd 101/75 (same dose) failed to increase DBP (-8 (-35.2- +19.2)mmHg; n=5), demonstrating tachyphylaxis. The IE response to the first administration of Sgd 101/75 (50 μmoles/kg) was maintained for at least 20 min, therefore tachyphylaxis was not investigated.

It is concluded that the rise in DBP due to Sgd 101/75 is probably mediated by $\alpha_1$-adrenoceptors because Sgd 101/75 acted as a partial agonist, as it does on the taenia (Ismail et al, 1981), but the contraction of the IE may involve $\alpha_{1s}$-adrenoceptors. Studies on the inferior eyelid smooth muscle *in vitro* are required to substantiate this.

Coates et al, (1982). Br. J. Pharmac., 75, 549-552
Ismail et al, (1981). Br. J. Pharmac., 72, 535-536P.

# BINDING OF ($^3$H)-PRAZOSIN TO HUMAN BLOOD PLATELETS

Roger Kerry, Ruth M. McKernan and Michael C. Scrutton, Department of Biochemistry, King's College, London, WC2R 2LS.

Aggregation of human blood platelets is mediated by interaction of adrenaline at $\alpha_2$-adrenoceptors (Grant & Scrutton, 1979; Hsu et al, 1979; Grant & Scrutton, 1980) although some evidence has been presented which indicates that a response mediated by $\alpha_1$-adrenoceptors can also occur in platelets from some donors (Grant & Scrutton, 1979). Radioligand binding studies using [$^3$H]-dihydroergocryptine and more recently [$^3$H]-yohimbine as radioligand have confirmed the presence of significant population of $\alpha_2$-adrenoceptors on this cell and have failed to demonstrate the presence of $\alpha_1$-adrenoceptors (Hoffman et al, 1979; Elliott & Grahame-Smith, 1980; Daigugi et al, 1981). In accord with this conclusion human platelets have been reported either to show no binding of [$^3$H]-prazosin (Daigugi et al, 1981) or to exhibit binding of this ligand having characteristics inconsistent with interaction at a membrane receptor (Motulsky & Insel, 1981).

The $\alpha_1$-adrenoceptor agonist, methoxamine, stimulates aggregation to sub-optimal concentrations of ADP in platelets obtained from 20% of the donors in our panel. We have therefore performed an analysis of the binding of [$^3$H]-prazosin to intact platelets which do, and do not, show the methoxamine response. The extent of specific binding of [$^3$H]-prazosin (as defined by the extent of binding in the presence and absence of 10 μM phentolamine) is significantly greater in methoxamine-sensitive platelets ($B_{max}$ = 32 ± 2 f.mol/$10^8$ platelets) than in methoxamine-insensitive platelets ($B_{max}$ = 9 ± 1 f.mol/$10^8$ platelets). The extent of [$^3$H]-dihydroergocryptine binding ($B_{max}$ = 75 ± 7 f.mol/$10^8$ platelets) is not significantly different for both platelet populations. Binding of [$^3$H]-prazosin to methoxamine-sensitive platelets is reversible and determination of $k_{on}$ (5.1 x $10^8$ $M^{-1}$ $min^{-1}$) and $K_{off}$ (2.5 x $10^{-1}$ $min^{-1}$) for the specific binding gives $K_D$ as 0.49 nM in reasonable agreement with the value for this parameter (0.25 nM) obtained by direct analysis of the binding isotherm. Studies using sub-type selective α-adrenoceptor antagonists have shown that binding of [$^3$H]-prazosin to methoxamine-sensitive platelets is inhibited far more effectively by indoramin ($K_I$ = 10 nM) and by prazosin ($K_I$ = 0.6 nM) than by yohimbine ($K_I$ = 2.3 μM) or rauwolscine ($K_I$ = 4.6 μM).

These data therefore provide some further indication of the presence of $\alpha_1$-adrenoceptors on platelets which respond to $\alpha_1$-adrenoceptor agonists. The restricted distribution of this response among human donors may explain the discrepancy between this study and the prior reports cited above.

Daigugi, M. (1981) Life Sci., 28, 2705-2717.
Elliot, J.M. & Grahame-Smith, D.G. (1980) Br. J. Pharmac., 70, 59P.
Grant, J.A. & Scrutton, M.C. (1979) Nature, Lond., 277, 659-661.
Grant, J.A. & Scrutton, M.C. (1980) Br. J. Pharmac., 71, 121-134.
Hoffman, B.B. et al (1979) Life Sci., 24, 1739-1746.
Hsu, C.Y. et al (1979) J. Pharmac. exp. Therap., 208, 366-375.
Motulsky, H.J. & Insel, P.A. (1981) Clin. Res., 29, 275A.

# SPECIES DIFFERENCES IN THE RESPONSE OF RAT, RABBIT AND GUINEA-PIG BLOOD PLATELETS TO ADRENALINE

Roger Kerry and Michael C. Scrutton, Department of Biochemistry, King's College, London, WC2R 2LS.

Blood platelets obtained from mammalian species exhibit marked diversity in their responses to adrenaline (cf. Scrutton & Wallis, 1981). The basis for these differences has been analysed by examining the effect of selective antagonists on the response to adrenaline and by characterising the response to selective adrenoceptor agonists.

Rabbit platelets do not respond to adrenaline as sole agonist but show an enhanced response to sub-optimal concentrations of other agonists, e.g. ADP, in the presence of adrenaline (cf. Drummond, 1976). This pro-aggregatory effect of adrenaline, which is mediated by an $\alpha_2$-adrenoceptor (Grant & Scrutton, 1980), is enhanced if the studies are performed in the presence of β-adrenoceptor blockade (10 μM propranolol). Conversely if α-adrenoceptors are blocked by addition of 10 μM phentolamine the response of rabbit platelets to other agonists, e.g. ADP, is inhibited by addition of adrenaline ($EC_{50}$ = 0.07 μM) or isoprenaline ($EC_{50}$ = 0.02 μM). This inhibitory effect can be blocked by ICI-118,551 ($IC_{50}$ = 12 μM) or butoxamine ($IC_{50}$ = 0.7 μM) but not by atenolol ($IC_{50}$ 100 μM) suggesting that it is mediated by $\beta_2$-adrenoceptors.

Rat platelets show neither aggregatory, pro-aggregatory nor inhibitory responses to adrenaline in the absence of antagonists. In the presence of 10 μM phentolamine addition of adrenaline or isoprenaline causes an inhibitory response mediated by $\beta_2$-adrenoceptors (Kerry & Scrutton, 1982). However in the presence of 10 μM propranolol adrenaline enhances the response to ADP ($EC_{50}$ = 3 μM). This effect is also observed on addition of the $\alpha_2$-adrenoceptor agonist, UK-14304 ($EC_{50}$ = 1.2 μM) but not of the $\alpha_1$-agonist methoxamine ($EC_{50} > 10$ μM). The pro-aggregatory response to adrenaline is blocked by the $\alpha_2$-adrenoceptor antagonist 781094 ($IC_{50}$ = 0.02 μM) but not by indoramin or prazosin ($IC_{50} > 10$ μM). Thus this response is mediated by an $\alpha_2$-adrenoceptor as in other species (Grant & Scrutton, 1980).

Guinea pig platelets resemble rat platelets in showing neither aggregatory, pro-aggregatory or inhibitory responses to adrenaline. However in contrast no such responses can be revealed in the presence of α- or β-adrenoceptor antagonists.

These data which confirm and extend those of Yu and Latour (1977) therefore indicate that both $\alpha_2$- and $\beta_2$-adrenoceptors are present on both rat and rabbit platelets. The lack of responsiveness of guinea pig platelets to adrenaline appears to result from the absence of functional adrenoceptors. Studies are in progress to determine whether these differences in responsiveness to adrenaline are reflected in the relative numbers of α- and β-adrenoceptors present on rabbit, rat and guinea pig platelets. These data have important implications for the use of small animal models to evaluate adrenoceptor antagonists as anti-thrombotic agents.

Drummond, A.H. (1976) in "Platelets in Biology and Pathology" (J.L. Gordon, ed.) Elsevier/North Holland Biomedical Press, pp. 203-239.
Grant, J.A. & Scrutton, M.C. (1980) Brit. J. Pharmacol., 71, 121-134.
Kerry, R. & Scrutton, M.C. (1982) Brit. J. Pharmacol., in press.
Yu, S.K. & Latour, J.G. (1977) Thrombosis and Haemostasis, 37, 413-422.

# INTERACTION OF HUMAN PLATELETS WITH α-ADRENOCEPTOR AGONISTS

Katherine A. Clare and Michael C. Scrutton, Department of Biochemistry, King's College, London, WC2R 2LS.

Interaction of adrenaline with $\alpha_2$-adrenoceptors on human blood platelets causes two distinct responses: (i) aggregation which appears to result from mobilisation of $Ca^{2+}$ to the cytosol (Owen & Le Breton, 1981); and (ii) inhibition of adenylate cyclase (Mills, 1975; Jakobs et al, 1976) which may facilitate, but is not required for, the aggregatory response. This situation might arise either where a single $\alpha_2$-adrenoceptor is coupled to both the $Ca^{2+}$ mobilisation system (and hence aggregation) and to adenylate cyclase as suggested for interaction of ADP with human platelets by Cusack & Hourani (1982); or where unique $\alpha_2$-adrenoceptors are coupled to these two functions as is observed for interaction of 5HT with the insect salivary gland (Berridge & Heslop, 1981).

We have assessed the affinities and efficacies of a wide range of putative α-adrenoceptor agonists for these two responses and on the basis of these data have classified these compounds into different groups as indicated in Table 1.

Table 1 Effect of various α-Adrenoceptor Agonists on Aggregation and Adenylate Cyclase Responses

| | Response | |
|---|---|---|
| Group | Aggregation | Adenylate Cyclase Inhibition |
| I | Full (partial) agonist: efficacy = $0 < \gtrsim 1$ | Full (partial) agonist: efficacy = $0 < \gtrsim 1$ |
| II | Partial agonist: efficacy $\simeq 0$ | Partial agonist: efficacy $> 0$ |
| III | Antagonist | Partial agonist: efficacy $> 0$ |
| IV | Partial agonist: efficacy $\simeq 0$ | Antagonist (or partial agonist: efficacy $\simeq 0$) |

In group I, but not in Groups II, III and IV, a correlation exists between the affinities for each response (correlation coefficent, 1.43). Such a correlation is also observed for compounds which act as antagonists for both the aggregatory response and for inhibition of adenylate cyclase (cf. also Lasch & Jakobs, 1979). No compound has yet been found which is effective as an agonist or an antagonist for aggregation but which has no effect on adenylate cyclase; or vice versa.

These data have failed to provide definitive evidence against the model in which a single receptor is linked to the two responses but do not appear simply consistent with this model.

Berridge, M.J. & Heslop, J.P. (1981) Brit. J. Pharmacol., 73, 729-738.
Cusack, N.J. & Hourani, S.M.O. (1982) Brit. J. Pharmacol., 76, 221-227.
Jakobs, K.H., Saur, W. & Schultz, G. (1976) J. Cycl. Nucleotide Res., 2, 381-392.
Lasch, P. & Jakobs, K.H. (1979) Naunyn Schmiedeberg's Arch. Pharmacol., 306, 119-125.
Mills, D.C.B. (1975) CIBA Foundation Symp. No. 35 (new series) pp. 145-167.
Owen, N.E. & Le Breton, G.C. (1981) Am. J. Physiol., 241, H613-666.

# THE EFFECT OF THROMBOXANE ANTAGONISM ON AGGREGATION AND RELEASE IN HUMAN PLATELETS

Roma A. Armstrong, R.L. Jones & N.H. Wilson, Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ.

Activation of human platelets *in vitro* can occur in two phases: primary and secondary aggregation. It is probable that primary aggregation involves the interaction with a specific receptor on the platelet surface (see MacIntyre, 1981). The existence of such a receptor for $TXA_2$ and $PGG_2/PGH_2$ is suggested by the following results:-

1. The ability of EP 045 (Jones *et al*, 1982) to antagonise platelet aggregation induced by AA, $PGH_2$, $TXA_2$ and a number of stable $TXA_2$ mimics, but not that induced by thrombin or ADP.

2. The stereo-specific binding of a radio-labelled $TXA_2$ mimic $^3H$ 15(S) 9,11-epoxymethano $PGH_2$ to a saturable site (2400 sites/platelet) with a high affinity (65 nM), and displacement of this binding by $PGH_2$, $TXA_2$, thromboxane mimics and antagonists in concentrations in which they are pharmacologically effective, but not by $PGF_{2\alpha}$, $D_2$, $E_2$ or $E_1$ (up to 14 μM).

3. The finding that exposure of human platelets to 11,9-epoxymethano $PGH_2$ (1 μM) for 60 min. at 37°C desensitises the platelet to a second exposure to 11,9-epoxymethano $PGH_2$ or to another $TXA_2$ agonist (including $PGH_2$ and $TXA_2$), but not to ADP or thrombin.

However secondary aggregation is associated with the release of dense granule constituents, and thus the liberation of platelet ADP and 5-HT which promote further aggregation of the platelets. It has been suggested that $TXA_2$ agonists can induce release independently of aggregation (Gerrard *et al*, 1977). We have investigated the possibility that there is a component of release induced by these agonists which is not inhibited by EP 045. Release of $^{14}C$ 5-HT from human platelets was induced by $PGH_2$ and 11,9-epoxymethano $PGH_2$ (1-15 μM). EP 045 (3-13 μM) was found to inhibit both 5-HT release and platelet aggregation in a parallel manner. A similar picture was seen with the carbacyclin, ZK 36374 (3-10 nM) which promotes $Ca^{2+}$ movement out of the platelet cytosol by increasing cAMP levels and with EDTA (0.01 M). Furthermore desensitisation to aggregation by 11,9-epoxymethano $PGH_2$ abolished release of 5-HT by a second exposure to the agonist.

We suggest that both aggregation and release induced by TXA agonists are receptor mediated processes in human platelets.

Gerrard, J.M. *et al* (1977) Am. J. Pathol. 86, 99-115.
Jones, R.L. *et al* (1982) Br. J. Pharmac. 76, 423-438.
MacIntyre, D.E. (1981) Platelets in Biology and Pathology 2 (ed. J.L. Gordon) 211-247.

160P

# ANTI-AGGREGATING ACTIVITY OF PROSTACYCLIN ANALOGUES IN WHOLE-BLOOD, PLASMA AND WASHED PLATELETS

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

Prostacyclin and its chemically-stable analogues carbacyclin and a 5-6 dihydro derivative 6β-$PGI_1$ are potent inhibitors of platelet aggregation in human platelet-rich plasma (PRP) determined in an optical aggregometer (Whittle et al., 1980). We have now compared the in vitro activity of prostacyclin and its analogues in PRP or a suspension of albumin-free washed platelets (using the optical aggregometer) and in whole-blood (using a platelet-counting technique) from both rabbit and man.

Rabbit and human blood was collected into siliconized plastic vessels containing trisodium citrate (0.315% final concentration). The PRP was collected following centrifugation (200 g for 15 min) and aliquots (0.5 ml) aggregated in a Born-type optical aggregometer (Payton) following incubation at 37°C by the addition of ADP or collagen. Platelets were isolated using the prostacyclin-washing technique described previously in detail (Vargas et al., 1982) and resuspended in albumin-free Tyrode's solution. Studies in aliquots of whole-blood utilized a platelet-counting technique in an Ultra-flo counter similar to that described by Lumley and Humphrey (1981), with aggregation (and hence fall in free platelet count) being induced by ADP or collagen. In all three systems, the platelets were pre-incubated for 1 min with the prostaglandins prior to aggregation.

In rabbit whole-blood and PRP, prostacyclin had a similar potency in inhibiting ADP-induced platelet aggregation, with $ID_{50}$ values of 6 ± 1 nM and 5.3 ± 1.3 nM (n=8) respectively, whereas the $ID_{50}$ in washed platelets was 4 ± 0.2 nM (n=8). In all three systems, the dose-inhibition curves for prostacyclin had identical slopes. Likewise in rabbit whole-blood and PRP, carbacyclin had comparable activity as an anti-aggregating agent, with $ID_{50}$ values of 139 ± 25 nM and 120 ± 20 nM (n=8), but was substantially more active in washed platelets ($ID_{50}$ 18 ± 2 nM, n=8; $P < 0.001$). The slopes of the dose-inhibition curves for carbacyclin were comparable in all three systems and were also comparable to those of prostacyclin and 6β-$PGI_1$. In contrast to carbacyclin, 6β-$PGI_1$ had comparable activity in PRP, washed platelets and whole-blood with $ID_{50}$ values of 1.2 ± 0.2 μM, 1.1 ± 0.2 μM (n=5) and 0.8 ± 0.3 μM, (n=3) respectively. Comparison of the potencies of these prostaglandins on human platelets showed a similar profile of activity. Thus prostacyclin had similar activity in washed platelets, PRP or whole-blood ($ID_{50}$ 2.4 ± 0.2 nM, 2.3 ± 0.5 nM and 2.4 ± 0.5 nM, respectively, n=9). However, carbacyclin was again more active in washed platelets than in PRP or whole-blood ($ID_{50}$ 19 ± 3 nM and 78 ± 1nM or 60 ± 14 nM, n= 4 respectively; $P < 0.01$).

Although prostacyclin is thought to bind to plasma protein which reduces its rate of chemical hydrolysis in plasma, the present studies indicate that such binding to blood components does not interfere with its biological activity in vitro since the anti-aggregating potency of prostacyclin was comparable in whole-blood, plasma or washed platelets. In contrast, the enhanced activity of carbacyclin but not 6β-$PGI_1$ in washed platelets indicate that these stable analogues bind to plasma components in a differential manner. Comparative studies of the anti-aggregating potency of prostacyclin analogues for structural-activity assessments should thus take into account any differences in binding to various blood components.

We thank Susan Knight and Paul Lidbury for excellent technical assistance.

Lumley, P., Humphrey, P.P.A. (1981). J. Pharmacol. Methods, 6, 153.
Vargas, J.R., Radomski, M. & Moncada, S. (1982). Prostaglandins, 23, 929.
Whittle, B.J.R. et al. (1980). Prostaglandins, 19, 605.

# PLASMA 'RCF' AND PROSTACYCLIN STIMULATING FACTOR IN HUMAN DIABETES

R.J. Griffiths , P.K. Moore, S. Ng Tang Fui[1]& A. Salter[1], Dept. of Pharmacology, Chelsea College, Manresa Road, London, SW3 6LX and [1]Unit of Metabolic Medicine, Guy's Hospital, London, SE1.

Reciprocal coupling factor (RCF) is the name previously given to the plasma component(s) which stimulate cytosolic prostaglandin (PG) inactivation and inhibit microsomal PG synthesis. In addition to RCF plasma contains a platelet-derived protein which stimulates prostacyclin ($PGI_2$) formation from vascular tissue and cultured endothelial cells. Plasma RCF concentration was elevated in rats made diabetic by intravenous injection of alloxan (Moore & Hoult, 1980). We have now studied plasma RCF and $PGI_2$ stimulating factor in human subjects with diabetes mellitus.

Blood (20ml) was obtained by venepuncture from 7 insulin dependent diabetics (mean age = 30.5 years), 7 diabetics with clinical evidence of microvascular disease i.e. retinopathy and/or nephropathy (mean age = 32.0 years) and 6 healthy controls (mean age = 28.5 years). All subjects were non-fasting males and denied taking aspirin-like drugs in the previous 10 days. Plasma was prepared by centrifuging anti-coagulated blood (1 vol. 3.8% w/v trisodium citrate : 9 vol. blood) at 1000g for 20 min. Plasma RCF was measured in standardised assays for PG metabolism (rat colon 100,000g supernatant incubated with 10µg/ml 9$\beta$ - $^3$H $PGF_{2\alpha}$, .5mM $NAD^+$, 37°C, 20min, measured by radiochromatography) and synthesis (bovine seminal vesicle microsomes in Tris-HCl buffer, pH 7.4 incubated with 10µg/ml arachidonic acid, 3mM reduced glutathione, 37°C, 60min, assayed by bioassay on the rat stomach strip). Vascular $PGI_2$ synthesis was determined in pieces of rat aorta (5-15mg) incubated at room temperature with 0.2ml Tris-HCl buffer (pH 7.4) or plasma for 5min. $PGI_2$ was assayed by its ability to inhibit ADP-induced human platelet aggregation.

Plasma from both diabetic and healthy subjects produced dose-dependent stimulation of rat colon PG breakdown and inhibition of seminal vesicle PG synthesis. The concentrations of plasma (expressed as % v/v incubation) required to produce 50% inhibition of synthesis (50% activation of breakdown in parenthesis) were 2.0 $\pm$ 0.2, n=16 (1.9 $\pm$ 0.3, n=8), 0.5 $\pm$ 0.07, n=16 (1.8 $\pm$ 0.2, n=13) and 5.1 $\pm$ 0.9, n=12 (4.2 $\pm$ 0.6, n=8) for healthy subjects and diabetics with and without vascular complications respectively. Rat aorta pieces incubated in Tris-HCl buffer generated 0.13 $\pm$ 0.02 ng/mg/5min, n=14 $PGI_2$ equivalents. All anti-aggregatory activity disappeared on boiling the incubate for 1min or on warming to 60°C for 10min. $PGI_2$ synthesis by rat aorta was stimulated following incubation with control plasma (0.34 $\pm$ 0.03 ng/mg/5min, n=10, $P< 0.05$) and plasma from diabetics without microvascular disease (0.38 $\pm$ 0.07 ng/mg/5min, n=10 $P< 0.05$). Plasma obtained from patients with retinopathy and/or nephropathy did not stimulate rat aorta $PGI_2$ synthesis (0.14 $\pm$ 0.02 ng/mg/5min, n=8).

The reduced plasma RCF and $PGI_2$ stimulating factor in diabetic subjects with microvascular disease may contribute to the raised platelet aggregability and increased risk of thrombosis in such patients.

We would like to thank the MRC for financial support.

Moore, P.K. & Hoult, J.R.S. (1980) Nature 288, 271

# EFFECT OF PROSTAGLANDINS $D_2$ AND $E_1$ ON ANAPHYLACTIC HISTAMINE RELEASE

C.T. Dollery, M. Ennis & C. Robinson, Department of Clinical Pharmacology, Royal Postgraduate Medical School, London W12 OHS

Prostaglandin $D_2$ ($PGD_2$) is the major cyclooxygenase product from stimulated mast cells and it is also detected in anaphylactic guinea-pig lung perfusates (Becker et al, 1981). It is therefore a potential candidate for a role in negative feedback regulation of mast cell mediator release. Holgate et al (1980) reported that although both $PGD_2$ and theophylline produced an increase in intracellular cyclic AMP, only theophylline caused an inhibition of mediator release from rat serosal mast cells. In view of the many differences between mast cells from different sites and species, we examined the action of $PGD_2$, $PGE_1$ and the phosphodiesterase inhibitor 3-isobutyl-methylxanthine (IBMX) in 3 different test systems. Two employed actively-sensitized (IgG model) guinea-pigs: the perfused isolated lung system and mast cells suspensions from enzymatically dispersed lungs, and for comparative purposes actively-sensitized (IgE model) rat peritoneal mast cells were the third test system.

Guinea-pigs (male, Dunkin-Hartley, ca 400 g) were sensitized with ovalbumin (50 mg sc and ip on day 1 and 50 mg ip on day 3) and used after 3-5 weeks. For perfusion studies, they were anaesthetised with pentobarbitone sodium (60 mg/kg ip) and were prepared for perfusion as described previously (Robinson et al, 1982). After 20 min equilibration ovalbumin (500 µg) was administered as a bolus injection via the pulmonary artery. Perfusate was collected in 30 sec fractions for the first 90 sec following anaphylaxis and then in 1 min fractions for an additional 8.5 min. Prostaglandin infusions were commenced simultaneously with the injection of ovalbumin. In experiments using IBMX, this was present in the perfusing medium throughout. Cell suspensions were prepared from collagenase dispersed guinea-pig lungs as described previously (Barrett et al, 1982). They were thereafter treated in the same manner as the rat peritoneal mast cells. Rats (either sex, Lister-Hooded, ca 180 g) were sensitized by the injection of 4000 L3 Nippostrongylus brasiliensis larvae subcutaneously into the thigh, the animals were used after 3-5 weeks. Rat peritoneal mast cells were obtained as previously reported (Pearce et al, 1979). The cells were incubated at 37°C for 5 min prior to antigenic challenge. All agents were added simultaneously with antigen. Histamine release was allowed to proceed for 10 min and was determined fluorimetrically using a Technicon autoanalyser.

Anaphylactic release of histamine from perfused lungs was significantly reduced from control values after anaphylaxis (9.95 $\pm$ 3.6 µg, n=4) by the infusion of either 5 µM $PGE_1$ (2.73 $\pm$ 0.6 µg, n=4) or 3 mM IBMX (0.29 $\pm$ 0.1 µg, n=6) but not 15 µM $PGD_2$ (10.80 $\pm$ 5.52 µg, n=3) into the perfusate. Histamine release from isolated guinea-pig lung mast cells was inhibited by $PGE_1$ (10 µM, ca 25%), $PGD_2$ (10 µM, ca 34%) and IBMX (2.5 mM, ca 64%). However only very high concentrations of the prostaglandins (50-100 µM) inhibited histamine release from rat peritoneal mast cells (ca 80%).

The differences in results obtained with isolated rat peritoneal and guinea-pig lung mast cell preparations provide further examples of mast cell heterogeneity, though the lack of effect of $PGD_2$ on perfused lungs is less easy to explain.

Barrett, K.E. et al (1982) Br.J.Pharmac. 75, Proc. Suppl. 1P.
Becker, EL. et al (1981) In: Biochemistry of the Acute Allergic Reactions, (A.R. Liss Inc, New York).
Holgate, S.T. et al (1980) J.Immunol. 124, 2093.
Pearce, F.L. et al (1979) In: The Mast Cell, its Role in Health and Disease, Eds Pepys, J. & Edwards, A.M. (Pitman, Tunbridge Wells) p 69.
Robinson, C. et al. Biochim.Biophys.Acta (in press).

# THE EFFECTS OF ANTI-INFLAMMATORY DRUGS ON PROSTAGLANDIN RELEASE FROM SUPERFUSED MINI-PIG SKIN

A. Chatelus, A. Civier, C.N. Hensby, H. Schaefer, B. Shroot & K.I. Williams, Centre International de Recherches Dermatologiques (CIRD), Sophia Antipolis, 06565 Valbonne Cédex, France.

Present techniques for the recovery of inflammatory mediators from skin are excessively invasive (eg. punch biopsy) or time-consuming (suction blisters). These drawbacks make accurate kinetic studies of mediator release difficult. We have recently described (Schalla et al 1982) a skin superfusion technique for studying prostaglandin (PG) release from human skin. The technique has now been adapted for use in the mini-pig whose skin pharmacological responses are similar to man (Hensby et al, 1982).

The abdominal skin of mini-pigs maintained under halothane anaesthesia was stripped of the horny layer at 6 separate sites using sellotape. Flat glass flow-chambers (diameter 2.5 cm, height 0.5 cm) were placed over the areas of exposed epidermis and held in place by vacuum. Tyrode's solution 1 ml/min, 37°C was superfused over the sites via the chambers and consecutive 15 min fractions were collected. The PG content of samples, after extraction, was estimated by GC/MS (Black et al, 1978). Output was then expressed as pg/min/$cm^2$ from the skin surface.

Initial experiments revealed that $PGF_2$ release over the first 15 min was 300-500 pg/min/$cm^2$. Within 45 min this had fallen to 150-250 pg/min/cm and remained in these limits for 6-8 h. Because of the initial high release, the fluid collected for the first 60 min was discarded. Samples for the next hour were taken to reflect basal output. Various anti-inflammatory drugs ($5 \times 10^{-5}$M to $5 \times 10^{-7}$M) were then added to the superfusing fluid for a three hour period. A "wash-out" period of 1-2 h with fresh Tyrode's completed the experiment. Both indomethacin and Benoxaprofen caused dose dependent inhibitions of PG synthesis. Indomethacin $5 \times 10^{-7}$M gave 75% reduction in basal release after 60 min whereas $1\text{-}5 \times 10^{-6}$M Benoxaprofen was needed. The reversible nature of the inhibition produced by both drugs was demonstrated by the return of PG production towards control values during the "wash-out" phase.

The technique described allows the simultaneous study of several concentrations of inhibitory drugs on skin PG synthesis <u>in vivo</u> whilst minimising surgical trauma.

Black, A.K. et al (1978) Br.J.Clin.Pharmac. 5, 431
Hensby, C.N. et al (1982) Int.J.Immunopharmac. 4, 352
Schalla, W. el al (1982) J.Invest.Dermatol. 78, 327

# ANALYSIS OF THE CONTRACTILE ACTION OF PROSTACYCLIN ON THE HUMAN BASILAR ARTERY IN VITRO

R.H. Lye, K.S.Paul & E.T. Whalley[1], Department of Neurosurgery, The Royal Infirmary, Oxford Road, Manchester M13 9WL & [1]Department of Pharmacology, University of Manchester, Manchester M13 9PT.

Prostacyclin($PGI_2$), which has been demonstated to be formed in human cerebral arterial endothelial cells (Abdel-Halim et al, 1980) has been suggested to be a physiological cerebral vasodilator (Boullin et al, 1979). Low ($10^{-10}$-$10^{-7}$M) and high ($10^{-6}$-$10^{-5}$M) concentrations of $PGI_2$ have been shown to produce concentration dependent relaxations and contractions respectively of both canine (Chapleau & White, 1979) and human (Paul et al, 1982) basilar arterial preparations in vitro under resting tension and when contracted with various spasmogenic agents. This study investigates possible mechanisms through which high concentrations of $PGI_2$ contract the human basilar artery in vitro under resting tension conditions.

Post-mortem basilar arteries were obtained and spiral strips mounted in 4 ml tissue baths filled with Krebs-Henseleit solution at 37°C and gassed with 95% $O_2$ and 5% $CO_2$. After a 2 h equlibration period under 1 g resting tension concentration-effect curves to $PGI_2$ were repeated at 90 min intervals in the absence and presence of the cyclo-oxygenase inhibitors indomethacin or flurbiprofen, the thromboxane synthetase inhibitor imidazole or the thromboxane(Tx) receptor antagonist EP045 (Jones et al, 1982).

Indomethacin (2.8, 5.6 & 14 μM), flurbiprofen (4, 8 & 16 μM) and EP045 (0.23, 2.3 & 11.5 μM) preincubated with the tissues for 45 min all produced concentration dependent significant ($P<0.05$ n=3-8) inhibitions of the contractile effects of $PGI_2$ ($10^{-6}$-$10^{-5}$M) without inhibiting the relaxant effects of $PGI_2$ ($10^{-9}$-$10^{-7}$M) compared to control tissues. Imidazole ($3.3 \times 10^{-4}$M) at a concentration previously demonstrated to inhibit production of $TxA_2$ from guinea-pig lung in vitro (Paul et al, 1982) had no effect on contractile or relaxant actions of $PGI_2$. Imidazole or indomethacin (2.8 - 14 μM) did not inhibit responses to standard concentrations of 5-hydroxytryptamine (5-HT) $10^{-7}$M, prostaglandin(PG)$F_{2\alpha}$ $10^{-7}$M, or U46619 $10^{-10}$M, which is a $TxA_2$ mimetic (Coleman et al, 1980). EP045 (0.23 - 11.5μM) produced concentration dependent significant ($P<0.05$, n=4) inhibitions of responses to standard concentrations of U46619, $PGF_{2\alpha}$, $PGE_2$ ($10^{-7}$M) and $PGD_2$ ($10^{-7}$M) but not to nor-adrenaline.

These results appear to suggest that the contractile action of high concentrations of $PGI_2$ on the human basilar artery in vitro may in part be mediated indirectly through the release of a cyclo-oxygenase product ( not $TxA_2$) which subsequently activates $TxA_2$ receptors on the tissue.

We are grateful to Dr. R.L. Jones for the generous gift of EP045.

Abdel-Halim, M.S. et al (1980) J. Neurochem. 34, 1331 -1333
Chapleau, C.E. & White, R.P. (1979) Prostaglandins 17, 573 -580
Coleman, R.A. et al (1980) Br. J. Pharmac. 68, 127P - 128P
Jones, R.L. et al (1982) Br.J. Pharmac. 76, 423 - 438
Paul, K.S. et al, (1982) J. Neurosurg. 57, (In Press)

# MECHANISM OF ABSORPTION OF THE STRONGLY ACIDIC DRUG PROXICROMIL

Carol A. Wilson, Michael G. Davis, David W. Payling[1] and Dennis A. Smith[1]
Department of Biology, Hatfield Polytechnic, Hatfield, Herts, and [1]Fisons plc, Pharmaceutical Division, Bakewell Road, Loughborough, Leics.

Proxicromil (FPL 57787, 6,7,8,9-tetrahydro-5-hydroxy-4-oxo-10-propyl-4H-naphtho [2,3-b] pyran-2-carboxylic acid) (Augstein et al.,1977) is well absorbed after oral administration (Smith & Neale,1980) despite its strong acidity (pKa 1.9). When the gastrointestinal tract of anaesthetised rats was ligated, and $^{14}$-C-proxicromil administered to specific regions by injection, appreciable uptake into the plasma occurred only from the duodenum, jejunum and ileum. Very little absorption of the compound was observed from the stomach. The distribution ratio (Scherrer & Howard,1977) of proxicromil was therefore measured using the model system of octanol and aqueous buffer (sodium ion concentration 0.155M). The log D value between octanol and aqueous buffer decreased with increasing pH from 3.7 at pH 3.6 to 1.8 at pH 6. This took place as a result of pH-dependent ionisation of the compound in accordance with its pKa. Above a pH of 6 the distribution ratio remained constant in this solvent system. Analysis of the octanol layer indicated that at pH values above 6 stoichiometric amounts of sodium ion and proxicromil were present indicating the formation between proxicromil and sodium ion of an ion pair which was sufficiently lipophilic to partition into the octanol. Decreasing the buffer content of sodium at pH 7.4 from 0.24 to 0.02M decreased the partitioning of proxicromil to log D 1.1.

The effect of pH on gastrointestinal absorption was investigated in vitro using everted rat intestine segments incubated in Krebs-Ringer bicarbonate buffer containing $^{14}$C-proxicromil (1.0mM). Sodium ion concentration was held constant at 0.155M. Below pH 6 an increase in uptake was observed with the decrease in degree of ionisation of the compound. Tissue concentrations after 30 minutes incubation increased from 15±1 $\mu molg^{-1}$ tissue at pH 6.5 to 27±3 $\mu molg^{-1}$ tissue at pH 4.5. At pH values above 6 the uptake was approximately constant (13±1 $\mu molg^{-1}$ tissue). Experiments were also carried out using anaesthetised rats with an isolated intestinal loop through which $^{14}$C-proxicromil (1.0mM) in solution in buffer (Sorensens or McIlvaines, sodium ion concentration 0.155M) was perfused at a rate of 1 $cm^3min^{-1}$. Uptake into plasma, monitored for 20 minutes, at a buffer pH of 5 was approximately 1.5 x that at pH 7 and 7 x that at pH 9 when calculated by either the area under the plasma curve or by the concentrations of radioactivity in plasma achieved at the end of the perfusion (pH5,17.7±3.4 $nmolml^{-1}$; pH7,11.8±1.9 $nmolml^{-1}$; pH9, 2.6±0.33 $nmolml^{-1}$). The increase in absorption with decreasing pH was therefore substantially less than would be expected if unionised drug concentration were the sole determinant of absorption rate since only a 7 x increase in absorption accompanied a 10,000 x rise in free drug concentration. Similar rates of drug uptake were observed at pH 5 when the gut was perfused using the segmented flow technique (Winne,1976) to reduce the unstirred layer thickness.

The results indicate that ion pair formation operates to allow absorption of the compound at pH values above 6. Since absorption of the compound occurs from regions of the gastrointestinal tract which have pH values above 6 it is suggested that ion pair formation, with naturally abundant cations such as sodium, plays a significant role in the absorption of proxicromil.

Augstein, J. et al (1977) Agents and Actions 7, 443
Smith, D.A. & Neale, M.G. (1980) Toxicol Letts. Special Issue 1, 064
Scherrer, R.A. & Howard, S.M. (1977) J.Med.Chem. 20, 53
Winne, D. (1976) Experientia 32, 1278

# THE PHARMACOKINETICS OF GLIPIZIDE IN MICE: EFFECTS OF INDUCTION AND INHIBITION OF DRUG METABOLISM

A.H. El Debani & I.H. Stevenson, Department of Pharmacology and Therapeutics, Ninewells Hospital and Medical School, Dundee, DD1 9SY.

Glipizide (N-cyclohexyl-N$^1$-p-(2-(-5 methylpyrazine-2-carboxamido-) ethylbenzene-sulphonylurea) is a potent, short acting sulphonylurea which has become established as an oral hypoglycaemic agent in maturity-onset diabetes. Although the drug is known to be extensively hydroxylated in man and animals by hepatic enzymes (Tommasini, 1975), many studies suffer from the disadvantage that they have been based solely on measurements of radioactivity following administration of labelled glipizide or have used radioimmunoassay, where the possibility of interference by metabolites occurs. This communication reports on the application of a specific HPLC method for glipizide to study the influence of phenobarbitone and SKF 525A pretreatment on the pharmacokinetics of glipizide in mice.

Normal male and female Swiss mice (37 - 45g) received either phenobarbitone sodium (60 mg/kg i.p. twice daily for four days) or SKF 525A (25 mg/kg i.p. 40 min prior to and again 2h after the administration of a single dose of glipizide (1 mg/kg i.p.). In each case saline controls were included. Groups of 3 animals were sacrificed by $CO_2$ asphyxiation at 1, 2, 3 and 4 hours after the glipizide dose with blood samples being collected by cardiac puncture into lithium heparin tubes. Glipizide concentration was estimated in plasma samples (100 $\mu$l) using a micro-modification of the specific HPLC method of Becker (1977).

The detection limit for the micro-method was 50 ng/ml using an injection volume of 6 $\mu$l, with day to day variation of the calibration curve being < 0.13% at 95% confidence limit. The three final plasma samples were used in calculation of elimination phase data and the pharmacokinetic parameters for glipizide, assuming a two-compartment model (Wahlin-Boll et al, 1982) are shown in Table 1.

| | GLIPIZIDE PHARMACOKINETIC PARAMETERS | | |
|---|---|---|---|
| | $t\frac{1}{2}\beta$(h) | Kel ($h^{-1}$) | Clearance ($L\ kg^{-1}\ h^{-1}$) |
| Saline | 2.31 | 0.30 | 0.04 |
| Phenobarbitone sodium | 0.54 | 1.28 | 0.88 |
| SKF 525 | 23.10 | 0.03 | 0.01 |

The data demonstrate clearly, using a specific method of assay, that the elimination kinetics of glipizide is markedly affected by induction and inhibition of metabolism. The differences found however may not depend solely on alteration in metabolism since some change in volume of distribution of glipizide occurred with drug treatment.

Becker, R. (1977) Arzeim. Forsch. 27, 102.
Tommasini, R. (1975) Curr. Med. Res. and Op. 3, Suppl.1.
Wahlin-Boll, E. & Almer, L.O. (1982) Clin. Pharm. 7, 362.

# SOME ASPECTS OF THE DISPOSITION AND METABOLISM OF 8-METHOXYPSORALEN IN EXPERIMENTAL ANIMALS

Z. Mohamed, J.D. Baty[1], S.F. Fan & I.H. Stevenson, Department of Pharmacology and Therapeutics and [1]Department of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee, DD1 9SY.

The successful treatment of psoriasis with photochemotherapy using oral 8-methoxypsoralen (8-MOP) and longwave ultraviolet radiation between 320 and 400 nm (UV-A) has been accompanied by a number of investigations into the pharmacokinetics of the drug (Pathak et al, 1974; Thune and Volden, 1977; Steiner et al, 1978; Hensby, 1978; Herfst et al, 1978; Wulf & Hart, 1979). We have studied the gross tissue distribution of radioactivity in mice following the administration of $^{3}$H-8-MOP in an attempt to determine which organs contain the highest concentration of radioactivity. The results indicate that 8-MOP and its metabolites achieved a higher concentration in liver (1.71% of the administered dose at 2hr), skin (1.48%) and fat (1.30%) relative to other tissues. Peak levels of radioactivity in tissues are reached within an hour and rapid decline is seen after 3 hours indicating a rapid rate of absorption and excretion of the drug. This finding is supported by the urinary and faecal excretion profile which shows that over 55% of the administered radioactivity is excreted within 5 hours of administration.

Little information is available concerning the metabolism of 8-MOP in man and animals. An interspecies difference in the route of metabolite excretion is evident. Biliary secretion of 8-MOP is high in the dog (Kolis et al, 1979) but 8-MOP and metabolites are eliminated almost completely via the urine in man (Pathak et al, 1977). In our study of the biliary secretion of $^{3}$H-8-MOP in a Wistar rat, approximately 5% of the administered radioactivity is excreted in bile in the 3.5 hours following drug administration. In another experiment bile was collected for 2.5 hours from a rat which was dosed orally with 8-MOP (20 mg/kg). Analysis of this bile by high performance liquid chromatography after enzymic hydrolysis indicated four possible metabolite peaks. Less than 0.4% of 8-MOP was excreted unchanged. A major metabolite was isolated and has been identified by mass spectroscopy and liquid chromatography as 8-hydroxypsoralen (8-HOP). Its mass spectra and that of synthetic 8-HOP were identical thus confirming 8-HOP as a metabolite of 8-MOP in the rat.

Hensby, C.N. (1978) Clin. Exp. Dermatol. 3, 355.
Herfst, M.J. et al (1978) Arch. Dermatol. Res. 262, 1.
Kolis, K.J. et al (1978) Drug Metab. Dispos. 7, 220.
Pathak, M.A. et al (1974) J. Invest. Dermatol. 62, 347.
Pathak, M.A. et al (1977) "Psoriasis: Second International Symposium" (E.M. Farber and A.J. Cox, eds) P262. Yorke Medical Book, New York.
Steiner, I. et al (1978) Acta Dermato-venerealogica 58, 185.
Thune, P. & Volden, G. (1977) Acta Dermato-venerealogica 57, 351.
Wulf, H.C. & Hart, J. (1979) Acta Dermato-venerealogica 59, 97.

# THE EFFECT OF ALINIDINE ON DRUG METABOLISM IN THE RAT

D.J. Back, H.S. Purba & C. Staiger[1]. Department of Pharmacology & Therapeutics, University of Liverpool, Liverpool L69 3BX and [1]Klinische pharmakologie, Klinikum der Universitat Heidelberg, 6900 Heidelberg, G.F.R.

Alinidine 2-[-N-allyl-N-(2,6 dichloro-4-phenyl)amino-2-imidazoline-hydrobromide, is a specific bradycardic drug decreasing heart rate by a direct action on the sinuatrial node (Arndts et al., 1981). Since alinidine is an imidazole derivative and a number of structurally related compounds have been shown to influence hepatic enzyme activity both in animals and man (Wilkinson et al., 1972; Rogerson et al., 1977; Serlin et al., 1979), we have investigated the effects of alinidine on drug metabolizing enzymes in the rat.

In vivo, groups of male Wistar rats were given alinidine (10 or 20 mg/kg) or saline i.p. 30 min before determination of pentobarbitone sleeping time (PST; 20 mg/kg). Also the effect of alinidine (20 mg/kg) on zoxazolamine paralysis time (ZPT; 60 mg/kg) and antipyrine clearance (5 μCi/kg; 15 mg/kg, [$^{14}$C]-antipyrine) was investigated. In vitro, the demethylation of aminopyrine (2.5 mM) was studied in the presence of alinidine (0.001, 0.01, 0.1, 1 and 10 mM) and deethylation of ethoxyresorufin (ERR, 250 nM; rats pretreated with β-naphthoflavone, 25 mg/kg/day for 3 days) with alinidine (10, 100, 250, 500 and 1000 nM). Lineweaver-Burk plots were constructed for aminopyrine N-demethylation kinetics using substrate concentrations of 0.25, 0.75, 1.5 and 2.5 mM and alinidine concentrations of 3.0 and 10.0 mM. In a chronic treatment study, rats were dosed with alinidine (20 mg/kg/day for 4 days i.p.) and microsomes prepared on the sixth day. Microsomal protein, cytochrome P-450 content, aminopyrine N-demethylase activity and ERR O-deethylase activity were measured.

After acute administration, alinidine (10 mg/kg) did not increase PST but significantly increased ZPT from 92.4 ± 30.9 to 175.2 ± 53.6 min (mean ± S.D.). At 20 mg/kg, alinidine increased PST from a control value of 27.6 ± 17.1 to 57.2 ± 8.2 min. Antipyrine half-live was significantly increased (control, 100.8 ± 12.9; pretreated, 186.0 ± 19.2 min) and clearance decreased (control, 2.17 ± 0.19; pretreated, 0.92 ± 0.08 ml/min) when rats were pretreated with 20 mg/kg alinidine. The addition of 10 mM alinidine to rat liver microsomes resulted in 42% inhibition of aminopyrine N-demethylase activity. ERR O-deethylase activity decreased by 50% in the presence of 250 nM alinidine (equimolar with substrate). After chronic alinidine pretreatment, cytochrome P-450, aminopyrine N-demethylase activity and ERR O-deethylase activity increased by 22.7%, 24.5% and 38.8% respectively.

We conclude that after acute administration, alinidine inhibits drug metabolism in the rat, affecting both cytochrome P-450 and P-448 enzymes. In contrast, chronic administration gave some evidence of induction of hepatic enzymes. Therefore during continuous alinidine administration both inhibition and induction of monooxygenase activity may occur. However, the doses of alinidine needed to produce these effects are very high in comparison with the normal therapeutic dose range of 20-80 mg (Harron et al., 1981).

Arndts, D. et al. (1981) Eur. J. Clin. Pharmacol. 21, 201-207.
Harron, D.W.G. et al. (1981) Lancet i, 351-353.
Rogerson, T.D. et al. (1977) Biochem. Pharmac. 26, 1039-1042.
Serlin, M.J. et al. (1979) Lancet ii, 317-319.
Wilkinson, C.F. et al. (1972) Biochem. Pharmac. 21, 3187-3192

# REPEATED DOSE INHALATION TOXICITY AND MUTAGENICITY-STATUS OF CR (DIBENZ-(b.f)-1,4 OXAZEPINE)

H F Colgrave, C G Lee, T C Marrs and B Morris, Pathology and Toxicology Section, CDE Porton Down, Salisbury, Wiltshire, SP4 OJQ.

CR, dibenz-(b.f.)-1,4 oxazepine is a sensory irritant similar to CS (o-chlorobenzylidene malononitrile) but more potent (Ballantyne, 1977a) and less acutely toxic (Ballantyne, 1977b). CR is neither teratogenic nor embryolethal (Upshall, 1974) and repeated application to the skin does not result in untoward effect (Marrs, 1982). Single doses of CR aerosol of up to 1.021 g $m^{-3}$ for 158 min do not produce any lesions in the lungs detectable by electron microscopy (Colgrave et al, 1979).

300 2 month old male golden hamsters and 290 SPF Porton mice were each divided into 3 test groups of 80, the remaining animals being designated controls. The test groups were exposed to aerosolized technical grade CR at Cts of 4223, 2031 and 1022 mg min $m^{-3}$, 5 days/week for 18 weeks. One year after the start of exposure the surviving animals were killed. The results of gross and histological post mortem examination were analysed using a statistical test for dose response. This test was performed both with and without inclusion of the high dose group since non-incidental findings might be influenced by mortality rate. Technical grade CR was examined for its ability to revert indicator strains of Salmonella typhimurium. Since this grade of CR is only about 95% pure, probably being contaminated with a manufacturing precursor, 2-aminodiphenyl ether, the latter was also tested. Both substances were also examined in three mammalian cell mutagenicity assays: the V79/HGPRT, L5178Y/TK +/- and micronucleus tests.

It was found in the exposed animals that there was a dose related decrease in survival ($P < 0.05$, both species). This effect was largely due to an increase in mortality in the high dose groups, which received total doses of 380,000 mg min $m^{-3}$. Few histological differences between the different dose groups were observed. Adrenal cortical, thyroid and pancreatic islet-cell adenomata were found in the hamsters, but the distribution appeared to be random. The mice showed evidence of a relationship between dose and chronic inflammation of the larynx when the dose response was analysed using the lower two dosed groups and the controls ($P < 0.001$). There was also a dose-related increase in the incidence of alveologenic carcinoma ($P < 0.05$) when the high dose group was excluded. However with both these lesions in the mice there was no statistically significant relationship between incidence and dose when all four groups were included in the analysis. Since the alveologenic carcinomata were detected in an incidental context the more appropriate statistical procedure was the inclusion of all the dosed groups and the controls.

In view of those findings and the fact that CR and its precursor were both non-mutagenic in all the assays used, it was concluded that technical grade CR was unlikely to be a carcinogen.

Ballantyne, B, (1977a) Med Annual, John Wright, Bristol
Ballantyne, B, (1977b) Toxicol 8 347
Colgrave, H F et al (1979) Brit J Exper Pathol 55 213
Marrs, T C et al (1982) Toxicol Lett in press
Upshall, D G, (1974) Toxicol appl Pharmacol 29 301.

# COMPARISON OF THE ACTIVITIES OF PEPTIDES RELATED TO THE ENKEPHALINS IN PHARMACOLOGICAL AND BINDING ASSAYS

A.D. Corbett, H.W. Kosterlitz, A.T. McKnight & S.J. Paterson, Unit for Research on Addictive Drugs, Marischal College, University of Aberdeen, Aberdeen, AB9 1AS

A number of endogenous opioid peptides have been isolated which have the sequence of [Met$^5$]enkephalin or [Leu$^5$]enkephalin within their structure. Considerable progress has been made in elucidating the mechanisms of biogenesis of these peptides; in particular, recent experiments have suggested the existence of three different precursor systems (Nahanishi et al, 1979; Gubler et al, 1982; Kakidani et al, 1982; Noda et al, 1982). Thus it is of interest to examine possible products of these precursors which may be generated during processing, for opioid activity in both pharmacological and binding assays.

The peptides were tested in four in vitro assay tissues: the vasa deferentia of mouse, rat and rabbit and the guinea-pig myenteric plexus-longitudinal muscle. In the assays, the activity of tissue peptidases was inhibited by a mixture of bestatin, L-leucyl-L-leucine, thiorphan and captopril (McKnight et al, 1982). The contractions of the guinea-pig myenteric plexus-longitudinal muscle, evoked by field stimulation of the intramural nerves, are readily inhibited by ligands which activate the μ- or κ-receptor, whereas the mouse vas deferens responds preferentially to δ-ligands; the vas deferens of the rat has a low sensitivity to κ-ligands whereas in the rabbit vas deferens κ-ligands but neither μ- nor δ-ligands are inhibitory. All the peptides used in this investigation had agonist but no antagonist action.

The potencies of the peptides to displace the binding of tritiated ligands were measured in homogenates of guinea-pig brain at 0°C. [$^3$H]-[D-Ala$^2$,MePhe$^4$,Gly-ol$^5$] enkephalin (1 nM) was used as a μ-ligand, [$^3$H]-[D-Ala$^2$,D-Leu$^5$]enkephalin (1 nM) as δ-ligand, and [$^3$H]-bremazocine (0.3 nM) as the κ-ligand after suppression of binding to μ- and δ-sites by addition of 100 nM unlabelled [D-Ala$^2$,MePhe$^4$,Gly-ol$^5$] enkephalin and [D-Ala$^2$,D-Leu$^5$]enkephalin.

The peptides which are C-terminal extensions of the native enkephalins exhibit a pattern of selectivity in both the pharmacological and binding assays which is different from the pentapeptides. [Leu$^5$]enkephalin is most active in the mouse vas deferens ($IC_{50}$ = 1.7 nM), less active in the guinea-pig ileum ($IC_{50}$ = 28.6 nM), much less active in the rat vas deferens ($IC_{50}$ = 550 nM) and inactive in the rabbit vas deferens ($IC_{50}$ >10,000 nM). [Leu$^5$]enkephalyl-Arg-Arg-Ile-Arg (dynorphin$_{1-9}$) and the heptadecapeptide dynorphin are most active in the guinea-pig ileum ($IC_{50}$ 2.36 and 0.29 nM, respectively), less active in the mouse vas deferens (6.5 and 0.91 nM) and rabbit vas deferens (6.1 and 3.0 nM) and inactive in the rat vas deferens ($IC_{50}$ >3000 nM). In the binding assays, [Leu$^5$]enkephalin is selective for the δ-binding site with a 10% cross-reactivity with the μ-binding site and is almost inactive at the κ-binding site. On the other hand, dynorphin$_{1-9}$ and dynorphin are highly selective for the κ-binding site with only a 5% cross-reactivity to the μ- and δ-binding sites.

Supported by grants from the MRC and U.S. NIDA (DA 00662).

Gubler, U. et al (1982) Nature 295, 206-208
Kakidani, H. et al (1982) Nature 298, 245-249
McKnight, A.T. et al (1982) Eur.J.Pharmac. In press
Nahanishi, S. et al (1979) Nature 278, 423-427
Noda et al (1982) Nature 295, 202-206

# NEUROMUSCULAR EFFECTS OF POLYMYXIN B: INTERACTION STUDIES IN THE CHICK BIVENTER CERVICIS MUSCLE

F.A. Wali (introduced by R.C. Elliott), Anaesthetics Unit, The London Hospital Medical College, Whitechapel, London E1 1BB.

Polymyxin B is a cyclic polypeptide antibiotic, which is isolated from Bacillus polymyxa (Ainsworth et al., 1949), and it has less toxic effect than the other antibiotics.

The mechanism of action of antibiotics is not entirely clear and it is not the same for individual antibiotic agents. Polymyxins are thought to act on the lipoprotein part of the microbial walls and membranes causing a disorientation and disruption of these membranes (Newton, 1956; Gale, 1963; Salton & Tomasz, 1974).

The purpose of this study was to investigate the effects of polymyxin B on twitch contractions and contractures produced by acetylcholine (ACh) and by tetraethylammonium (TEA) in the isolated chick biventer cervicis (BVC) nerve-muscle preparation.

Polymyxin B(9.4 units, 1.31 μg) produced a transient increase in the amplitude of the twitch contractions, elicited by repetitive nerve stimulation at 0.2 Hz with 5V and 0.5 ms pulse duration, by 37%±1.2, n=6, for about 4 min. ACh(0.55-5.5 mM) and TEA (2.4-12.0 mM) produced concentration-dependent contractures in the chick BVC muscle. These responses were reduced by polymyxin B(9.4 units). The mean (±SEM) ED 50s for the contractures produced by ACh in the control Krebs solution and in polymyxin B were 0.22±0.06 mM and 3.8±0.04 mM, n=6, $P < 0.02$, respectively. The mean (±SEM) ED50s for the contractures produced by TEA in the control Krebs solution and in polymyxin B were 5.0±0.05 mM and 7.8±0.02 mM, n=6, $P < 0.05$, respectively. Recovery of the responses occurred in less than ½ h.

It has been reported (Singh, Harvey & Marshall, 1978), that polymyxin B in high concentrations (0.4 mM, about 400 μg, 2880 units) produced neuromuscular blockade in the mouse phrenic nerve-hemidiaphragm preparations. The neuromuscular blockade produced was similar to that produced by lack of $Ca^{2+}$ or excess of $Mg^{2+}$. Transmission was blocked probably due to a $Ca^{2+}$ binding effect of polymyxin, since the neuromuscular blockade was reversed by calcium. Other antibiotics, e.g. streptomycin, neomycin, have been reported to act post-synaptically and reduce the sensitivity of the postsynaptic membrane to ACh or direct electrical stimulation (Elmqvist & Josefsson, 1962).

In the present experiments polymyxin B(9.4 units), seemed to facilitate the twitch contractions in the chick BVC muscle. The depression in twitch contractions observed by Singh et al. (1978), may be attributed either to a species difference or to the dose ranges used (400 μg in the mouse v. 1.31 μg in the chick muscle).

In conclusion, polymyxin B produces a transient increase in the twitch contractions, but it reduces the contractures produced by ACh and TEA in the chick BVC muscle. Polymyxin B may have both pre-and postsynaptic actions at the chick neuromuscular junction.

Ainsworth, G.C., Brown, A.M. & Brownlee, G. (1949). Nature, 160, 263
Elmqvist, D. & Josefsson, J.O. (1962). Acta. Physiol. Scand. 54, 105-110
Gale, E.F. (1963). Pharmacol. Rev. 15, 481-530
Newton, B.A. (1956). Bact. Rev. 20, 14-27
Salton, Y.N., & Tomasz, A. (1974). Ann.N.Y. Acad. Sci. 235, 1-620
Singh, Y.N., Harvey, A.L. & Marshall, I.G. (1978). Anesthesiology, 48, 418-424

# THE EFFECTS OF PRETREATMENT ON THE RESPONSE CHARACTERISTICS OF CARBON FIBRE MICROVOLTAMMETRIC ELECTRODES

J.A.STAMFORD & Z.L.KRUK, Department of Pharmacology, The London Hospital Medical College, Turner St, London E1 2AD.

Carbon fibre microelectrodes suitable for unit recording have been described by Armstrong-James and Millar (1979). These microelectrodes were also found to be suitable for assay of catecholamines by High Speed Cyclic Voltammetry (HSCV) (Armstrong-James et al, 1981). In order to minimise damage on penetration into neural tissue, the tips were routinely etched in chromic acid to a conical shape (Armstrong-James et al, 1980). Reconditioning of 'poisoned' microelectrodes was achieved using a method qualitatively similar to that of Gonon et al (1981).

Microelectrodes were prepared as described by Armstrong-James & Millar (1979) and the protruding carbon fibre was cut to a length of c.50um. The microelectrodes were then etched in concentrated chromic acid. Finally, microelectrodes were electrochemically conditioned in phosphate buffered saline by applying a sawtooth waveform (0 to +2V, 75 Hz) for 30 seconds. After each treatment step the response of the microelectrodes to DA and DOPAC was examined by HSCV with an asymmetrical test waveform (-0.75 to +1.25V, 75Hz) once per second. This technique is similar to that of Kruk et al (1981).

Unetched microelectrodes gave well defined peaks for DA but not for DOPAC, which produced ill defined oxidation and reduction currents. Etching led to sharp peaks for both DA and DOPAC and considerably improved reproducibility. The sensitivity to DA was increased without any change for DOPAC. Etching made the DA peaks occur approximately 60 to 70 mV earlier. Etching also appeared to increase the size of the background current, presumably due to an increase in surface area (Plotsky, 1982). Electrochemical conditioning appeared to have little effect on responses. However the procedure seemed to increase adsorption of the compounds as they proved difficult to wash off the electrode surfaces. The stability of the electrochemical background signal was also reduced. Scanning electron microscopy has not shown evidence of cracking or pitting of the surface. Thus an effect on surface chemical groups seems most likely.

In conclusion it can be said that etching in chromic acid improves the electrochemical responsiveness of the microelectrodes and that electrochemical conditioning does not cause any useful further improvement in freshly etched microelectrodes.

Armstrong-James, M & Millar, J. (1979) J. Neurosci. Meth. 1, 279-87.
Armstrong-James, M et al. (1980) J. Neurosci. Meth. 2, 431-2.
Armstrong-James, M et al. (1981) J. Neurosci. Meth. 4, 385-406.
Gonon, F.C et al (1981) Anal. Chem. 53, 1386-9.
Kruk, Z.L et al (1980) Life Sci. 27, 2093-8.
Plotsky, P.M. (1982) Brain Res. 235, 179-84.

# THE POTENTIATION BY KETAMINE OF THE EFFECTS OF GABA ON THE RAT SUPERIOR CERVICAL GANGLION IN VITRO

H.J. Little, University Department of Pharmacology, South Parks Road, Oxford, OX1 3QT, U.K.

It has been reported previously that the depolarisation of the superior cervical ganglion produced by γ-aminobutyric acid (GABA) is considerably increased by the dissociative anaesthetic ketamine (Little, 1982), and it was suggested that this might contribute to the reported anticonvulsant actions of this anaesthetic. Further investigation of the mechanism of this potentiation has now been carried out.

The method of Brown and Marsh (1974) was used for recording extracellular potential changes from the superfused ganglion. Doses of GABA were applied at 15 min intervals and the amplitude of the responses expressed as percentages of the first control response or, in the dose response curve experiments, as percentages of the maximum.

The following are the percentage changes seen 15 and 30 min after ketamine was added to the superfusion medium. The GABA concentration was 9.7 μM. (This is the $ED_{50}$ concentration as derived from dose response curves. The mean absolute amplitude of responses to this concentration was 385 μV, s.e.m. = 41, n = 6).

| Ketamine concentration (n = 6 throughout) | | | |
|---|---|---|---|
| 18.3 μM | 128 ± 11, | 128 ± 10 |
| 36.5 μM | 133 ± 17, | 130 ± 19 |
| 73 μM | 167 ± 26, | 147 ± 19 |
| 180 μM | 184 ± 20, | 201 ± 29 |
| 365 μM | 161 ± 13, | 162 ± 17 |

The log dose-response curve to GABA was shifted to the left by ketamine (180 μM), the $ED_{50}$ being decreased by 3.8 μM and the maximum increased to 115% (s.e.m. = 9, n = 7), P 0.05.

Ketamine has been shown to inhibit GABA uptake (Azzaro and Smith, 1977) so to determine whether this contributed to the effect seen studies were made, firstly, after uptake inhibition by nipecotic acid and, secondly, using 3-aminopropane sulphonic acid (3-APS), a GABA agonist which is not taken up by the GABA uptake system. A concentration of 1 mM nipecotic acid was found to cause the maximum increase in the responses to 4.85 μM GABA and in the presence of this concentration ketamine (180 μM) caused further increases of up to 139% (s.e.m. = 13, n = 6). Ketamine also considerably potentiated the responses to APS. At 180 μM, after 45 min, the results were as follows: APS 720 nM, 221% (s.e.m. = 35, n = 5); APS 1.8 μM ($ED_{50}$), 168% (s.e.m. = 12, n = 5); APS 7.2 μM, 135% (s.e.m. = 16, n = 6).

The effect of ketamine in the responses to GABA was not altered by the benzodiazepine antagonist Ro 15-1788 at 3.34 μM. (This concentration antagonises the potentiation of GABA responses by chlordiazepoxide on this tissue). Ketamine did not affect the responses to GABA when a state of desensitization was present. The latter was achieved by applying GABA, 97 μM, at intervals of 7½ min.

This evidence suggests that the effect of ketamine on the responses to GABA is not due to inhibition of uptake, nor is it due to action at benzodiazepine receptors or an effect on desensitization. It is possible that there is an action at the same site as the barbiturates, which also potentiate GABA responses on this preparation.

Azzaro, A.J. & Smith, D.J. (1977) Neuropharm. 16, 349-356.

Brown, D.A. & Marsh, S. (1974) J. Physiol. 246, 24-26P.

Little, H.J. (1981) Br. J. Pharmac. 74, 880-881P.

# USE-DEPENDENT EFFECTS OF CALCIUM ENTRY BLOCKING DRUGS ON THE ELECTRICAL AND MECHANICAL ACTIVITIES OF GUINEA-PIG TAENIA CAECI

T.B. Bolton K. Kitamura & N. Morel*, Department of Pharmacology, St. George's Hospital Medical School, London SW17 ORE.

Although blockade of ionic channels in the cell membrane is believed to be the main mechanism of action of calcium entry blocking drugs, intracellular effects have been postulated (e.g. Weston, 1978; Jetley & Weston, 1980). We have studied the effects of several calcium entry blocking drugs on the electrical activity of taenia to see whether we could detect effects on tension at concentrations which were without effect on electrical activity measured either by the double sucrose gap or microelectrode technique. The actions of nifedipine (10nM-0.1μM) diltiazem (0.1-10μM) and D600 (0.1-10μM) were qualitatively similar and differed only in their speed of onset and potency. Their effects on action potentials (spikes) and contractions evoked by depolarizing current pulses were studied. All drugs were without effect on the membrane resistance as measured by their effect on the size of hyperpolarizing electrotonic potentials evoked by current pulses.

Higher concentrations reduced both the size and the frequency of spikes and their associated contractions in the sucrose gap. Intracellular records revealed a reduction in the rate of rise of the spike, spike size, and in the frequency of spikes. Lower concentrations were without detectable effect on the size of initial spikes of a train evoked by long (> 10s) depolarizing pulses, but the amplitude and frequency of later spikes in the train were severely reduced. Initial tension was little affected but progressively declined during the train. If test spikes and their associated contraction were evoked by short depolarizing current pulses applied at intervals, the depressant effects of these drugs were intensified for a period following a 10-20s train of spikes, indicating that the effects on electrical activity and tension were use-dependent as described in cardiac muscle (McDonald et al, 1980). The use-dependency of the block probably also explains why the tonic component of high-K contractures was more sensitive to these agents than initial, phasic tension (Godfraind & Dieu, 1981).

These results indicate than an effect at lower concentrations may only become apparent during electrical activity and opening of ionic channels. In whole smooth muscle tissues, channel blockade would affect spike propagation and so reduce smooth muscle tension.

N.M. is a research assistant of F.N.R.S. (Belgium).

Godfraind, T. & Dieu, D. (1981). J. Pharmacol. exp. Ther. 217, 510-515.
Jetley, M. & Weston, A.H. (1980). Br. J. Pharmac. 68, 311-319.
McDonald, T.F. et al (1980). Pflügers Arch. 385, 175-179.
Weston, A.H. (1978). pp. 15-22 in "Adrenoceptors" ed. Szabadi, E. et al. Elsevier.

# ON THE MECHANISMS OF CONTRACTION AND RELAXATION OF CIRCULAR SMOOTH MUSCLE FROM GUINEA-PIG STOMACH TO FIELD STIMULATION

Brenda Costall, R.J. Naylor & Connie C.W. Tan, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP.

Analyses of changes in the tension of circular smooth muscle of guinea-pig stomach to exogenously applied neurotransmitter stubstances and their antagonists have allowed a characterisation of adrenoceptor mechanisms of possible relevance to gastric emptying in vivo (Costall et al., 1981; Sahyoun et al., 1982a). Here, we extend the studies to allow a comparison with the interactions of endogenously released neurotransmitters.

Circular smooth muscle preparations from the cardia, fundus, body and antrum of guinea-pig stomach, bathed in 15 ml oxygenated (95% $O_2$, 5% $CO_2$) Krebs-Henseleit solution containing 100 mg/l ascorbic acid at 37°C (responses recorded isometrically), were subject to field stimulation (FS, 0.125-10 Hz, 0.05-0.1 ms, 25-40 V, applied for 30 s on a 5 min. cycle; all responses abolished by tetrodotoxin, $10^{-7}$ M). FS caused contractions of preparations from the body, antrum (0.125-10 Hz), fundus (0.125-1.0 Hz) and cardia (0.125-2.0 Hz); relaxations developed at higher frequencies only in tissues of the fundus (2.0-10 Hz, subsequent to contraction) and cardia (5-10 Hz, independent of contractile responses). All contractions were atropine ($10^{-9}$ M) sensitive, even converted to marked (cardia, fundus) or moderate (body) relaxations at $10^{-8}$ M atropine (antral tissue never relaxed to atropine, even at 7 x $10^{-8}$ M). FS-induced contractions of tissues from the cardia, fundus, body and antrum were resistant to antagonism by prazosin ($10^{-8}$ - $10^{-6}$ M), haloperidol ($10^{-8}$ - $10^{-6}$ M) and methysergide ($10^{-8}$ - $10^{-6}$ M); however, contractions of antral preparations, but not those from the cardia, fundus and body, were yohimbine sensitive (50% reduction at $10^{-8}$ M, increased at $10^{-7}$ and $10^{-6}$ M).

In subsequent investigations of relaxation responses, atropine (7 x $10^{-8}$ M), was routinely included in the Krebs-Henseleit solution to obviate contraction effects. Relaxation responses of cardia and body preparations, but not those of the fundus, were reduced by prazosin ($10^{-7}$ - $10^{-6}$ M) and propranolol ($10^{-7}$ - $10^{-6}$ M) (a combined treatment was additive) or by high concentrations of haloperidol (5 x $10^{-6}$ and $10^{-5}$ M, significant in body only), but not by methysergide or yohimbine.

A comparison of FS and exogenous drug effects shows similarities (contractions of all tissues via cholinergic mechanisms, additionally at $\alpha_2$-adrenoceptor mechanisms in antral tissue; relaxations in cardia and body mediated partly via $\alpha_1$- and $\beta$-adrenoceptor mechanisms) and differences ($\alpha_2$-adrenoceptor dependent contractions of fundus and body preparations revealed by exogenous catecholamine treatment but not by FS; $\alpha_1$- and $\beta$-adrenoceptor dependent relaxations of the fundus and antrum revealed by exogenous treatments but, again, not by FS). The value of both approaches can be exemplified by data obtained for metoclopramide. Thus, metoclopramide fails to antagonise relaxations induced in any stomach preparation by exogenous administrations (Sahyoun et al., 1982b) or by FS. However, whilst studies using exogenous administrations failed to show an interaction of metoclopramide with the contractile effect of any added substance, acetylcholine included, metoclopramide was clearly shown in present studies to enhance the contraction to FS of all tissues ($10^{-8}$ - $10^{-6}$ M) via atropine ($10^{-8}$ M) sensitive mechanisms.

Costall, B. et al. (1981). Br. J. Pharmac. 74, 179P-180P.
Sahyoun, H.A. et al. (1982a). J. Pharm. Pharmac. 34, 27-33.
Sahyoun, H.A. et al. (1982b). Naunyn-Schmiedeberg's Arch. Pharmac. 319,8-11.

# ADENOSINE DEPENDENCE IN GUINEA-PIG ILEUM IN VITRO

H.O.J. Collier & J.F. Tucker, Department of Pharmacology, Chelsea College, Manresa Road, London SW3 6LX.

Incubation of guinea-pig isolated ileum at 22°C for up to 24 h with clonidine or normorphine induces, in the final cholinergic motoneurone (FCMN) of the myenteric plexus, dependence on either drug (Collier et al, 1981a & b). Using a comparable method, we have studied the induction in the FCMN of dependence on adenosine, through activation of an inhibitory purinoceptor, of type R, subtype $A_1$ (Paton, 1981), for which a selective antagonist, 8-phenyltheophylline (8-PT), is available (Griffith et al, 1981).

After incubation at 22°C for 16-21 h with 4 µM adenosine in Krebs solution containing 70 µM hexamethonium, the preparation was set up at 37°C in fluid of the same composition and challenged 30-40 min later with 10 µM 8-PT. This precipitated a mean withdrawal contracture of 91.3 ± 4.5 s.e. mean percent of the maximal response to acetylcholine ($n$ = 7). The mean response to 8-PT of control preparations incubated at the same time without adenosine and set up and tested in parallel was much less (8.6 ± 4.4%; $n$ = 7; $P<0.001$). Inclusion of 1 µM 8-PT in the incubation fluid containing 4 µM adenosine significantly inhibited dependence induction (mean contracture: 19.8 ± 6.1%; $n$ = 7; $P<0.001$). Fresh preparations, set up at 37°C in 4 µM adenosine in hexamethonium-Krebs and challenged 30-40 min later with 10 µM 8-PT gave a mean contracture of 13.1 ± 5.3% ($n$ = 13), which was significantly less than that of test preparations incubated with adenosine at 22°C for 18-21 h before setting up ($P<0.001$). Preparations incubated for 16-21 h in 4 µM adenosine, when transferred to an adenosine-free solution, developed a spontaneous withdrawal contracture that adenosine suppressed. Tetrodotoxin (1.5 µM) or hyoscine (50 nM) suppressed both precipitated and spontaneous withdrawal contractures. Omission of hexamethonium from the incubation fluid did not prevent dependence induction.

These findings provide a first example of induction of dependence by continued activation of an inhibitory purinoceptor. The findings with hexamethonium, tetrodotoxin and hyoscine suggest that the adenosine dependence develops in the FCMN. Since activation of $A_1$ purinoceptors probably inhibits adenylate cyclase (Van Calker et al, 1979), dependence may be due to a compensating hypertrophy of a cyclic AMP system of the neurone.

We thank Miles Laboratories Ltd. and Sandoz Ltd. for gifts of apparatus and drugs.

Collier, H.O.J. et al (1981a) Br.J.Pharmac. 73, 443-453
Collier, H.O.J. et al (1981b) Br.J.Pharmac. 73, 921-932
Griffith, S.G. et al (1981) Eur.J.Pharmac. 75, 61-64
Paton, D.M. (1981) J.Auton.Pharmac. 1, 287-290
Van Calker, D. et al (1979) J.Neurochem. 33, 999-1005

# THE INHIBITORY EFFECTS OF INTRAGASTRIC FPL 52694 ON ACID SECRETION IN THE CONSCIOUS FISTULA DOG

S.P. Canfield & B.P. Curwain, Department of Physiology, St. Mary's Hospital Medical School, London W2 1PG.

FPL 52694 is a mast cell stabilising agent shown to inhibit pentagastrin-induced gastric acid secretion (Pigas) in anaesthetised, pylorus ligated rat and dog (Nicol et al, 1981) when given i.v. We have studied the effect of FPL 52694 when applied to the gastric mucosa topically during stimulation of acid output by i.v. infusion of pentagastrin (2ug $kg^{-1} h^{-1}$) or histamine (Higas, 30ug $kg^{-1} h^{-1}$). Experiments were carried out on 5 male beagles with gastric fistulae. Stimulant was infused throughout the experiment and gastric juice collected by gravity drainage in 15 min samples. Measurements made were: volume; osmolarity; and output of ions ($H^+$, $Na^+$ & $Cl^-$). After 90 min the fistula was closed and FPL 52694 (4.35 mg $ml^{-1}$ in distilled $H_2O$) injected through the fistula into the stomach as 4 x 10 ml aliquots over 30 min. The fistula was then opened, the stomach allowed to drain and juice collected for a further 60 min. The results shown are mean + s.e. mean values at 30 min after draining FPL 52694 and are expressed as % fall compared with mean values for 30 min period pre-FPL 52694, except for $Na^+$ where output rate has been used.

During Pigas, FPL 52694 caused a reduction of $H^+$ output ($H^+$out) of 88 ± 5.3% (n=5) and $Cl^-$out of 75 ± 8.2%. $H^+$out was inhibited more than $Cl^-$out ($P<0.05$). Volume of secretion (Vout) was reduced by 70 ± 10.8% and osmolarity was reduced by 22 ± 4.7%. There was a large but very variable increase in $Na^+$out from 13.7 ± 1.1 to 124 ± 73umol $min^{-1}$. This was not significant at 5% level but was seen in all 5 dogs. A similar pattern was seen during Higas. $H^+$out fell by 72 ± 10.2%, $Cl^-$out 38 ± 19.1%, Vout 28 ± 19.1% and osmolarity by 12 ± 4.4%. The difference between the inhibition of $H^+$out and $Cl^-$out was significant ($P<0.05$). $Na^+$out increased from 9.1 ± 2.5 to 65 ± 12.8 umol $min^{-1}$ and this increase was significant ($P<0.01$). For comparison, Pigas was also inhibited with cimetidine (4umol $kg^{-1}$ i.v.). At 30 min post-injection, inhibitions were: $H^+$out 72 ± 10.9%; $Cl^-$out 72 ± 9.3%; Vout 68 ± 9.4%; and osmolarity 8 ± 4.7%. $Na^+$out increased from 9.0 ± 2.1 to 11.4 ± 2.8umol $min^{-1}$, a non-significant change.

Clearly FPL 52694 applied to the gastric mucosa causes a marked inhibition of both Pigas and Higas and is characterised by a greater reduction of $H^+$out than $Cl^-$out, a fall in the osmolarity of the juice and a large increase in $Na^+$out. This is different from the pattern seen with cimetidine. The results with FPL 52694 are similar to those seen with intragastric NaF in the anaesthetised cat (Bond & Hunt, 1956; Reed & Smy, 1980). The major effect of FPL 52694 is an inhibition of HCl production with a smaller, apparent further reduction of $H^+$ which could be due to addition of $NaHCO_3$ to the juice or exchange of $H^+$ for $Na^+$ across the mucosa.

Bond, A.M. & Hunt, J.N. (1956) J.Physiol. 133, 317-329P
Fozzard, J.R. & Leach, G.D. (1968) Europ.J.Pharmac. 2, 239-249P
Nicol, A.K. et al (1981) J.Pharm.Pharmacol. 33, 554-556P
Reed, J.D. & Smy, J.R. (1980) J.Physiol. 301, 39-48P

# THE EFFECT OF TWO ANAESTHETIC AGENTS ON THE ACTION OF 5-HT ON PENTAGASTRIN-STIMULATED ACID SECRETION IN RAT

S.P. Canfield & A.D. Hughes, Department of Physiology, St. Mary's Hospital Medical School, London W2 1PG

Urethane is a widely used anaesthetic in gastric secretory studies in rat but is associated with elevated plasma adrenalin (Armstrong et al, 1982), which may inhibit acid output and also with reduced gastrointestinal blood flow (Bell, 1977). We have compared pentagastrin-stimulated acid secretion (Pigas) and its inhibition by 5-HT during anaesthesia with urethane and also with pentobarbitone which does not elevate plasma adrenalin.

Wistar rats (200-400g) were deprived of food but not water for 18h and then anaesthetised with urethane (1.75g $kg^{-1}$ i.p.) or pentobarbitone sodium (60mg $kg^{-1}$ i.p.). Stomachs were perfused with 0.9% saline at 37°C (1.5 ml $min^{-1}$) using a modified Ghosh & Schild technique. Gastric perfusate was collected for 15 min periods and titrated to pH7. Pentagastrin (100ug $kg^{-1}$ $h^{-1}$) was infused via a jugular vein throughout the experiment and 5-HT given by injection into a tail vein once a secretory plateau had been established. Peak inhibition is expressed as % preceeding response averaged over 30 min prior to injection of 5-HT. Values are mean ± s.e. mean and tests of significance were made using the Mann-Whitney U-test.

In pentobarbitone rats mean Pigas was 179 ± 12umol $H^+$ $h^{-1}$ (n=15). 5-HT (5-20 ug $kg^{-1}$ i.v.) caused a dose-dependent inhibition of Pigas, 10ug $kg^{-1}$ producing 63 ± 9.2% (n=6) inhibition. In urethane anaesthetised animals Pigas was 92 ± 8umol $H^+$ $h^{-1}$ (n=17). In 11 of these animals Pigas was inhibited by 5-HT. At 10ug $kg^{-1}$ i.v.) inhibition was 39 ± 9.9% (n=8). In the remaining six animals 5-HT (10ug $kg^{-1}$ i.v.) increased acid output by 145 ± 8.6% (n=6). Comparison of Pigas in these two urethane groups showed that the six animals in which 5-HT increased secretion had significantly lower ($P<0.01$) Pigas (51 ± 3.8umol $H^+$ $h^{-1}$) than the group where secretion was inhibited (113 ± 10umol $H^+$ $h^{-1}$) and both urethane groups had significantly lower Pigas ($P<0.01$) than the pentobarbitone animals. Our Pigas results with urethane are comparable with other published values (Whittle & Main, 1973). 5-HT never increased Pigas in pentobarbitone animals.

5-HT in doses of 1-10mg $kg^{-1}$ i.p. has been reported to suppress acid secretion in rats (Thompson, 1977) but in this study much lower doses (5-20ug $kg^{-1}$ i.v.) comparable with doses in rats having cardiovascular effects (Fozzard & Leach, 1968) and effects upon intestinal ion transport (Hardcastle et al, 1981), caused significant inhibition of Pigas. Both Pigas and the effect of 5-HT on Pigas was influenced by the anaesthetic agent used. These effects were both quantitative and qualitative in respect of the action of 5-HT. The possibility of the stimulatory action of 5-HT in some urethane-anaesthetised animals being related to effects on mucosal blood flow is being investigated.

Armstrong, J.M. et al (1982) Proc. Br. Pharm. Soc. Stockholm
Bell, G.J. et al (1977) Br. J. Pharmac. 61, 126P
Hardcastle, J. et al (1981) J. Physiol. 320, 41-56
Fozzard, J.R. & Leach, G.D.H. (1968) Europ. J. Pharm. 2, 239-249
Thompson, J.H. (1971) Res. Comm. Chem. Path. 2, 687-781
Whittle, B.J.R. & Main, I.H.M. (1973) Br. J. Pharmac. 49, 534-542

# EFFECTS OF THEOPHYLLINE AND NEOBIPHYLLIN ON THE PULMONARY VASCULAR RESPONSE TO PAF-ACETHER IN GUINEA-PIG LUNG

F.M. Cunningham, J. Morley and L.E. Wood, Department of Clinical Pharmacology, Cardiothoracic Institute, Fulham Road, LONDON SW3 6HP.

PAF-acether has been shown to cause extravasation of protein in the perfused guinea pig lung *in vitro*, and a platelet dependent bronchoconstriction *in vivo*. These properties are appropriate to a mediator of asthma and acute inflammation (Vargaftig et. al., 1980; Basran et. al. (1982). Aminophylline and Neobiphyllin have been shown to inhibit PAF-acether induced extravasation of plasma protein in guinea pig skin (Cunningham and Wood, 1982). The effects of theophylline and Neobiphyllin on PAF-acether induced protein extravasation and increased perfusion pressure in the guinea pig lung have now been examined.

Guinea pig lungs were perfused *in situ* via the pulmonary artery, at a constant flow rate of 6ml/min, with Kreb's Henseleit bicarbonate buffer at 37C containing bovine serum albumin (4% w/v), Evan's blue dye (2.5% w/v), 125I-fibrinogen (5nCi/ml), and gassed with 95% O2, 5% CO2 (Boardman et. al., 1982). Protein extravasation was monitored continuously by means of a collimated crystal scintillation detector with an automated monitoring system. A calibrated stand pipe, open to the atmosphere, was used to measure perfusion pressure. PAF-acether was administered by close arterial infusion. For drug studies, Neobiphyllin or theophylline were present in the perfusion medium at a fixed concentration.

PAF-acether (10ng/ml) caused a significant ($p < 0.05$) increase in perfusion pressure over a five minute period commencing 45secs after injection. The pressure increase was reduced in the presence of theophylline (50 ug/ml) and Neobiphyllin (100ug/ml) ($p < 0.05$), although the effect of Neobiphyllin was more prolonged. A significant ($p < 0.05$) decrease in basal perfusion pressure was observed when theophylline and Neobiphyllin were present in the medium perfusing the lungs. Despite the increased 125I-fibrinogen content of the lungs, observed after 30mins in the presence of the methylxanthines, the increased extravasation in response to administration of PAF-acether was reduced in the presence of Neobiphyllin ($p < 0.05$) or theophylline.

This study demonstrates that methylxanthines impair increased plasma protein extravasation induced by the mediator PAF-acether, and this phenomenon may contribute to the efficacy of these drugs in asthma.

Basran, G.S., Dewar, A., Morley, J., Page, C.P., Paul, W. & Wood, L. (1982). Brit J Pharmacol. 77 (Suppl.) 437P.

Boardman, L.E., Morley, J. & Sanjar, S. (1982). Brit J Pharmacol. 75 (Suppl.) 144P.

Cunningham, F.M. & Wood, L.E. Brit J Pharmacol. 77 (Suppl). 550P.

Vargaftig, B.B., Lefort, J., Chignard, M. & Benveniste, J. (1980). European J Pharmacol. 65, 185-192.

# PERMEABILITY CHARACTERISTICS OF PLATELET-ACTIVATING FACTOR IN GUINEA-PIG SKIN

A. Dewar, J. Morley, C.P. Page & W. Paul, Department of Clinical Pharmacology, Cardiothoracic Institute, Fulham Road, LONDON SW3 6HP.

PAF-acether (1-o-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine) is the most potent platelet aggregating agent known. However, in addition to its proposed role in platelet aggregation, PAF-acether has the properties of a mediator of allergy and inflammation, being produced by a variety of inflammatory cell types (Vargaftig et al., 1981) and able to elicit inflammatory responses in experimental animals (Humphrey et al., 1982, Page & Paul, 1982) and man (Basran et al., 1982).

Using isotopic techniques, we have previously reported the acute effects of PAF-acether on plasma protein extravasation (PPE), and platelet and red blood cell accumulation following intradermal injection in the guinea-pig (Page & Paul, 1982). Here, we have extended our studies to include a definition of the time course of PAF-acether induced PPE as well as histological and ultrastructural features of the response.

Following intradermal injection of PAF-acether (10 ng/site), the PPE response was almost complete within 30 min and no further increase occurred after 1h, even following superinjection of prostaglandin E2 (PGE2) (1.5 nmol). The acute PPE response (0-30 min) to PAF-acether (1 ng/site) was significantly ($p < 0.025$) potentiated by concomitant administration of PGE2 (1 nmol/site) and significantly ($p < 0.005$) reduced by isoprenaline (5 nmol/site; $n = 5$).

Histological sections of skin sites removed from guinea-pigs (injected i.v. with colloidal carbon) 40 min after i.d. PAF-acether (100 ng/site) showed morphological evidence of oedema (notably, separation of collagen bundles) and leakage of carbon particles through inter-endothelial cell gaps as seen under the electron microscope. These observations are consistent with the data obtained using 125-I-albumin. Skin sites injected with PAF-acether more than 1h prior to i.v. colloidal carbon showed no leakage of carbon particles.

The present data indicate that PAF-acether is a potent permeability factor in guinea-pig skin, which increases vascular permeability as a consequence of gap formation between endothelial cells, analogous to that produced by histamine-like mediators (Majno et. al., 1967).

Basran, G.S., et al (1982) Amer. Rev. Respir. Dis. 52 (suppl.), 125.
Humphrey, D., et al (1982) Lab. Invest. 46, 422-427.
Majno, G.M. et al (1967) Circulation Res. 21, 833-847.
Page, C.P. and Paul, W. (1982) Clin. Rheumatol. 1, 144.
Vargaftig, B.B. et al (1981) Ann. N.Y. Acad. Sci. 370, 119-137.

# EFFECT OF VERAPAMIL AND LANTHANUM ON PAF-INDUCED AGGREGATION OF RABBIT PLATELETS IN VITRO

Shahnawaz Khan and Anthony D. Smith, Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 7PN.

Platelet Activating Factor (PAF), a phospholipid structurally identified as 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine, is the most potent aggregating agent of blood platelets in animals and humans (Cazenave et al., 1979, McManus et al., 1981, Chesney et al., 1982, Vargaftig et al., 1981). The aggregation caused by PAF proceeds through an arachidonic acid-independent pathway and is not inhibited by indomethacin or other NSAI drugs (Chignard et al., 1979, Cazenave et al., 1979, Vargaftig et al., 1981). Cazenave et al., (1979) reported that PAF-induced aggregation is blocked by membrane active drugs such as chlorpromazine, lidocaine, methylsurgide, and imipramine.

We have found that verapamil, a slow $Ca^{2+}$ channel inhibitor, blocks PAF action on rabbit platelets in vitro. Verapamil in concentrations of $10^{-6}$ M and above showed dose dependent inhibition of PAF activity. $10^{-4}$ M verapamil completely blocked the action of $8 \times 10^{-9}$ M PAF while a dose of $1.5 \times 10^{-4}$ M verapamil was required for complete inhibition of $1.6 \times 10^{-8}$ M PAF. These concentrations of PAF caused irreversible aggregation in platelet rich plasma when used in the absence of verapamil.

When verapamil was added to PAF-aggregated platelets, it caused disaggregation. The disaggretated platelets were refractory to further addition of the same concentration of PAF and required a much higher concentration (40 fold) of PAF for further aggregation. The verapamil-disaggregated platelets could be aggregated by $10^{-6}$ M A23187. Verapamil also inhibited PAF-induced release of 5HT from labelled platelets.

Lanthanum ions, reported as $Ca^{2+}$ antagonist (Robbilee and Shepro, 1976) also inhibited PAF in a dose dependent manner but failed to disaggregate the PAF-aggregated platelets. $La^{3+}$ ($10^{-4}$ M) blocked verapamil-induced disaggregation of PAF-aggregated platelets. It has recently been reported (Shaw and Lyons, 1982) that TMB-8, which is thought to inhibit intracellular $Ca^{2+}$ transport, inhibits PAF-stimulated 5HT release.

These results suggest the involvement of calcium in activation of platelets by PAF and utilization of different pools of calcium by PAF and A23187. The disaggregation of aggregated platelets is suggestive of some mechanism of action of verapamil other than simple inhibition of calcium ion translocation.

Cazenave JP, Benveniste J, Mustard JF (1979) Lab Invest 41: 275
Chignard M, Le Couedie JP, Tence M, Vargaftig BB, Benveniste J (1979) Nature 279: 799
Chesney CM, Pifer DD, Byers LW, Muirhead EE (1982) Blood 59 (3): 582
McManus LM, Hanahan DJ, and Pinckard RN (1981) J Clin Invest 67: 903
Robbilee LS and Shepro D (1976) Biochim. Biophys. Acta 436: 448
Shaw JD and Lyons RM (1982) Biochim. Biophys. Acta 714: 492
Vargaftig BB, Chignard MM, and Benveniste J (1981) Biochem. Pharmacol. 30: 263
Vargaftig BB, Chignard MM, Benveniste J, Lefort J and Wal F (1981) Annals. N.Y. Acad. Sc. 370: 119

# EVIDENCE THAT PLATELET ACTIVATING FACTOR DOES NOT MEDIATE COLLAGEN-INDUCED AGGREGATION OF HUMAN ASPIRIN-TREATED PLATELETS

Barbara Nunn, Beecham Pharmaceuticals Research Division, Great Burgh, Yew Tree Bottom Road, Epsom, Surrey, KT18 5XQ, England.

The suggestion (Chignard et al, 1980) that 1-O-octadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (Platelet Activating Factor, PAF) acts as a mediator of collagen-induced aggregation has been investigated by examining responsiveness to collagen of platelets made totally but selectively insensitive to PAF.

PAF, stored at $-20^{O}$ in ethanol, was dried under nitrogen and redissolved in 154 mM NaCl containing 0.25% (w/v) bovine serum albumin. Human platelet-rich plasma (PRP) was prepared as previously described (Nunn and James, 1980) and mixed with 0.1 volumes 4 mM aspirin, freshly dissolved in 154 mM NaCl, to prevent prostanoid synthesis. Some 10-15 min after aspirin-treatment (by which time responsiveness to 1-2 mM arachidonate had been lost), half of each preparation was desensitised to PAF by gentle mixing with 0.01 volumes 10 µM PAF (final concentration, 0.1 µM) twice at room temperature. An interval of 3-5 min was allowed between the two additions. After a further 3-5 min, the PRP was mixed with 0.005 volumes 200 µM PAF (final concentration, 1 µM) and left at room temperature for at least 15 min before being studied in the aggregometer. The control half of each preparation was processed similarly except that the addition was 154 mM NaCl each time.

Aggregation in response to collagen was measured as previously described (Nunn and James, 1980). $Log_{10}$ concentration-response curves were obtained in the control and PAF-desensitised halves and the concentration of collagen producing a 50% maximal response (EC50) read by interpolation. The effect of PAF-desensitisation was expressed as a dose-ratio calculated by dividing the EC50 for the desensitised half by the corresponding control EC50.

The control half of each preparation always aggregated in response to 0.05 µM PAF (threshold concentration). The PAF-desensitised halves gave no response to 50 µM PAF, and hence were at least one thousand times less sensitive than normal. In contrast, dose-response curves for collagen in the two halves of each preparation were essentially superimposable. For example, the EC50s were 4.0 ± 0.5 µg/ml and 3.8 ± 0.7 µg/ml (mean ± s.e. mean, n = 5) in the control and PAF-desensitised halves respectively. The dose-ratio was 0.93 ± 0.09 (mean ± s.e. mean, n = 5).

These results show that loss of sensitivity to PAF did not cause any loss of sensitivity to collagen in human aspirin-treated PRP. Hence it would appear that PAF does not mediate "prostanoid-independent" aggregation in response to collagen. The present study is concerned only with PAF acting at the external surface of the platelet, and does not of course exclude a role for PAF as an intraplatelet mediator.

I am grateful to Professor J.J. Godfroid of Paris University for supplying the Platelet Activating Factor.

Chignard, M. et al (1980) Br. J. Haemat. 46, 455-464
Nunn, B. and James, F.J. (1980) Br. J. clin. Pharmac. 9, 239-245

# EFFECT OF Ro 31-1118, A NOVEL $\beta_1$-SELECTIVE ADRENOCEPTOR ANTAGONIST, ON PLATELET AGGREGATION

D. Bradshaw, P.H. Franz & A.L. Sugden, Department of Pharmacology, Roche Products Limited, Welwyn Garden City, Hertfordshire AL7 3AY.

A number of $\beta$-adrenoceptor blocking drugs have been shown to be capable of inhibiting platelet aggregation although this effect does not appear to be directly related to blockade of the $\beta$-adrenoceptor (Bucher and Stucki, 1969; Weksler et al, 1977; Bouvenot et al, 1980). Ro 31-1118 (1-[4-(2-(4-fluorophenethyloxy)ethoxy)phenoxy]-3-isopropylamino-2-propanol hydrochloride) is a novel $\beta$-adrenoceptor antagonist with a high degree of selectivity for the $\beta_1$-receptor. The present studies were undertaken in order to investigate the effect of Ro 31-1118 on platelet aggregation.

Platelet aggregation was assessed in human platelet-rich plasma by measuring the changes in optical density induced by an aggregating agent according to the method of Born (1962). Aggregation was induced by the addition of either adrenaline, adenosine 5'-diphosphate (ADP) or collagen and concentrations which induced an approximately 80% maximal response were used for determinations with the test compounds. For each plasma sample tested the effects of Ro 31-1118, propranolol and aspirin were assessed by determining the concentration of each compound causing 50% inhibition of aggregation ($IC_{50}$). Results were obtained for 6 plasma samples for each aggregating agent and the mean $IC_{50}$ values ($\pm$ S.E.M.) obtained for the three compounds are shown in Table 1. For adrenaline and ADP the results refer only to the second phase of aggregation, there being no effect of the compounds on the first phase.

Table 1 $IC_{50}$ values for inhibition of platelet aggregation

| Aggregating agent | $IC_{50}$ values ($\mu$M) | | |
|---|---|---|---|
| | Ro 31-1118 | Propranolol | Aspirin |
| Adrenaline | 32.4 $\pm$ 7.5 | 43.3 $\pm$ 9.5 | 33.5 $\pm$ 9.0 |
| ADP | 32.8 $\pm$ 4.9 | 36.1 $\pm$ 4.5 | 33.8 $\pm$ 8.5 |
| Collagen | 33.5 $\pm$ 6.2 | 41.8 $\pm$ 7.4 | 40.8 $\pm$ 8.5 |

Comparison of the $IC_{50}$ values using Student's paired t-test for statistical analysis indicated no significant difference between the three compounds in their ability to inhibit platelet aggregation.

In conclusion, Ro 31-1118, a novel $\beta_1$-adrenoceptor antagonist, is an inhibitor of platelet aggregation with a potency equivalent to that of propranolol and aspirin.

Born, G.V.R. (1962) Nature, 194, 927 - 929.
Bucher, H.W. & Stucki, P. (1969) Experientia 25, 280 - 282.
Bouvenot, G. et al (1980) Meditteranean Med. 218, 9 - 14.
Weksler, B.B. et al (1977) Blood, 49, 185 - 196.

# FORSKOLIN IS A POTENT INHIBITOR OF HUMAN PLATELET AGGREGATION

M.B.H. Youdim, Rappaport Medical Research Center, Department of Pharmacology, Faculty of Medicine, Technion, P.O.Box 9649, Haifa, Israel.

Measures taken to increase cyclic AMP levels in platelets, be it by stimulation of synthesis via receptors or by interference with its degradation, e.g. use of phosphodiesterase inhibitors, will lead to a pronounced increase in the resistance of platelets to aggregate (Luscher and Massini, 1975). Forskolin (7β-acetoxy-8,13,-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-one) can independently of receptors, guanyl nucleotides or the guanine nucleotide regulatory protein, directly activate the catalytic subunit of adenylate cyclase in a large number of intact tissues (Seamon et al, 1981; Seamon and Daly, 1981; Cuthbert and Spayne, 1982). We have, therefore, studied the action of forskolin on platelet activation.

Platelet aggregation as induced by ADP, noradrenaline (NA), serotonin (5-HT) and thrombin was examined using citrated human platelet-rich plasma (PRP). Aggregation was quantified photometrically with a Chrono-log double channel aggregometer (Youdim et al, 1981) as the maximal rate of change in light transmission through a sample (0.45ml) stirred (1000RPM) at 37°C on the addition of aggregation inducers (10μM) alone or in combination with forskolin (3 min preincubation). Forskolin was dissolved in 96% alcohol. The amount of alcohol added to the PRP was less than 0.2% and was without effect on aggregation.

Forskolin was very effective in preventing platelet aggregation induced by ADP, NA, 5-HT and thrombin. The $IC_{50}$s for inhibition of platelet aggregation using the latter inducers were in the range of 2-5μM which are similar to those required to activate half-maximally the catalytic subunit or adenylate cyclase (5-10μM) (Seamon et al, 1981). The inhibition of platelet aggregation by forskolin is time-dependent. Forskolin (8μM) caused maximum inhibition of ADP (10μM) induced aggregation with 5 min preincubation. Similar results were obtained using other platelet activating substances. The ADP (10μM) induced aggregation could be reversed by forskolin (8μM).

It is well established that measures taken to increase intracellular cyclic AMP will prevent the onset of platelet aggregation (Moncada, 1982). However, the mechanism by which cyclic AMP stabilizes the membrane is not known. The availability of the compound forskolin, which can directly increase cyclic AMP in the intact cell leading to stabilization of platelet membrane may prove useful in elucidating mechanism of action of the latter. It may also be a pointer in the development of drugs which inhibit platelet aggregation.

Dr. R. Epstein and Dr. J. Daly are gratefully acknowledged for the generous samples of forskolin.

Cuthbert, A.W. & Spayne, J.A. (1982) Br.J.Pharmac. 76, 33-35.
Luscher, E.F. & Massini, P. (1975) In: Biochemistry and Pharmacology of Platelets. Eds. K. Elliott & J. Knight. Elsevier, Amsterdam pp.5-23.
Moncada, S. (1982) Br.J.Pharmac. 76, 3-31.
Seamon, K. & Daly, J.W. (1981) J.biol.Chem. 10, 9799-9801.
Seamon, K., Padgett, W. & Daly, J.W. (1981) Proc.Natl.Acad.Sci. USA 78, 3363-3367.
Youdim, M.B.H. et al (1981) Br.J.clin.Pharmac. 12, 535-542.