THE IMPORTANCE OF KININASE II IN THE INACTIVATION OF BRADYKININ IN THE RABBIT

E.J. Clark, S.D. Brain*and T.J. Williams, Section of Vascular Biology, MRC Clinical Research Centre, Watford Road, Harrow, HA1 3UJ, U.K.

Bradykinin can be metabolised in vivo by kininase I (carboxypeptidase N) and kininase II (angiotensin converting enzyme). We have investigated the importance of these enzymes in metabolising bradykinin by studying the effect of specific inhibitors for each pathway on the oedema inducing activity of bradykinin in rabbit skin.

Local oedema formation was measured in NZW rabbits as the extravascular accumulation of intravenously-injected ^{125}I-albumin in response to intradermally-injected bradykinin (0.1ml injection volume). The kininase II inhibitor captopril (Cap) when mixed with bradykinin (Bk) potentiated oedema in a dose dependent manner (see Table 1). The presence of captopril (10^{-10}mol/0.1ml) caused a ten-fold increase in the potency of bradykinin as an oedema-inducing agent. As a consequence of its vasodilator activity PGE_2 increased the potency of bradykinin 100-fold and in the presence of both captopril (10^{-10}/0.1ml) and PGE_2 ($3x10^{-10}$mol/0.1ml), the increase was >1,000-fold. In contrast, the kininase I inhibitor DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid (MERGETPA, Plummer and Ryan 1981) had no effect on bradykinin-induced oedema.

Table 1. Effect of enzyme inhibitors on the activity of bradykinin

Agent (mol/0.1ml)	Oedema (µl plasma/site) mean±s.e.m n=5 rabbits
Bk (10^{-11}mol)	36.6 ± 11.1
Bk (10^{-11}mol) + Cap (10^{-11}mol)	49.6 ± 14.3
Bk (10^{-11}mol) + Cap (10^{-9}mol)	84.8 ± 27.0
Bk (10^{-11}mol) + MERGETPA (10^{-7}mol)	40.9 ± 11.1
Cap (10^{-9}mol)	6.7 ± 0.6
MERGETPA (10^{-7}mol)	9.4 ± 0.8
Phosphate buffered saline (PBS)	7.6 ± 0.7

Experiments were carried out to investigate the effects of the enzyme inhibitors on the activity of bradykinin incubated in plasma. The oedema inducing activity of bradykinin (10^{-10}mol/0.1ml) was assayed in the presence of PGE_2 ($3x10^{-10}$mol/0.1ml) added after incubation (5 min at 37°C) in PBS or rabbit plasma; Bk in PBS 90.1±10.2µl, Bk in plasma 31.5±1.9µl. Captopril (10^{-10}mol/0.1ml) prevented the loss of the activity of bradykinin when added to the plasma at the beginning (102.9±18.0µl) $p<0.05$ paired t test but not at the end (42.5±8.1µl) of the incubation period (mean s.e.m. n=5). In similar experiments MERGETPA at doses up to (10^{-8}mol/0.1ml) had no effect on the loss of the activity of bradykinin in plasma.

These results show the importance of kininase II in metabolising bradykinin in both the skin and plasma of the rabbit and show that angiotensin converting enzyme inhibitors such as captopril can thus enhance the oedema inducing activity of bradykinin. The failure of the kininase I inhibitor MERGETPA to affect the breakdown of bradykinin in these experiments suggest that the conversion of bradykinin to bradykinin des Arg is a less important metabolic pathway. However this pathway could become more important when angiotensin converting enzyme inhibitors are present.

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Plummer, T.H. & Ryan, T.J. (1981) Biochem. Biophys. Res. Comm. 98, 448.

IN VIVO pA_2 STUDIES USING NALOXONE INFUSIONS REVEAL MULTIPLE SITES MEDIATING OPIOID DEPRESSION OF RESPIRATION

M. Yeadon & I. Kitchen, Department of Biochemistry Division of Pharmacology & Toxicology, University of Surrey, Guildford, Surrey, GU2 5XH, U.K.

Drugs of the 4-anilinopiperidine series (fentanyls) are used as anaesthetic analgesics (Janssen, 1982) but all suffer from the undesirable side effect of respiratory depression. It is generally believed that these compounds depress respiration via the μ opioid receptor (Hassen et al., 1984) though there is evidence for the involvement of other receptor types (Ling et al., 1985 ; Hurle et al., 1985). We report here in vivo apparent pA_2 studies for naloxone antagonism of fentanyl induced respiratory depression which suggest the involvement of at least two opioid receptor types.

Ventilatory parameters were measured in urethane-anaesthetised (1.5g/kg i.p.) male Wistar albino rats (300-450g) using a tracheal cannula connected via a Fleisch (OO) pneumotachometer to a Grass volumetric pressure transducer. Agonists were administered into the right jugular vein in 0.1ml saline and naloxone was given as an i.v. bolus (50, 200 or 800μg/kg) followed by a continuous i.v. infusion (1.06, 4.25 or 17.0μg/kg/min, respectively) into the left jugular. Pharmacokinetic studies with naloxone confirmed that steady state blood naloxone concentrations of 17, 69 and 275nM were obtained respectively using this infusion protocol. Alfentanil, sufentanil and fentanyl produced qualitatively similar responses consisting of an apnea of immediate onset and dose-related duration. In addition, both tidal volume and respiratory frequency, and thus minute volume were depressed in a dose-related manner. The relative potencies of the drugs to cause a 50% depression in minute volume 15s after dosing and to produce an apnea of 6s duration were dissimilar and were 0.99 (sufentanil), 1.3 (alfentanil) and 3.0 (fentanyl). Quantitative studies of naloxone antagonism showed that apparent pA_2 for antagonism of apnea was the same for the three drugs. However, pA_2 values for antagonism of minute volume depression differed significantly through the fentanyl series. In bilaterally vagotomised animals, the apnea response was abolished and the pA_2 values for naloxone antagonism of minute volume depression were altered (Table 1).

Table 1 Antagonism by naloxone of respiratory depression in intact and vagotomised rats

	INTACT		BILATERALLY VAGOTOMISED
	Apnea	Minute Volume	Minute Volume
Sufentanil	9.06 ± 0.06	9.11 ± 0.06	8.45 ± 0.05
Alfentanil	9.05 ± 0.05	8.83 ± 0.04	8.59 ± 0.02
Fentanyl	9.02 ± 0.04	8.63 ± 0.05	8.75 ± 0.05

(Values are mean ± s.e.mean of 6-8 determinations of pA_2 made at 3 different blood naloxone concentrations.)

For all pA_2 determinations, the slope of the Schild plot did not differ significantly from unity. In conclusion, the identical pA_2 values for antagonism of apnea for the three fentanyl drugs suggest an identical receptor interaction for this effect. However, the difference in pA_2 values for antagonism of minute volume depression may indicate differential interactions of the drugs with a heterogeneous opioid receptor population in producing this effect.

Hassen, A.H., Feuerstein, G. & Faden, I. (1984) Neuropharmacol. 23, 407-415
Hurle, M.A., Mediavilla, A. & Florez, J. (1985) Neuropharmacol. 24, 597-606.
Janssen, P.A.J. (1982) Acta anaesth. Scand. 26, 262-268.
Ling, G.S.F., Spiegel, K., Lockhart, S.H. & Pasternak, G.W. (1985) J. Pharmacol. Exp. Ther. 232, 149-155.

ANTAGONISM OF CARBENOXOLONE GASTRIC PROTECTION IN RATS BY POTASSIUM-SPARING DIURETICS, SPIRONOLACTONE AND AMILORIDE

S.L. Perks*, B.Y.C. Wan & P.C. Thornton, Departments of Biochemistry and Pharmacology, Biorex Laboratories Ltd., Canonbury Villas, London N1 2HB.

Carbenoxolone (CBX) has mineralocorticoid-like side-effects which can be managed by the use of diuretics. However, the potassium-sparing diuretics spironolactone (SP) and amiloride (AM) also antagonise the ulcer-healing by CBX in man (Doll et al., 1968; Reed et al 1980). The mechanism of this antagonism has not been fully evaluated.

The present study investigates the concomitant administration of SP, AM and non-potassium-sparing diuretics bendrofluazide (BF) and frusemide (FR) on the protective effect of CBX and an analogue cicloxolone (CCX) on ethanol-induced gastric erosions. A structurally-unrelated ulcer-healing agent sofalcone (SN) whose actions are similar to CBX was included for reference. The effects of the ulcer healing agents on water-induced diuresis were also studied.

Gastric erosions were induced with ethanol using groups of 6 male Biorex Wistar rats (Wan and Gottfried, 1985). Dose levels used (mg/kg) were; CBX, CCX and SN, 70 po, AM and SP 10 s.c., FR and BF 5 and 10 po, respectively.

Table 1: Inhibition of ethanol-induced gastric erosions (%)

	Alone	+ AM	+ SP	Alone	+ BF	+ FR
CBX	80***	43	46	67*	69**	41*
CCX	80***	21	33	79*	76***	66***
SN	40	61**	29	-	-	-

Mann-Whitney U test against controls *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

These results agree with those observed clinically. Both SP and AM inhibit the gastric protective action of CBX and of CCX but the protection of SN was not affected. The non-potassium-sparing diuretics BF and FR did not reduce the protective action.

The mineralocorticoid-like antidiuresis of both CBX and CCX was partially reversed by all 4 diuretics. SN was without effect on water, sodium and potassium excretion.

It may be possible that mineralocorticoid-like actions of CBX and CCX are integral to their gastric protection. SN is also protective to the gastric mucosa and its mode of action has been suggested as increasing PGE_2 concentrations by inhibiting the degrading enzyme 15-hydroxyprostaglandin dehydrogenase (Muramatsu et al 1984). This action is also shared by CBX but may not represent its sole mode of action in gastric protection.

Doll, R., Langman, M.J.S & Shawdon, H.H. (1968) Gut **9**, 42-45.

Muramatsu, M., Tanaka, M. et al. (1984) Biochem. Pharmacol. **33**, 2629-2633.

Reed, P.I., Lewis, S.I, et al. (1980) Scand. J. Gastroent **15**, Suppl 65, 51-55.

Wan B.Y.C. & Gottfried, S. (1985) J. Pharm. Pharmacol. **37**, 739-740.

MODULATION OF THE $GABA_A$ RECEPTOR BY PROPOFOL

T.G. Hales* & J.J. Lambert (Introduced by I.H. Stevenson). Neuroscience Research Group, Department of Pharmacology and Clinical Pharmacology, University of Dundee, Ninewells Hospital and Medical School, Dundee, DD1 9SY.

Extracellular recordings made from the cat spinal cord suggest that the general anaesthetic 2,6-di-isopropylphenol (propofol) enhances synaptic inhibition mediated by GABA (Lodge & Anis, 1984). The steroidal general anaesthetic alphaxalone has been shown to modulate the $GABA_A$ receptor of cultured rodent central neurones (Barker et al, 1987) and bovine chromaffin cells (Cottrell et al, 1987). In the present study the actions of propofol, in a fat emulsion formulation, on the $GABA_A$ receptor ionophore complex were investigated under voltage clamp conditions using bovine chromaffin cells in culture.

Whole-cell (holding potential = -60mV) and outside-out patch (holding potential = -80mV) recordings (Hamill et al, 1981) were made at 20-22°C on isolated chromaffin cells maintained in culture for 1-7 days (Cottrell et al, 1987). Propofol at 1.7, 8.4 and 16.8μM in the medium, reversibly potentiated submaximal whole cell currents, evoked by locally applied GABA (100μM), by 274 ± 45% (n=5 ± S.E.M.), 592 ± 141% (n=4) and 964 ± 140% (n=4) of the control respectively. In contrast, no change in the amplitude of GABA evoked currents was detected during administration of the fat emulsion vehicle (intralipid) alone. The potentiation of the GABA-evoked current, induced by propofol (1.7 μM), occurred without any significant change in reversal potential, and was insensitive to the benzodiazepine antagonist Ro15-1788 (300nM).

Locally applied propofol (600 μM), in the absence of GABA, evoked an inward whole-cell current which was reversibly suppressed by bicuculline (1μM). Bath application of propofol established the threshold concentration for this direct effect to be approximately 8μM. With internal and external Cl^- concentrations of 30.2 and 148.0 mM respectively, the reversal potential of the response to propofol was -36.4 ± 1.8 mV (n = 4), which is similar to that found for GABA under the same ionic conditions (-34.7 ± 1.4 mV, n = 5) and close to the theoretical Cl^- equilibrium potential of -40.0 mV. Additionally, on outside-out membrane patches, 30μM propofol elicited single channel currents with a similar reversal potential to those gated by GABA. Such currents were reversibly antagonised by 1μM bicuculline.

In conclusion, like alphaxalone, the general anaesthetic propofol both potentiates GABA-evoked responses and directly activates the $GABA_A$ receptor itself.

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Barker, J.L. et al (1987) J. Physiol. 386, 485-501.
Cottrell, G.A. et al (1987) Br. J. Pharmac. 90, 491-500.
Hamill, O.P. et al (1981) Pflügers Arch. 391, 85-100.
Lodge, D. & Anis, N.A. (1984) Br. J. Anaesth. 56, 1143-1151.

NEUROKININ RECEPTOR AGONISTS MAY DEPOLARIZE SPINAL MOTONEURONES VIA TWO DISTINCT MECHANISMS

S.J. Ireland*, C.C. Jordan and I.K. Wright, Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 0DJ, U.K.

In the isolated hemisected spinal cord of the neonatal rat, neurokinin-induced depolarizing ventral root potentials appear to be mediated via more than one receptor type (see Wienrich et al., 1987). Both NK_1-like and NK_3-like binding sites are present in the spinal cord of the adult rat, the latter apparently being restricted to the dorsal horns (Ninkovic et al., 1985; Hunter et al., 1987). We have compared the ability of the NK_1-selective agonist substance P methyl ester (SPOMe; Watson et al., 1983) and the NK_3-selective agonist succinyl-[Asp^6,MePhe^8]-SP(6-11) (senktide; Wormser et al., 1986) to evoke ventral root depolarisation in the rat spinal cord and sought to establish whether the responses were due to a direct action on motoneurones.

Ventral root potentials were recorded from lumbosacral spinal cords removed from 1-8 day old C.D. rats (Glaxo) (see Brown et al., 1985). Spinal cords were hemisected in the saggital plane; some were quadrisected by means of a further (horizontal) cut perpendicular to the first. The identity and concentration of peptides was determined as described previously (Brown et al., 1985).

In both hemisected (HSC) and quadrisected (QSC) spinal cords, senktide ($1x10^{-8}$ - $3x10^{-7}$M) or SPOMe ($3x10^{-8}$ - $1x10^{-6}$M) caused concentration-related depolarisation. Mean EPMR (± s.e.) values senktide: SPOMe (SPOMe=1.0) were 0.34±0.42 (HSC) and 0.24±0.045 (QSC) (n=4). Responses to approximately maximally-effective concentrations of senktide ($3x10^{-7}$M) or SPOMe ($1x10^{-6}$M) were of similar amplitude. In the presence of tetrodotoxin (TTX, $5x10^{-7}$M) responses to senktide were markedly attenuated; in HSC, the response at $1x10^{-5}$M, was only 12.0±4.7% of the control maximum (n=4), whilst in QSC the corresponding value was 6.3±2.0% (n=4). Against SPOMe, TTX caused parallel rightward displacement of the concentration-response curve (mean concentration ratios 4.7±1.3 (n=4), HSC; and 2.8±0.5 (n=4), QSC). High magnesium medium ($MgCl_2$, $2x10^{-2}$M, no added $CaCl_2$) produced effects similar to those of TTX. The response to senktide ($1x10^{-5}$M) was reduced to 7.3±2.3% (n=4; HSC) or 4.8±2.4% (n=4; QSC) of the control maximum. The concentration-response curve to SPOMe was displaced to the right (mean ratios 8.5±1.4 (n=4), HSC; and 4.6±0.5 (n=4), QSC). On HSC, the N-methyl-d-aspartate (NMDA) antagonist 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid ($1x10^{-4}$M) caused rightward displacement of the concentration response curves to senktide (mean concentration ratio 46.4±12.9, n=4) and NMDA (52.7±5.2, n=4) but it had little effect on depolarisation induced by SPOMe (concentration ratio 2.0±0.7, n=4) or quisqualic acid (1.4±0.4, n=4).

It is concluded that, in the neonatal rat spinal cord, the depolarization response to SPOMe can be mediated via a direct action at motoneurones, while that to senktide is due, almost exclusively, to an indirect action. The apparent paradox that the location of receptors activated by senktide does not parallel that of NK_3-like binding sites needs to be resolved.

Brown, J.R. et al. (1985) in 'Tachykinin antagonists': Hakanson, R. & Sundler, F., (ed.), pp.355-365. Elsevier, Amsterdam.
Hunter, J.C. et al, (1987) Neurosci. Lett. 78, 12-16.
Ninkovic, M. et al. (1985) Eur. J. Pharmac. 106, 463-464.
Watson, S.P. et al. (1983) Eur. J. Pharmac. 87, 77-84.
Wienrich, M. et al. (1987) in 'Substance P and Neurokinins' : Henry et al. (eds) pp. 328-330. Springer Verlag, New York.
Wormser, M. et al. (1986) EMBO Journal 5, 2805-2808.

L-GLUTAMATE - ACTIVATED SINGLE ION CHANNELS IN CELLS ACUTELY DISSOCIATED FROM ADULT RAT HIPPOCAMPUS

A.J. Gibb, MRC Receptor Mechanisms Research Group, Dept. of Pharmacology, Univ. College, Gower Street, London WC1E 6BT. (Introduced by D. Colquhoun).

Much of the evidence in support of glutamate being a neurotransmitter in mammals comes from *in vivo* experiments, or from *in vitro* brain slice and receptor binding studies. In contrast, glutamate-activated ion channels have so far been studied using cultured neonatal or embryonic neurones (Cull-Candy & Usowicz, 1987; Jahr & Stevens, 1987). This study concerns glutamate-activated ion channels in cells dissociated from adult rat hippocampus (Kay & Wong, 1986).

Recordings were made in a nominally Mg^{2+} free solution (in mM: NaCl, 118; KCl, 5; $CaCl_2$, 2.5; $NaHCO_3$, 25; $NaHPO_4$, 1) bubbled with 95% O_2 and 5% CO_2. Whole cell recordings demonstrated that cells respond to glutamate (20μM - 1mM) with a maintained current during agonist application. Single channel currents were evoked by glutamate (1 μM). Five different conductance levels can be detected in the presence of glutamate (Figure 1) although often only 2 or 3 of the 5 levels are evident in any one patch.

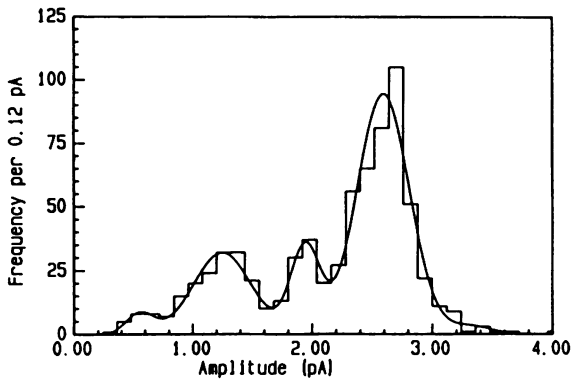

Figure 1. **Histogram of 719 current amplitudes measured from an inside-out patch held at +80mV. The curve shows the sum of five Gaussian components of amplitude (pA) 0.55±0.12, 1.2±0.25, 1.9±0.12, 2.6±0.22 and 3.3±0.18 with relative areas 0.03, 0.23, 0.12, 0.60 and 0.02 respectively: 74% of transitions were between the shut level and an open level, and 26% were between open levels.**

Channel closed time distributions were best fitted by the sum of four exponential components with time constants of 0.13±0.04ms, 0.90±0.12ms, 7.36±0.68ms and 107±13ms (mean ± SEM, n=4, E_m= -80mV). Gap times showed no obvious voltage dependence. In contrast both open time and burst length distributions were voltage-dependent and were best fitted with two exponential components. Burst length distributions had time constants of 0.17±0.04ms and 1.7±0.07ms at -80mV and 0.19±0.07ms and 4.4±0.8ms at -40mV. This voltage dependence of open time and burst length may be attributable to channel block (Nowak *et.al.*,1984) by residual Mg^{2+} ions present in the recording solution.

Cull-Candy, S.G. & Usowicz, M.M. (1987) *Nature* 325, 525-528.
Jahr, C.E. & Stevens, C.F. (1987) *Nature* 325, 522-525.
Kay, A.R. & Wong, R.K.S. (1986) *J. Neurosci. Meth.* 16, 227-238.
Nowak,L., Bregestowski,P., Ascher,P., Herbet,A. & Prochiantz,A. (1984) *Nature*, 307, 462-465.

DIFFERENTIAL MODULATION BY GLYCINE AND L-GLUTAMATE OF [^{3}H]-TCP BINDING TO THE NMDA RECEPTOR

J. Bénavidès*, J.P. Rivy and B. Scatton. Laboratoires d'Etudes et de Recherches Synthélabo (LERS), 31 Ave P.V. Couturier, 92220 Bagneux, FRANCE.

It has been recently shown in electrophysiological studies that low concentrations of glycine potentiate the effects of N-methyl-D-aspartate (NMDA) in cultured mouse brain neurons (Johnson and Ascher, 1987). However, the mechanism whereby glycine modulates NMDA receptor mediated responses is as yet unknown. In an attempt, to characterize the molecular basis of this phenomenon, we have studied the possible modulation by glycine of the binding of ^{3}H-TCP (N-(1- 2-thienyl cyclohexyl)piperidine), a ligand which recognizes the ionic channel associated to the NMDA receptor in an agonist dependent fashion (Loo et al., 1986).

^{3}H-TCP binding was measured in rat forebrain membranes prepared as described previously (Loo et al., 1986) except that 0.5% Triton X100 was included in the second washing step and that 0.1 mM EGTA was present in the incubation medium.

^{3}H-TCP (2.5 nM) binding to brain membranes reached equilibrium in less than 20 min and displayed monophasic association kinetics with a t 1/2 of 2 min. In the presence of 10^{-4}M L-glutamate, association was slower (t 1/2 = 4 min) and biphasic and the equilibrium was only reached after 4 hrs of incubation but at much higher ($\cong$ 3 fold) binding levels. Glycine (10^{-4}M) had no effect on the association of TCP in the absence of glutamate (t 1/2 = 2 min) but increased the association rate in the presence of glutamate (t 1/2 = 2.8 min, cf. 4 min). At equilibrium, glycine potentiated by 50% the glutamate enhancement of ^{3}H-TCP binding. Under basal conditions, dissociation of bound ^{3}H-TCP occured at a very high rate (t 1/2 = 1 min). In the presence of 10^{-4}M glutamate this process became slower (t 1/2 = 38 min) and exhibited biphasic kinetics. Dissociation kinetics were no further modified by 10^{-4}M glycine. The alterations of ^{3}H-TCP binding induced by L-glutamate and glycine (at equilibrium) were due to increases in affinity (Kd = 69, 30, 21 nM for basal, 10^{-4}M glutamate and 10^{-4}M L-glutamate + 10^{-4}M glycine, respectively) with no changes in binding capacity. The enhancement of ^{3}H-TCP (2.5 nM) binding by glycine was concentration-dependent (EC_{50} = 50 nM in the presence of 10 µM L-glutamate and 1 µM in the presence of 0.1 µM L-glutamate). No glycine effect was detected in the presence of the NMDA receptor antagonist 2-APV (10 µM). Finally, the glycine potentiation of the L-glutamate effect was resistent to strychnine and was mimicked by L-alanine and L-serine whilst GABA, taurine and β-alanine were without effect.

The present results suggest that glycine allosterically interacts with the ^{3}H-TCP binding site in the NMDA receptor associated ionic channel via a strychnine insensitive mechanism. The dependency of the glycine effect on the presence of L-glutamate suggests a preferential effect of glycine on the activated, open state, of the NMDA receptor.

Johnson, J.W. and Ascher, P. (1987). Nature 325, 529.
Loo, H. et al. (1986). Eur. J. Pharmacol. 123, 467.

IFENPRODIL, A POTENT NON-COMPETITIVE NMDA ANTAGONIST WITH A NOVEL MECHANISM OF ACTION

C.J. Carter*, F. Noel, J. Benavidès, J.P. Rivy, F. Thuret, K.G. Lloyd, S. Arbilla, S.Z. Langer, B. Zivkovic and B. Scatton. Laboratoires d'Etudes et de Recherches Synthélabo (LERS), 31 Ave. P.V. Couturier, 92220 Bagneux, FRANCE.

Ifenprodil (Vadilex®) is currently in clinical use as a cerebral anti-ischaemic agent. It has been shown to markedly reduce the neuronal necrosis produced by middle cerebral artery occlusion in cats (MacKenzie et al, 1984), and its actions were originally interpreted in terms of its adrenolytic and central vasoconstrictor properties. We have since found that this agent is a potent N-methyl-D-aspartate (NMDA) antagonist in a number of in vitro and in vivo animal models. Thus, ifenprodil dose-relatedly and totally antagonizes the effects of NMDA (80 µM) on cyclic GMP production in immature rat cerebellar slices in vitro with an IC_{50} of 0.4 µM (cf. 2-APV = 12 µM; CPP = 4 µM). This antagonism is non-competitive. Ifenprodil also blocks the NMDA-induced liberation of ^{3}H-acetylcholine from rat striatal slices with an IC_{50} of 1.6 µM (cf. 2-APV = 27 µM; CPP = 8 µM). On the immature rat hemisected spinal cord preparation ifenprodil (10^{-9}-10^{-7}M) dose-relatedly but partially antagonises the depolarizing effects of NMDA with a maximal inhibition of 30% of 10^{-7}M. In binding experiments on rat cortical membranes, ifenprodil (10^{-7}-10^{-4}M) displaces the competitive NMDA antagonist 3((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid ([^{3}H]CPP) (Olverman et al, 1986) in a shallow manner. At 10 µM, ifenprodil reduces [^{3}H]CPP Bmax from 3.84 to 2.34 pmoles/mg protein, with no change in Kd. Ifenprodil (10^{-9}-10^{-3}M) dose relatedly but partially displaces (60% inhibition at 10^{-3}M) the non-competitive NMDA channel ligand [^{3}H]-TCP (Loo et al, 1984) (Hill number = 0.3). In contrast, ifenprodil is able to totally prevent the increase in [^{3}H]-TCP binding provoked by glutamate (10^{-5}M) in a non-competitive manner. In vivo, ifenprodil antagonises the release of striatal dopamine evoked by the local infusion of NMDA (10^{-3}M) in the rat (ID_{50} = 1.5 mg/kg ip) and blocks the harmaline-evoked increase in cerebellar cyclic GMP levels in mice (ED_{50} = 6 mg/kg ip).

Ifenprodil is thus a potent non-competitive NMDA antagonist. Its partial blockade of the depolarizing effects of NMDA (compared to total antagonism by competitive antagonists or channel blockers) and its interaction with both the [^{3}H]-CPP and [^{3}H]-TCP binding sites suggest a distinct and novel site of action within the NMDA receptor complex. The cerebral anti-ischaemic properties of ifenprodil may be related to its antagonism of the effects of NMDA.

Loo, P., Braunwalder, A., Lehmann, J. and Williams, M. (1984) J. Pharmacol. 123, 467-468.

MacKenzie, E.T., Gotti, B., Nowicki, J.P. and Young, A.R. (1984) in 'Neurotransmitters and the Cerebral Circulation': MacKenzie, E.T., Seylaz, J. and Bès, A. (eds), pp. 219-243, Raven Press, New York.

Olverman, H.J., Monaghan, P.J., Cotman, C.W. and Watkins, J.C. (1986) Eur. J. Pharmacol. 131, 161-162.

RECEPTOR AUTORADIOGRAPHY WITH L[^{3}H]GLUTAMATE REVEALS THE AXONAL TRANSPORT OF GLUTAMATE BINDING SITES IN RAT VAGAL AFFERENTS

D. Lodge*, M. Cincotta, S.J. Lewis, A.J.M. Verberne, B. Jarrott, R.J. Summers and P.M. Beart, University of Melbourne, Clinical Pharmacology, Austin Hospital, Heidelberg, VIC 3084 and †Department of Pharmacology, Parkville, VIC 3052, Australia.

The nodose ganglion (NG) and the associated vagus nerve (X) have proved a convenient model system to analyse receptor synthesis and subsequent axonal transport (Laduron, 1984). Recent evidence from our laboratory that perikarya of vagal afferents neurones within the NG are sensitive to excitotoxic amino acids (Verberne et al., 1987), when taken with evidence that L-glutamate may be a transmitter of baroreceptor afferents (Talman et al., 1984), prompted a study of glutamate receptor dynamics in the NG/X system.

In Sprague-Dawley rats (225-250g), anaesthetized with an amylobarbitone-methohexitone (30 & 16.7 mg/kg i.p. respectively) mixture, the left NG was exposed and X was tightly ligatured approximately 1 cm distal to the ganglion. In some animals, a double ligature (0.5 cm apart) was employed. Control rats received a loosely-fitting ligature at the same location or a sham operation. After a 24h recovery, rats were reanaesthetized and each NG/X was removed and frozen in mounting medium at -20^{0}C overnight. Longitudinal sections (10 um), including the NG and X, were thaw-mounted on to glass microscope slides for receptor autoradiography with L[^{3}H]glutamate (Cincotta et al., 1987). Tissue sections were incubated for 10 min in 50 mM Tris-citrate pH 7.0, containing 2.5 mM Ca^{2+} and 20 mM Cl^-, with 1 uM L[^{3}H]glutamate. Non-specific binding was defined with 0.1 mM L-glutamate. After rapid washing and drying, the sections were apposed to [^{3}H]sensitive film for 40 days.

In sham-ligated X, combined densitometry and histology demonstrated that the majority of L[^{3}H]glutamate binding was associated with the NG and was near background in X. Binding sites within NG appeared to be unevenly distributed with some punctate areas showing intense labelling. After ligation, binding remained in NG, but was also found in X proximal and distal to the ligature(s). Further studies employing direct scintillation spectrometry demonstrated that all subtypes of glutamate receptors were present in the NG/X system: N-methyl-D-aspartate (1 mM), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (1 uM) and kainate (1 mM) displaced 26%, 16% and 57% of specific L[^{3}H]glutamate binding respectively. Similarly, placement of a ligature on the central branch of X revealed accumulation of receptors both proximal and distal to this ligation.

These findings establish that glutamate receptors are normally present in the NG of the rat and are consistent with current concepts of perikaryal synthesis, axonal transport and recycling of neurotransmitter receptors (Laduron, 1984). The functional significance of the presence of glutamate binding sites on the perikarya, peripheral and central terminals of vagal afferent neurones remains to be established.

Cincotta, M. et al. (1987) Neurosci. Letts. Suppl. 27, 63.
Laduron, P.M. (1984) Biochem. Pharmacol. 33, 897-903.
Talman, W.T. et al. (1984) Fed. Proc. 43, 39-43.
Verberne, A.J.M. et al. (1987) Eur. J. Pharmacol. 139, 365-367.

*Permanent address: Department of Physiology, Royal Veterinary College, London NW1 OTU, England.

NORADRENALINE AND R(α)-METHYLHISTAMINE INHIBIT THE RELEASE OF [^{3}H]-HISTAMINE FROM RAT CEREBRAL CORTICAL SLICES

S.J. Hill* & C.S. Young. Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

There is now strong evidence that histamine may have a role as a neurotransmitter or neuromodulator in the mammalian CNS (Prell & Green, 1986). Immunohistochemical studies have shown that there are diffuse projections of ascending histaminergic neurones to rat cerebral cortex (Pollard & Schwartz, 1987). Presynaptic histamine H_3-receptors controlling transmitter release at histaminergic nerve terminals have been demonstrated recently in this brain region (Arrang et al., 1983, 1987). We now report that noradrenaline can also inhibit histamine release from rat cerebral cortical slices.

The release of [^{3}H]histamine evoked by depolarisation with KCl was measured essentially as described by Arrang et al. (1983), except that rat (Wistar, males, 250g) brain slices were incubated in Krebs medium containing 50mM KCl for 15 min, prior to preincubation with 0.4μM [^{3}H]histidine in normal Krebs medium (40 min), in order to stimulate histamine synthesis. [^{3}H]histidine-labelled slices (50μl) were then added to microfuge tubes containing 450μl Krebs medium at 37°C. Antagonists were added 22 min and agonists 7 min prior to stimulation with KCl (2 or 40mM) for a further 7 min. Incubations were terminated by rapid centrifugation and [^{3}H]histamine in the supernatants was isolated by ion-exchange chromatography on Amberlite GG50 resin as described by Arrang et al. (1983).

Depolarisation with KCl (40mM) produced a 4.1 ± 0.4 fold (n=16) increase in [^{3}H]histamine release which was reduced by 81 ± 2.9% in calcium-free medium containing 0.5mM EGTA (3 experiments). Incubation of slices with S(α)-fluoromethylhistidine (20μM, Kollonitsch et al., 1978), an inhibitor of histidine decarboxylase, prevented the synthesis and release of [^{3}H]histamine in rat cerebral cortical slices. The H_3-agonist R(α)-methylhistamine (1μM, Arrang et al., 1987) produced a 74 ± 1.3% inhibition (n=4) of the KCl-evoked release of [^{3}H]histamine which was completely inhibited by the H_3-antagonist thioperamide (1μM, n=3, Arrang et al., 1987). Noradrenaline also inhibited histamine release (IC_{50} = 2.1 ± 0.7μM, n=4) producing a maximal inhibition of 62 ± 2.7 % (n=13). The response to noradrenaline (20 or 40μM) was significantly inhibited ($p<0.05$) by phentolamine (2μM) and yohimbine (2μM) but not by propranolol (2μM) (n=3 experiments in each case).

The results of this study suggest that both histamine H_3-receptors and α-adrenoceptors are present on histaminergic nerve terminals in rat cerebral cortex and can inhibit the release of histamine.

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Arrang, J.-M. et al. (1983) Nature, 302, 832-837.
Arrang, J.-M. et al. (1987) Nature, 327, 117-123.
Kollonitsch, J. et al. (1978) Nature, 274, 906-908.
Pollard, H. & Schwartz, J.-C. (1987) Trends Neurosci., 10, 86-89.
Prell, G.D. & Green, J.P. (1986) Ann. Rev. Neurosci., 9, 209-254.

5-HT RECEPTOR ANTAGONISTS PREVENT THE INHIBITORY EFFECT OF SYSTEMIC AND LOCAL INJECTION OF BUSPIRONE IN THE RAT HIPPOCAMPUS

M.J. Rowan, J.J. O'Connor* & R. Anwyl[1], Department of Pharmacology and Therapeutics and [1]Department of Physiology, Trinity College, Dublin 2, Ireland.

Buspirone is a non-benzodiazepine anxiolytic agent with high affinity for 5-HT1A binding sites in the brain (Glaser & Traber, 1983; Peroutka, 1985). Recently it has been shown to mimic the inhibitory effects of 5-HT in the rat hippocampal slice (Rowan & Anwyl, 1986; Andrade & Nicoll, 1987). This led to the hypothesis that buspirone may act as an agonist or partial agonist at 5-HT1A receptors. The purpose of the present experiments was to look at the neurophysiological effects of buspirone in the hippocampus in vivo and investigate its receptor action by means of known 5-HT receptor antagonists.

Stimulating wire electrodes and a cannula to which recording wire electrodes were attached, were implanted in the dorsal hippocampus of male Wistar rats (250-350g) under pentobarbitone anaesthesia (60 mg/kg). Animals were allowed one week for recovery before recordings were taken in a restraining hammock. Stimulation and recording was carried out in the stratum radiatum of the CA1 region. Low frequency stimuli (0.05Hz) of 0.3-0.9mA were used to evoke small amplitude (2mV) excitatory postsynaptic potentials (e.p.s.ps). Buspirone and saline were applied both via the i.p. route and via the cannula directly into the hippocampus (i.h.). All antagonists were injected i.p., 30 minutes prior to buspirone administration.

Acute i.p. injection of buspirone (3 mg/kg) produced a marked reduction in the amplitude of the e.p.s.p. which was maximal at 60 min (Table 1). Local application of 1 µg buspirone into the hippocampus produced a similar inhibitory effect which reached a peak 5 min after injection. Saline (i.p. and i.h.) had no effect. Pretreatment with either the non-selective 5-HT receptor antagonist, methysergide, or spiroxatrine, a relatively selective 5-HT1A receptor antagonist (Alexander & Wood, 1987), blocked the inhibitory effect of both i.p. and i.h. buspirone. Ketanserin, a 5-HT2-receptor antagonist, had no effect.

Table 1 Effect of 5-HT receptor antagonists on the inhibition of the e.p.s.p. produced by i.p. or i.h. buspirone (e.p.s.p. amplitude expressed as mean ± s.e. mean % control for n rats)

		Buspirone i.p. (3 mg/kg)	Buspirone i.h. (1µg)
Saline	(1 ml/kg)	51 ± 7 (4)	54 ± 9 (4)
Methysergide	(5 mg/kg)	89 ± 13 (3)*	105 ± 14 (3)*
Ketanserin	(1 mg/kg)	63 ± 11 (4)	69 ± 13 (4)
Spiroxatrine	(1 mg/kg)	96 ± 9 (6)*	98 ± 12 (4)*

*$P < 0.05$ level, compared to animals pretreated with saline.

These findings provide further evidence that buspirone exerts inhibitory effects in the hippocampus which may be mediated through 5-HT1A receptors.

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Alexander, B.S. & Wood, M.D. (1987) Brit. J. Pharmacol. 91, Proc. Suppl. 449P.
Andrade, R. & Nicoll, R.A. (1987) Naunyn Schmiedeberg's Arch. Pharmacol. 336, 5-10.
Glaser, T. & Traber, J. (1983) Eur. J. Pharmacol. 88, 137-138.
Peroutka, S.J. (1985) Biol. Psychiat. 20, 971-979.
Rowan, M.J. & Anwyl, R. (1986) Eur. J. Pharmacol. 132, 93-96.

RESPONSES OF MOTONEURONES TO 5-HYDROXYTRYPTAMINE (5-HT) AND 5-HT RECEPTOR AGONISTS IN THE HEMISECTED SPINAL CORD OF THE NEONATE RAT

L.A. Connell* & D.I Wallis, Department of Physiology, University College Cardiff, CF1 1XL.

The lumbar spinal cord was removed from 3 - 8 day old rats, hemisected, and superfused with oxygenated modified Krebs. Recordings were made of changes in the potential measured between a ventral root and the surface of the cord (Connell & Wallis, 1987).

5-HT, 5-carboxamidotryptamine (5-CT) and (+) α methyl-5-HT (αMe-5HT) (10-4 to 10-6M) evoke concentration-related depolarization of motoneurones (Connell & Wallis, 1987). Under control conditions the potencies of 5-CT and αMe-5-HT do not differ significantly from each other or from 5-HT. However, in order to make a reliable comparison of potency, it was important to know whether uptake mechanisms and breakdown by Monoamine Oxidase (MAO) were having an effect upon the concentration of the indoleamines in the vicinity of the motoneurones.

Superfusion for 1h with the 5-HT uptake inhibitor citalopram (10-7M) potentiated the amplitude of submaximal responses to 5-HT and reduced the EC50, while maximal responses were unaffected (Table 1). Responses to 10-5M 5-HT were

	CONTROL	CITALOPRAM 10-7M	PARGYLINE 5x10-4M
5-HT EC50	20.5 ± 1.2μM (n=20)	3.6 ± 1.1μM (n=8)*	12.7 ± 4.1μM (n=7)
MAXIMUM	0.97 ± 0.5mV	1.39 ± 0.4mV	0.98 ± 0.11mV

Table 1. Effect of citalopram and pargyline on responses to 5-HT (* P<0.001).

increased from 29 ± 5% to 64 ± 5% (n=9, P<0.001) by citalopram. The EC50 for 5-CT was not significantly altered by citalopram (control 28 ± 4.6μM, n=7, citalopram 31.8 ± 10.8μM, n=8) but responses to 10-5M 5-CT were potentiated (25 ± 4% to 43 ± 5%, n=9, P<0.01). The EC50 for αMe-5-HT was also not significantly altered by citalopram (control 31.2 ± 5.6μM, n=8, citalopram 14.4 ± 5.2μM, n=3), but responses to 10-5M αMe-5-HT were potentiated (27 ± 5% to 52 ± 6%, n=4, P<0.05).

Superfusion for 30min with the MAO inhibitor pargyline (5x10-4M) had no significant effect on either submaximal or maximal responses to 5-HT, or on the EC50 (Table 1). Submaximal responses to 5-CT and αMe-5-HT were not potentiated by pargyline

It is concluded that uptake processes lower the effective concentration of 5-HT within the cord and lead to an overestimate of the EC50. αMe-5-HT and 5-CT may also interact with the uptake system. Destruction by MAO does not appear to alter the responses to these indoleamines significantly.

Connell L.A & Wallis D.I, 1987, J.Physiol. 390, 44P.

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5-HT DEPOLARIZATIONS OF RABBIT CERVICAL SYMPATHETIC AXONS: MEDIATION BY 5-HT_{3B} RECEPTORS

P.Elliott* & D.I.Wallis, Department of Physiology, University College, P.O.Box 78, Cardiff, CF1 1XL.

5-HT depolarizations of rabbit superior cervical ganglia may be mediated by a subclass of 5-HT_3 receptors (5-HT_{3A}) whereas those of sympathetic postganglionic nerve terminals, triggering noradrenaline release, may involve 5-HT_{3B} receptors (Richardson & Engel,1986). 5-HT_3 receptors can be differentiated by the antagonists MDL 72222 and SDZ 206-830 (Compound I) with pA_2 values of 7.7 and 13.1 on 5-HT_{3A} compared to 8.9-9.3 and 10.1 on 5-HT_{3B} receptors, respectively (Richardson et al.,1985). Here we describe a 5-HT receptor mediating depolarizations of preganglionic cervical sympathetic nerve axons.

Depolarizations of desheathed rabbit cervical sympathetic trunks were measured by extracellular recordings of the d.c. potential across a silicone grease-sealed partition. The preparations were continuously superfused with Krebs solution at room temperature (20-24°C). Cumulative dose-response (CDR) curves for 5-HT (1μM -1mM) had a mean maximum of 227 ± 39 μV (n=30, mean ± s.e.mean) with an ED_{50} of 24 ± 6.9 μM. Depolarizations by selective 5-HT receptor agonists were compared. The mean depolarizations for 100 μM of each was: 5-HT (157 ± 41 μV), 2-Me-5-HT (134 ± 36 μV), (+)alpha Me-5-HT (71 ± 23 μV), 5-CT (36 ± 9 μV) (n=4). The mean maxima of CDR curves for 5-HT and 2-Me-5-HT (1μM -1mM, n=4) were 519 ± 167 μV and 317 ± 63 μV, and the ED_{50}s were 60 ± 37 μM and 63 ± 36 μM, respectively. The high potency of 2-Me-5-HT suggests that the depolarizations are mediated via 5-HT_3 receptors (Richardson & Engel,1986).

Ketanserin (10^{-8}-10^{-6}M) and methiothepin (10^{-8}-10^{-6}M) had little or no action on 5-HT depolarizations. The 5-HT_3 antagonists MDL 72222 and ICS 205-930 were tested on repeated CDR curves to 5-HT and pA_2 values estimated by Schild plots (Arunlakshana & Schild,1959). Control experiments suggested that repeated CDR curves were shifted to the right; although no correction has been made for this, the overestimate of pA_2 values is likely to be less than 0.5. Schild plots for MDL 72222 (1 -100nM) gave an apparent pA_2 of 9.2 ± 0.2 with a slope of 0.94 ± 0.18 (n=12) and for ICS 205-930 (0.1 -3nM) the apparent pA_2 was 10.33 ± 0.29 with a slope of 1.08 ± 0.27 (n=11).

The pA_2 for MDL 72222 suggested that depolarizations are mediated by 5-HT_{3B} receptors. To test this hypothesis SDZ 206-830 at 0.001, 0.1 and 10nM was tested on depolarizations to 100 μM 5-HT. The reductions of 5-HT responses by 0.1 and 10 nM SDZ 206-830 were significant when compared to control experiments ($p < 0.005$). The relatively low potency of SDZ 206-830 also suggests the involvement of 5-HT_{3B} receptors in depolarization of rabbit cervical sympathetic axons.

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Arunlakshana,O. & Schild,H.O. (1959) Br.J.Pharmac.14:48-58
Richardson,B.P. & Engel,G. (1986) TINS 9(9):424-428
Richardson,B.P. et al (1985) Nature 316:126-131

5-HT_1 AGONISTS REDUCE 5-HT RELEASE IN RAT HIPPOCAMPUS IN VIVO AS DETERMINED BY BRAIN MICRODIALYSIS

T.Sharp*, S.Bramwell, L.Maskell and D.G.Grahame-Smith.
MRC Clinical Pharmacology Unit, Radcliffe Infirmary, Woodstock Road, Oxford, OX2 6HE.

It is generally believed that 5-HT_1 agonists with selectivity for either the 5-HT_{1A} or 5-HT_{1B} subtype reduce brain 5-HT neurotransmission via autoreceptor activation. Since putative 5-HT_{1A} agonists such as 8OHDPAT may act on somatodendritic and not nerve terminal 5-HT autoreceptors to produce this effect (Middlemiss, 1984), methods promising to directly measure brain 5-HT release in vivo should be valuable to study these drugs. Using brain microdialysis, Brazell et al (1985) reported decreased cortical extracellular 5-HT in response to the putative 5-HT_{1B} agonist RU 24969. We therefore attempted to determine whether this in vivo method is also sensitive to the actions of various putative 5-HT_{1A} agonists.

Male Sprague Dawley rats (260-300g) were anaesthetized with chloral hydrate and a dialysis loop was stereotaxically implanted into frontal cortex or ventral hippocampus. The perfusion medium was artificial CSF with and without 10^{-6} M citalopram, a 5-HT uptake blocker. Perfusates were collected every 20 min for analysis of 5-HT and 5-HIAA using HPLC-EC (Sharp et al, 1987).

Basal 5-HT levels in cortical perfusates continously declined over time and were not further reduced by 8OHDPAT (1 mg/kg). However, a stable baseline of reliably detectable amounts of 5-HT was obtained in hippocampal perfusates when citalopram was present in the perfusion medium. Under these conditions 8OHDPAT caused a marked dose-dependent (5, 10, 100, 250 µg/kg sc) decrease in 5-HT output (maximum effect -78.5 ± 4.4%, 40 min post-drug). The putative 5-HT_{1A} agonists gepirone (5 mg/kg sc) and ipsapirone (5 mg/kg sc) also reduced 5-HT, by 55.6 ± 2.8% (N=4) and 54.7 ± 2.4% (N=3) respectively. Additionally, RU 24969 produced a dose-dependent (0.25, 1, 2.5, 5 mg/kg sc) reduction in hippocampal output of 5-HT with a similar magnitude of effect to the 5-HT_{1A} agonists. In comparison to their effects on 5-HT, the agonists weakly decreased extracellular 5HIAA.

These data obtained using brain microdialysis suggest that systemically administered putative 5-HT_{1A} and 5-HT_{1B} agonists markedly inhibit 5-HT release in rat ventral hippocampus. The microdialysis method should be a useful tool for further studies on brain 5-HT autoreceptor function in vivo.

Brazell, M.P. et al (1985) Br. J. Pharmac. 86, 209-216
Middlemiss, D.N. (1984) Naunyn-Schmiedebergs' Arch. Pharmac. 327, 18-22.
Sharp, T. et al, (1987) Life Sciences 41, 869-872.

CHRONIC MAO A BUT NOT MAO B INHIBITION DECREASES 5-HT_{1A} MEDIATED INHIBITION BY FORSKOLIN-STIMULATED ADENYLATE CYCLASE

A.J. Sleight*, C.A. Marsden and M.G. Palfreyman[1], Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH and [1]Merrell-Dow Research Institute, Cincinnati, Ohio, USA.

5-Hydroxytryptamine (5-HT) inhibits forskolin-stimulated adenylate cyclase activity through activation of the 5-HT1A receptor sub-type (De Vivo & Maayani, 1986). The aim of this study was to determine whether chronic inhibition of MAO A or B decreases the ability of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OHDPAT), a selective 5-HT1A agonist, to inhibit forskolin-stimulated adenylate cyclase activity and whether this could be correlated to a down-regulation in the binding parameters of [^{3}H]8-OHDPAT to the 5-HT1A binding site.

Adenylate cyclase activity was measured as previously described (De Vivo & Maayani, 1986). In untreated rats the EC_{50} for the 8-OHDPAT induced inhibition of forskolin-stimulated adenylate cyclase was calculated to be 1.2×10^{-8}M using a submaximal concentration of forskolin (10^{-7}M). The putative 5-HT1A receptor antagonist, spiroxatrine (10^{-7}M) (Nelson & Taylor, 1986) attenuated the effect of 8-OHDPAT on forskolin-stimulated adenylate cyclase activity.

Groups of 12 rats were given daily injections of either saline, the selective MAO A inhibitor clorgyline (1 mg/kg p.o.), the selective MAO B inhibitor selegiline (1 mg/kg p.o.) or the non-selective inhibitors E- -fluoromethylene-m-tyrosine (MDL 72394; 0.25 mg/kg p.o.) or tranylcypromine (5 mg/kg p.o.) for 21 days. 72 hours after the end of drug administration, the animals were killed and the hippocampi, cortices and frontal cortices removed. MAO activity was determined in the cortex, and the effect of 10^{-9}M, 10^{-8}M and 10^{-7}M 8-OHDPAT on forskolin-stimulated adenylate cyclase activity in the hippocampus. Three Scatchard analyses of [^{3}H]8-OHDPAT binding to the 5-HT1A binding site were performed per treatment group using frontal cortices pooled from 2-3 rats.

The KD and Bmax values for [^{3}H]8-OHDPAT binding in saline treated animals were found to be 4.04 $\pm$ 0.4 nM and 14.55 $\pm$ 0.2 fmol/mg protein respectively. None of the treatments caused any significant change in the KD value while only MDL 72394 and clorgyline significantly decreased ($P<0.05$) the Bmax value (9.29 $\pm$ 0.8 and 10.47 $\pm$ 0.6 fmol/mg respectively). Chronic MAO A (clorgyline) or MAO A+B inhibition (MDL 72394 or tranylcypromine) reduced the ability of 8-OHDPAT to inhibit forskolin-stimulated adenylate cyclase activity (rank order of potency: MDL 72394 = tranylcypromine > clorgyline). Chronic MAO B inhibition (selegiline) had no effect on the 8-OHDPAT induced inhibition of forskolin-stimulated adenylate cyclase activity.

The results indicate that chronic inhibition of MAO A but not MAO B results in decreased 8-OHDPAT binding sites and desensitization of the 8-OHDPAT inhibition of forskolin-stimulated adenylate cyclase activity. Tranylcypromine, however, reduced the adenylate cyclase response without altering the number of binding sites but this drug is a reversible inhibitor of MAO A and B *in vivo*. Therefore, after chronic treatment and 72 hours withdrawal, the 5-HT_{1A} binding may have sufficient time to recover while changes in the receptor coupled adenylate cyclase take longer to be reversed (Vetulani et al, 1976).

A.J. Sleight is a SERC CASE student in conjunction with Merrell-Dow and C.A. Marsden is a Wellcome Trust Senior Lecturer.

De Vivo, M. & Maayani, S. (1986) J. Pharmacol. Exp. Ther. 238, 248-253.
Nelson, D.L. & Taylor, W.E. (1986) Eur. J. Pharmacol. 124, 207-208.
Vetulani, J., Stawarz, R.J. & Sulser, F. (1976) J. Neurochem. 27, 661-666.

THE EFFECTS OF PHORBOL ESTERS AND LITHIUM ON 5-HT RELEASE IN RAT HIPPOCAMPAL SLICES

S.M.P. Anderson, P.P. Godfrey and D.G. Grahame-Smith, MRC Unit of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford, OX2 6HE

The biochemical targets of lithium relating to its therapeutic actions on manic-depressive disorders are currently unknown. There is evidence that Li^+ affects central 5-HT function, perhaps by altering release of 5-HT from the presynaptic terminals (Katz et al, 1968; Grahame-Smith and Green, 1974; Treiser et al, 1981). Recently it has been shown that neurotransmitter release can be enhanced by activators of protein kinase C (PKC) such as phorbol esters (PMA and PDBU) and synthetic diacylglycerols (dioctanylglycerol, DC8) (Malenka et al, 1986). This suggests that the second messengers derived from inositol phospholipids may be involved in the release mechanism. Since lithium may also interfere with phosphoinositide turnover in brain (Godfrey et al, 1986) we have investigated the interactions between 5-HT release, Li^+ and PKC, in rat hippocampal slices.

Male Sprague-Dawley rats (150-250g) were used; some were pretreated twice daily for 3 days with LiCl or NaCl (3mMol/kg in saline, s.c.). Hippocampal tissue was prepared from paired LiCl and NaCl treated rats. The slices (250-250μm) were preloaded with [^{3}H]-5-HT (10nM; specific activity 28.3 mCi/mmol) in oxygenated Hepes-ringer buffer containing 10μM pargyline. The slices were then superfused in the above buffer containing 0.5μM citalopram at 0.5 ml/min. A 4 minute pulse of buffer containing 35mMK^+ was given after 28 min.; phorbol esters and DC8 were added 12 min prior to K^+; lithium (0.1-10mM) and the PKC inhibitor staurosporine were added at the start of the perfusion. Fractions were collected every 4 min and [^{3}H]-5HT measured by liquid scintillation counting.

Rats which received 3d LiCl showed no change in basal 5-HT release but K^+-evoked release was reduced by 40±5% compared with controls. When 0.1-10mM LiCl was present in the superfusion medium K^+-stimulated 5-HT release was reduced in a dose-dependent manner. Addition of PDBU (10nM-1μM) or PMA (100 nM) to the medium of control tissue produced increases in K^+-stimulated 5-HT release of 30±5% and 28±3% respectively. The inactive phorbol ester, 4β phorbol (1μM) had no effect. When phorbol esters were included in the superfusion medium of Li treated rats, K^+-evoked release of 5-HT was significantly higher, 40±7%, than in controls. DC8 (100μM) produced similar effects to phorbol esters, again 4-β phorbol had no effect. The increase in K^+-stimulated 5-HT release in both control or lithium-treated rats produced by the phorbol esters was blocked by staurosporine (1μM). Control tissue exposed to 1 mM LiCl for 74 min before addition of phorbol esters showed results similar to those after 3d lithium treatment.

We conclude that lithium inhibits 5-HT release from hippocampal tissue, that phorbol ester overcomes this block by an action on PKC and that these changes may be related to the effects of lithium on the phosphatidyl inositol cycle.

Grahame-Smith, D.G., and Green, A.R. (1974). Br. J. Pharmac. 52, 19-20.
Treiser, S.L. et al (1981). Science 213, 1529-1531
Katz, R., et al (1968). Science 162, 466-467
Malenka, R.C. et al (1986). Nature 321, 175-177
Godfrey, P.P. et al (1986). Br. J. Pharmac. 117P

IDENTIFICATION OF 5-HT_3 RECOGNITION SITES IN N1E-115 NEUROBLASTOMA CELLS WITH [^{3}H]ICS 205-930

D. Hoyer* & H.C. Neijt. 1) Preclinical Research, SANDOZ Ltd, CH-4002 Basel Switzerland. 2) Department of Veterinary Pharmacology, Pharmacy and Toxicology, University of Utrecht, P.O. Box 80.176, NL-3508 TD Utrecht, The Netherlands.

In contrast to 5-HT_1 and 5-HT_2 receptors, the 5-HT_3 receptor has not up to now been identified by radioligand binding. [^{3}H]ICS 205-930 (ICS) recognition sites were analysed in membranes prepared from murine neuroblastoma N1E-115 cells. [^{3}H]ICS bound rapidly and reversibly to high affinity sites: Bmax = 40 ± 5 fmoles/mg protein, pK_D = 9.20 ± 0.5 (n =11). Saturation and competition experiments (table 1) carried out with a large variety of drugs suggested the existence of a single class of [^{3}H]ICS recognition sites which displayed the pharmacological profile of a 5-HT_3 receptor.

Table 1: Affinities (pK_D -log M, n≥3) of drugs for [^{3}H]ICS sites.

Drug	pK_D	Drug	pK_D	Drug	pK_D
ICS 205-930	9.14	MDL 72222	8.21	mCPP	6.99
BRL 43694	8.89	GR 38032F	7.87	5-HT	6.69
quipazine	8.68	metitepine	7.41	2-methyl-5-HT	6.24
BRL 24924	8.50	mianserin	7.25	phenylbiguanide	6.01

Potent 5-HT_3 receptor antagonists showed nanomolar affinities for [^{3}H]ICS binding sites: ICS (Richardson et al, 1985), BRL 43694, BRL 24924 (Fake et al, 1987), quipazine, MDL 72222 (Fozard, 1984), GR 38032F (Brittain et al, 1987). The rank order of affinity of agonists was compatible with that of a 5-HT_3 receptor. Drugs acting at 5-HT_1, 5-HT_2, α- and ß-adrenoceptors, dopamine and histamine receptors (methysergide, ketanserin, propranolol, phentolamine, sulpiride, SCH 23390, cimetidine) were essentially inactive at 10 µmol/l. The binding of [^{3}H]ICS 205-930 was stereoselective and was not affected by guanine nucleotides. GppNHp (.1mM) did not affect the binding of agonists, suggesting that 5-HT_3 recognition sites are not coupled to G proteins. The interactions of agonists and antagonists with [^{3}H]ICS recognition sites were competitive: in saturation experiments performed with [^{3}H]ICS, the presence of 5-HT or MDL 72222 caused an apparent increase of K_D while Bmax was not decreased. This is compatible with surmountable antagonism. These data demonstrate that [^{3}H]ICS is a suitable ligand for the identification of 5-HT_3 recognition sites in membrane preparations. These findings are consistent with the electrophysiological data showing 5-HT_3 receptors to be present on N1E-115 cells (Neijt et al, 1986). This is the first successful identification of 5-HT_3 recognition sites using radioligand binding in a cell line.

Brittain R.T. et al, (1987) Br. J. Pharmacol. 90, 87P.
Fake C.S. et al (1987) Br. J. Pharmacol. 91, 335P.
Fozard J.R. (1984) Naunyn Schmied. Arch. Pharmacol. 326, 36-44.
Neijt H.C. et al, (1986), Eur. J. Pharmacol. 127, 271-274.
Richardson B.P. et al, (1985) Nature, 316, 126-131.

ISOLATION AND WASHING OF HUMAN PLATELETS WITH NITRIC OXIDE

*M.W. Radomski, R.M.J. Palmer, N.G. Read and S. Moncada, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.

The vascular endothelium releases prostacyclin and nitric oxide (NO) which are potent vasodilators (Moncada et al., 1976; Palmer et al., 1987). Nitric oxide and prostacyclin are also inhibitors of platelet aggregation (Radomski et al., 1987a). In addition, NO is an inhibitor of platelet adhesion (Radomski et al., 1987b). Prostacyclin protects platelets from damage during isolation, washing and storage for up to 96 h (Radomski & Moncada, 1983). Prostacyclin washed platelets (PWP) remain functional when stored for up to 96h after preparation. We have now investigated whether NO also has a protective effect on the morphological and functional integrity of human washed platelets.
Blood was collected from healthy volunteers who had not taken any medication in the previous two weeks. Platelets were isolated and washed with prostacyclin according to the method of Radomski and Moncada (1983) or with NO (NOWP) following the procedure shown in Table 1.

Table 1

Step	Objective	NO (μM)	Tyrode's solution	Centri-fugation	Time (min)	Temp (C°)
1	Collect blood	10	-	-	3	22
2	Obtain PRP	10	-	280	10	22
3	Sediment platelets	10	-	950	5	22
4	Sediment platelets	10	+	850	5	37
5	Final suspension	-	+	-	-	37

The recovery of NOWP from blood in the final suspension was 72 ± 4%, (mean ± s.e.m., n=8) and that of PWP was significantly higher (83 ± 5%, mean ± s.e.m., n=6, $p < 0.05$). The addition of NO (10 μM) throughout the procedure resulted in an increase in cGMP to a maximum of 7.9 ± 1.8 pmole/10^8 platelets, (mean ± s.e.m., n=3). This returned to basal levels within 5 min of resuspending the platelets in Tyrode's solution alone. No increases in cAMP were observed in NOWP.
The sensitivity of NOWP to collagen, ADP, U46619 and thrombin was maximal 5 min after preparation while that of PWP increased over a period of two hours. Thereafter, there was no significant difference ($p > 0.05$, n=8) in the aggregating responses of PWP and NOWP to these agents. The aggregating response of NOWP to collagen (2 $\mu g.ml^{-1}$) declined after 24 h storage at 4°C, whereas that of PWP remained stable for 48 h. Ultrastructural examination showed no difference between PWP and NOWP at 24 and 48h, although at 96h PWP were better preserved.
These results suggest that NO protects platelets from damage during isolation and washing, through an increase in cGMP.

Moncada, S. et al. (1983). Nature 263, 663-665.
Palmer, R.M.J. et al. (1987). Nature, 327, 524-526.
Radomski, M. & Moncada, S. (1983). Thromb. Res., 30-383-389.
Radomski, M.W. et al. (1987a). Br. J. Pharm., 92, 181-187.
Radomski, M.W. et al. (1987b). Lancet, in press.

DIFFERENCES IN THE INTERACTION OF EDRF AND NITRIC OXIDE WITH ION EXCHANGE RESINS

Clive J. Long*, Kazuhisa Shikano and Barry A. Berkowitz. Department of Pharmacology, Smith Kline and French Laboratories, Philadelphia, PA 19479, USA.
(Introduced by M. N. Perkins).

Evidence has been presented which associates the relaxant actions of the endothelium derived relaxant factor (EDRF) with nitric oxide solution (NO). Both substances are unstable in biological media and the half-life of both may be extended by superoxide dismutase, implying a similar mechanism of degradation. Of interest, Palmer et al., (1987) assayed the perfusate of stimulated cultured endothelial cells and, under the conditions used, have detected the presence of NO in quantities sufficient to induce relaxation of the bioassay tissue. Cocks et al. (1985) have shown that the relaxant actions of EDRF may be reduced by interposing an anion exchange resin between the perfused endothelial cells and the bioassay tissue; we have extended these findings to elucidate the actions of a number of resins on the activity of NO.

Superfused endothelium-removed rabbit aortic rings were used as the bioassay for EDRF- and NO- induced relaxations. EDRF relaxation was induced by A23187 (1nM-1μM) stimulation of $3x10^6$ perfused cultured endothelial cells immediately upstream of a precontracted bioassay tissue. Various resins (0-500mg) were included between the endothelial cells and the bioassay tissue. Both anion exchange (AG1 and 1',2'amino) and Hb-agarose reduced the relaxant response to EDRF but cation exchange (carboxyl) and reverse phase (C18) resins did not. NO solution was prepared by incubating distilled water with NO gas and was a potent relaxant of the bioassay, the relaxation induced was reduced only by Hb-agarose. C18, AG1, 1',2'amino and carboxyl resins were ineffective, in confirmation of the bioassay data.

It is clear that anion exchange resins interact with the EDRF to reduce the relaxant response to this agent; however they are ineffective at reducing the NO-induced relaxation and on this evidence the EDRF and NO appear to act as separate species. This laboratory (Shikano et al., 1987) has previously demonstrated that the EDRF and NO differ in terms of comparative pharmacology. In addition, Cocks et al. (1987) have recovered relaxant activity from the lyophilisate of an EDRF-containing solution, indicating that the EDRF is unlikely to be gaseous.

We conclude that whilst EDRF might possess a nitrogen oxide group (or a similar entity) as a component of the structure, there is emerging chemical and biological evidence that the EDRF is not identical to NO.

Cocks, T. M. et al. (1985) J. Cell. Physiol 123, 310-320.
Cocks, T. M. et al. (1987) IUPHAR abstracts S72.
Palmer, R. M. J. et al. (1987) Nature 327, 524-526.
Shikano, K. et al. (1987) Br. J. Pharmacol. In Press.

100P

ENDOTHELIAL DERIVED RELAXING FACTOR (EDRF) INHIBITS PLATELET ADHESION TO BOVINE ENDOTHELIAL CELLS

J.M. Sneddon,* T. Bearpark & J.R. Vane.
The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ.

As well as relaxing vascular smooth muscle, EDRF inhibits platelet aggregation (Azuma et al 1985, Radomski et al 1987). Thus, EDRF production may serve as a local defence mechanism in response to a thrombogenic stimuli. Because EDRF is unstable, and is inactivated by heptoglobulin-haemoglobin complexes in plasma (Edwards et al 1986) it is unlikely to influence platelet function in the bloodstream. However, EDRF may influence platelet reactivity at the endothelial cell (EC) surface.

We show here that EDRF release reduces the adhesion of thrombin-stimulated platelets either to bovine aorta EC or to cultured bovine aortic endothelial cells (BAEC). Segments of bovine aorta clamped between two plates, the upper drilled with holes to form incubation chambers in which the EC surface formed the base or confluent monolayers of BAEC, were incubated for 10 min with Krebs'buffer containing 10 μM indomethacin. The buffer was replaced with a suspension of 500 μl of washed indomethacin-treated human platelets pre-labelled with [3H] adenine, and incubated with 0.3 u/ml thrombin for 20 min. The platelet suspension was aspirated, the endothelial surface washed with 3 x 1 ml buffer and the residual radio-activity determined. Additions of other substances to thrombin-stimulated platelets were made 5 min after thrombin. Without thrombin, 2.8 $\pm$ 0.7% (n = 9) of the applied radioactivity remained on the BAEC. With thrombin, adhesion increased to 20 $\pm$ 2% (n = 6) and similarly for EC. Thrombin-induced adhesion was significantly reduced by superoxide dismutase (SOD), which stabilizes and prolongs the activity of EDRF. The action of SOD was potentiated by bradykinin which releases EDRF from BAEC, and by the selective cGMP phosphodiesterase inhibitor M & B 22,948. Platelet adhesion was substantially increased by haemoglobin which inactivates EDRF. The results are summarized in table 1.

Table 1

	% of thrombin-induced adhesion	
	Intact Aorta	Cultured BAEC
Control (Thrombin 0.3u/ml)	100 (8)	100 (9)
SOD (66u/ml)	72 $\pm$ 8 (8)	47 $\pm$ 7 (6)
SOD + Bradykinin (400ng/ml)	39 $\pm$ 8 (8)	53 $\pm$ 7 (6)
Haemoglobin (10 μM)	121 $\pm$ 12 (9)	192 $\pm$ 42 (6)
Haemoglobin + SOD	122 $\pm$ 11 (6)	181 $\pm$ 58 (3)
M & B 22,948 1 μM	31 $\pm$ 6 (9)	44 (2)
M & B + SOD	19 $\pm$ 4 (8)	31 (2)

SOD, bradykinin or haemoglobin did not influence the binding of thrombin-stimulated platelets to empty culture dishes or de-endothelialized aorta.

We conclude that BAEC release superoxide anions and EDRF and that EDRF release is potentiated by bradykinin and preserved by SOD. The actions of M & B 22,948 are in line with the known stimulation of guanyl cyclase by EDRF.

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Azuma,H.,Kishikawa,M.,Sekizaki,S.(1985) Br.J.Pharmac.,88,411
Cocks,T.M.,Angus,J.A.,Campbell,J.H.,Campbell,G.R.(1985)J.Cell.Physol.,123,310
Edwards,D.H.,Griffith,T.M.,Ryley,H.C.,Henderson,A.H.(1986)Cardiovasc.Res.,20, 549
Radomski,M.W.,Palmer,R.M.J. & Moncada,S.(1987) Br.J.Pharmac.,92,181

DIFFERENTIAL BIOASSAY OF ENDOTHELIUM-DERIVED RELAXING FACTOR AND NITRIC OXIDE ON RABBIT AORTA AND GUINEA-PIG TRACHEA

G.J. Dusting*, M.A. Read & A.G. Stewart, Department of Physiology, University of Melbourne, Parkville, Victoria 3052, Australia.

Endothelium-derived relaxing factor (EDRF) is a potent local vasodilator mediator that inhibits platelet aggregation and synergises with prostacyclin in this respect (Moncada et al, 1987; Dusting et al, 1987). EDRF, like the organic nitrate vasodilators, elevates cyclic GMP in vascular smooth muscle. Furchgott (1987) suggested that EDRF was identical to nitric oxide, and Palmer et al. (1987) showed by chemiluminescence that nitric oxide released from cultured endothelial cells accounted for the EDRF activity detected on strips of rabbit aorta. Using a superfusion cascade to take advantage of the principles of parallel bioassay (Vane, 1969), we now show that EDRF can be distinguished from nitric oxide on guinea-pig isolated trachea.

Bovine aortic endothelial cells, cultured on microcarrier beads, were loaded into columns (0.5-1 cm) and perfused with Krebs solution containing indomethacin (3 μM). The effluent of the columns superfused a cascade of three bioassay tissues, consisting of three de-endothelialized, rabbit aortic strips (Read & Dusting, 1987; Palmer et al, 1987) or two de-endothelialized rabbit aortic strips with a strip of guinea-pig trachea (epithelium removed) mounted between them. Aortic strips were contracted submaximally with phenylephrine (1 μM), and tracheal strips were precontracted by the addition of histamine (1 μM) to the cell effluent. Infusion of bradykinin (1-100 nM) produced no direct effect on the bioassay tissues, but when infused through the cell column it released EDRF. This caused relaxations of the rabbit aortae that diminished on successive tissues, when compared to those produced by glyceryl trinitrate as a stable standard (Read & Dusting, 1987). Haemoglobin (1 μM), infused directly over the bioassay tissues, abolished bradykinin-induced relaxations, but did not affect glyceryl trinitrate responses. Methylene blue (20 μM, over the tissues) also inhibited or abolished bradykinin-induced relaxations, and reduced those of glyceryl trinitrate. These effects are consistent with the binding or inactivation of EDRF, and are not due to direct effects on guanylate cyclase.

On cascades which included guinea-pig trachea, nitric oxide solution (0.1-10 μl) caused dose-dependent relaxations of all tissues, which were greatest on the uppermost aorta. In contrast, infusions of bradykinin (30-100 nM, through the cells) produced relaxations matching those of nitric oxide (10 μl) on the uppermost aorta, but caused no effect on the tracheal strip, and had weaker effects than nitric oxide on the lower aorta. Bradykinin (30 nM), infused directly over the tissues simultaneously with nitric oxide (10 μl), did not alter the effect of the latter on any tissue. Thus EDRF appears to be inactivated or bound by the tracheal tissue, to a greater extent than nitric oxide. Clearly, EDRF does not have identical biological activity to nitric oxide, although it could contain an active NO moeity attached to a carrier molecule.

Supported by the National Heart Foundation of Australia.

Dusting, G.J., Macdonald, P.S., Read, M.A. & Stewart, A.G. (1987) In: Sixth Int. Symp.Vasc.Neuroeffector Mechanisms. Ed. J.A. Bevan, H. Majewski, R.A. Maxwell & D.F. Story, ICSU Press (In press).

Furchgott, R.F. (1987) In: Mechanisms of Vasodilatation IV. Ed. P.M. Vanhoutte, Raven Press; New York. (In press).

Moncada, S., Palmer, R.M.J. & Higgs, E.A. (1987) In: Thrombosis and Haemostasis 1987. Ed. J. Vermylen, H.R. Lijnen & J. Arnout, Leuven Univ. Press.

Palmer, R.M.J., Ferrige, A.G. & Moncada, S. (1987) Nature 327, 524-526.

Read, M.A. & Dusting, G.J. (1987) Clin. exp.Pharmac.Physiol. (In press).

Vane, J.R. (1969). Br.J.Pharmac. 35, 209-242.

RELEASE OF ENDOTHELIUM DERIVED RELAXING FACTOR (EDRF) AND PLATELET INHIBITORY ACTIVITY BY THE PERFUSED RABBIT AORTA

H. Bult*, H. Fret, A.G. Herman & R. Van den Bossche, University of Antwerp (UIA), Division of Pharmacology, B2610 Wilrijk.

Radomski et al. (1987) reported that cultured endothelial cells release a platelet inhibitory activity (PIA) with characteristics of EDRF. Release of EDRF, but not of PIA was detectable when endothelium of the freshly isolated rabbit thoracic aorta (RbA) was stimulated with acetylcholine (Ach) in the presence of indomethacin (INDO, Bult et al., 1987). This discrepancy was investigated further.

The perfusion of the RbA and the continuous bioassay of EDRF were performed as described (Bult et al., 1987). EDRF was expressed as percentage relaxation of the abdominal aortic segment. Aliquots of perfusate (200 µl) were collected on 100 µl of a suspension of washed rabbit platelets (PS), 60 s before and after Ach injection into the lumen of the RbA. The platelets were immediately stimulated with 0.3 µM 9,11-dideoxy-9α,11α-methano epoxy-prostaglandin F_{2alpha} (U46619), and aggregation was measured 1 min later. It was expressed as percentage of the control aggregation with 200 µl effluent collected before Ach injection. Prostacyclin (PGI_2) was assessed by radioimmunoassay of 6-oxo-$PGF_{1\alpha}$ (ir-6oxo).

Ach (0.003 to 30 nmol) caused a dose-dependent release of EDRF, ir-6oxo and PIA. Release of the latter two was abolished by indomethacin (INDO, Table 1). In order to raise the EDRF content in the effluent, the RbA was laterally compressed to an external width of 1.5 mm. It led to a major shift of the dose-relaxation curve and a small increase of the maximum relaxation (table 1), indicating that more EDRF was present in the effluent. In spite of this, the effluent failed to suppress platelet aggregation when PGI_2 formation was blocked. However, pretreatment of the PS with a subthreshold concentration of PGI_2 revealed the presence of a labile PIA (table 1). When the time course of the release of PIA by the compressed RbA was studied in greater detail, a PIA was present 45 s after Ach injection. At that time EDRF also reached a peak concentration in the effluent.

Table 1. EDRF, PIA and ir-6oxo in effluent obtained 60 s after injection of 30 nmol Ach in six rabbit thoracic aortae before and after compression.

Aorta	INDO µM	PGI_2 in PS	EDRF % relaxation	platelet inhibition (%)	ir-6oxo pg/ml
Normal	0	-	55 ± 3	89 ± 3*	6400 ± 1900
Normal	3	-	54 ± 6	3 ± 9	47 ± 15
Compressed	3	-	66 ± 4	-5 ± 12	30 ± 8
Compressed	3	+	60 ± 8	64 ± 14*	51 ± 4

Results given as mean ± s.e. mean; * $P < 0.05$, paired Student's t-test.

In conclusion, these findings are all consistent with the idea that EDRF may suppress platelet activation (Radomski et al., 1987). There appears to be no fundamental difference with respect to the anti-platelet activity of the EDRF released by fresh or cultured endothelial cells. However, the amounts of EDRF generated by the RbA are apparently often below the threshold for platelet inhibition.

Bult, H., Jordaens, F., Herman, A.G., Van Hove, C. & Verbeuren, T.J. (1987) Thromb. Haemostas. 58, 272 (Abstract).

Radomski, M.W., Palmer, R.M.J. & Moncada, S. (1987) Br. J. Pharmac. 92, 181-187.

ENDOTHELIUM DERIVED RELAXING FACTOR INHIBITS PLATELET AGGREGATION IN VIVO

J.C. Hogan, M.J. Lewis, A.H. Henderson, Departments of Pharmacology & Therapeutics and Cardiology, University of Wales College of Medicine, Heath Park, Cardiff.

Endothelium derived relaxing factor (EDRF) inhibits platelet aggregation in vitro (Furlong et al., 1987) being similar in this respect to sodium nitroprusside (NaNP) (Mellion et al., 1981). To date the demonstration of in vivo EDRF effects has been hampered by the lack of an assay for its in vivo action and the toxicity of agents known to either release EDRF e.g. calcium ionophore or increase its effects e.g. superoxide dismutase. However, release of EDRF by carbachol, a muscarinic agonist in use clinically, has now been shown to inhibit platelet aggregation in vitro (Bhardwaj and Moore, 1987). In this study we investigate the effects of carbachol-induced EDRF release and NaNP on platelet aggregability and platelet cGMP levels in vivo in the rabbit.

The ear artery and vein of New Zealand White rabbits were cannulated under lignocaine local anaesthesia. Drugs were administered by vein (iv) and blood sampled from the artery (1.8ml) into a syringe containing 0.2ml 3.2% trisodium citrate as anticoagulant and the cGMP phosphodiesterase inhibitor M&B22948 (dissolved in 1% triethanolamine) to give a final blood concentration of 10^{-4}M. Carbachol (50mcg) was given iv to stimulate EDRF release. When required the EDRF inhibitors methylene blue (M.Blue) or haemoglobin (Hb) were given iv as 2.5cc of a 1% soln. over 10 minutes, and 0.3ml of 10^{-3}M soln. over 30 secs respectively. NaNP was given as an infusion of 20 µg/min over 30 minutes. Blood was sampled before, and ca.5 minutes following administration of carbachol or M.Blue and at the end of the infusion of NaNP. ADP induced platelet aggregation was measured immediately in whole blood by impedance aggregometry and platelet cGMP levels using a commercially available radioimmunoassay kit. Results are given in the tables below and expressed as the mean±SD of increase in impedance (Ω) two minutes after addition of ADP. cGMP levels are expressed as pmol/10^9 platelets.

Aggregometry

	Carbachol	Carbachol + M.Blue	Carbachol + Hb	NaNP
Pre treatment	39.7 ± 7.3	46.0 ± 9.0	28.4 ± 4.1	30.4 ± 5.0
Post treatment	21.1 ± 5.3	50.7 ± 12.4	31.6 ± 7.1	18.5 ± 8.4
n.	8	6	7	6
p.	< 0.01	n.s.	n.s.	< 0.001

Platelet cGMP

	Carbachol	Carbachol + M.Blue	Carbachol + Hb	NaNP
Pre treatment	4.13 ± 1.44	4.81 ± 2.56	4.79 ± 2.32	4.35 ± 2.28
Post treatment	13.04 ± 6.11	5.97 ± 2.13	6.15 ± 1.79	7.84 ± 1.46
n.	6	6	6	6
p.	< 0.01	n.s.	n.s.	< 0.025

ADP-induced platelet aggregation is reduced by carbachol in vivo and this action is inhibited by M.Blue and Hb. Furthermore this action is similar to that of NaNP, both resulting in elevated platelet cGMP levels. We suggest that this action of carbachol is mediated by EDRF and that this demonstrates EDRF activity in vivo.

Bhardwaj R, and Moore PK (1987) Br. J. Pharmac. 91, 402P.
Furlong B et al. (1987) Br. J. Pharmac. 90, 687-692.
Mellion et al. (1981) Blood, 54, 946-955.

RELEASE OF AND RELAXATION INDUCED BY ENDOTHELIUM-DERIVED RELAXING FACTOR IN ATHEROSCLEROTIC RABBIT AORTA

T.J. Verbeuren*, F.H. Jordaens, A-E. Van Hoydonck & A.G. Herman, Dept. of Medicine, Univ. of Antwerp (UIA), Universiteitsplein 1, B-2610 Wilrijk, Belgium.

Several studies have illustrated that experimental atherosclerosis inhibits the endothelium-dependent relaxations in isolated animal arteries (see Verbeuren and Herman, 1987). Recent data shows that also in the human coronary artery, atherosclerosis reduces the relaxations to acetylcholine and substance P (Bossaller et al., 1987; Berkenboom et al., 1987). Theoretically, several steps involved in the endothelium-mediated relaxations may be affected by the atherosclerotic process; previous studies illustrated that in the atherosclerotic arteries (1) the endothelium is still present (Verbeuren et al., 1986), (2) the release of the endothelium-derived relaxing factor (EDRF) is only decreased in severely affected arteries (Verbeuren et al., 1986; Sreeharan et al., 1986; Verbeuren and Herman, 1987) and (3) also relaxation of the smooth muscle cells is only decreased in severely affected arteries (Verbeuren et al., 1986).

The present study was designed to investigate the release and the vascular effects of the EDRF in atherosclerotic rabbit thoracic aortas; in view of the recent conclusion that EDRF and nitric oxide (NO) may be the same (Palmer et al., 1987), the effect of NO was also investigated. Rabbits were fed either a control or a 0.3% cholesterol-containing diet during 16 weeks. Isometric tension was recorded in segments of control and atherosclerotic aortas and the release of EDRF was analyzed on detector aortas using a bio-assay technique (Verbeuren et al., 1986). In the atherosclerotic aortas, the endothelium-dependent relaxations to acetylcholine, substance P and Ionophore A23187 were nearly absent. Despite this absence of endothelium-mediated relaxation, acetylcholine still induced release of EDRF from the atherosclerotic tissues, albeit less than from the control aortas (Table 1). If the EDRF released from control or atherosclerotic aortas was analyzed on atherosclerotic detector aortas than less relaxation was obtained than in control detector tissues. In thoracic aortas of control rabbits, NO evoked concentration-dependent, rapidly reversible relaxations; such relaxations were markedly reduced in the atherosclerotic aortas.

Table 1 : Release of EDRF from rabbit aortas expressed as % relaxation noted in the control detector bio-assay tissue.

Acetylcholine (x 10^{-8} g)	Control	Atherosclerosis
0.08	9.5 ± 3.5	0*
1.25	29.2 ± 9.2	4.4 ± 2.7*
20	63.8 ± 8.2	28.4 ± 7.9*
320	97.2 ± 6.9	56.0 ± 9.3*

$p<0.05$

We conclude that in the atherosclerotic rabbit thoracic aorta (1) less EDRF is produced by and/or released from the endothelial cells; (2) the EDRF which is liberated evokes less relaxation and (3) NO (EDRF?) also evokes less relaxation.

Berkenboom, G., Depierreux M., & Fontaine J. (1987) Br.J.Pharmacol. 92, 113-120.
Bossaller, C. et al. (1987) J. Clin. Invest. 79, 170-174.
Palmer, R.M.J., Rerrige, A.G. & Moncada, S. (1987) Nature 327, 524-526.
Sreeharan, N. et al. (1986) Can. J. Physiol. Pharmacol. 64, 1451-1453.
Verbeuren, T.J. & Herman, A.G. (1987) in 'Mechanisms of Vasodilatation': Vanhoutte P.M. (ed.), In Press.
Verbeuren, T.J. et al. (1986) Circulation Res. 58, 552-564.

EFFECT OF CAPTOPRIL ON THE RELEASE OF PROSTACYCLIN INDUCED BY BRADYKININ (BK) FROM GUINEA-PIG LUNGS OR ENDOTHELIAL CELLS

G. de Nucci, T. Warner* & J.R. Vane.
William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ.

Captopril inhibited the release of prostacyclin (measured as 6-oxo-$PGF_{1\alpha}$) induced by bradykinin (BK) or angiotensin I from EC in culture, suggesting that activation of angiotensin converting enzyme (ACE) is essential for the release of prostacyclin induced by those peptides (Sawada et al., 1986). We have tested the effect of captopril on the release of prostacyclin from guinea-pig isolated lungs and also on the release of endothelium derived relaxing factor (EDRF) and prostacyclin from bovine aortic endothelial cells (BAEC).

Male Dunkin-Hartley guinea-pigs (300-350 g) were anaesthetised with pentobarbitone (60mg/kg) i.p. After mid-thoracotomy the pulmonary artery and trachea were cannulated and the lungs were removed and perfused through the pulmonary artery with warmed (37°C) and gassed (95%O_2/5%CO_2) Krebs'solution at a constant rate of 5ml/min (Piper & Vane, 1969). Activity of ACE was measured by bioassay using a cat jejunum for BK metabolism (Ferreira & Vane, 1967) and a rat colon for angiotensin conversion (Regoli & Vane, 1964). Prostacyclin was measured as 6-oxo-$PGF_{1\alpha}$ by radioimmunoassay (Salmon, 1978). BAEC were cultured on microcarrier beads and prepared in a column with a bioassay cascade of four rabbit aortas to measure EDRF release (Gryglewski et al., 1986).

Captopril (10 μM, infused for 20 min) inhibited both the metabolism of BK and the conversion of angiotensin I in guinea-pig isolated lungs (n=4). It enhanced the release of 6-oxo-$PGF_{1\alpha}$ induced by an infusion of BK (0.2 μM) from 5.3 $\pm$ 0.9 ng/ml to 11.8 $\pm$ 1.7 ng/ml, ($p<0.01$, n=6). Captopril did not alter the release of 6-oxo-$PGF_{1\alpha}$ from guinea-pig isolated lungs induced by arachidonic acid (13 μM) (control : 13.9 $\pm$ 1.4 ng/ml captopril-treated: 18.8 $\pm$ 4.9 ng/ml, $p>0.1$, n=6). Neither angiotensin I nor angiotensin II (5 μM, n=5) induced release of 6-oxo-$PGF_{1\alpha}$ from guinea-pig isolated lungs.

Captopril did not affect the release by BK or ADP of 6-oxo-$PGF_{1\alpha}$ or of EDRF from BAEC. These results demonstrate that ACE activity is not required for the release of 6-oxo-$PGF_{1\alpha}$ or EDRF induced by BK. They also support our previous results on isolated lungs of guinea-pigs exposed to O_2, whereby a decrease of ACE activity was accompanied by an increase in 6-oxo-$PGF_{1\alpha}$ output induced by BK (de Nucci et al., 1986).

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de Nucci, G. et al (1986). European J. Pharmacol. 126, 11-20.
Ferreira, S.H. & Vane, J.R. (1967). Br. J. Pharmac. Chemother. 30, 417-424.
Gryglewski, R.J. et al (1986). Brit. J. Pharmac. 87, 685-694.
Piper, P.J. & Vane, J.R. (1969). Nature 223, 29-35.
Regoli, D. & Vane, J.R. (1964). Br. J. Pharmacol. Chemother. 23, 351-359.
Salmon, J.A. (1978). Prostaglandins 15, 383-397.
Sawada, S. et al (1986). Japanese Circ. J. 50, 242-247.

PHOSPHOLIPASE C RELEASES ENDOTHELIUM DERIVED RELAXING FACTOR (EDRF) FROM BOVINE AORTIC ENDOTHELIAL CELLS (BAEC)

R. Gryglewski*, G. de Nucci, T. Warner, T. Bearpack & J. R. Vane.
William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ.

Khan & Furchgott (1987) and Ignarro (1987) proposed that EDRF was nitric oxide (NO) and Palmer et al. (1987) showed that porcine endothelial cells stimulated by bradykinin released NO, which accounted for the EDRF activity. However, the mechanism by which EDRF is released is yet to be understood. In this abstract we report that activation of phospholipase C (PLC) is involved in the release of EDRF from BAEC.

BAEC were cultured on microcarriers. The cell column and the bioassay cascade were assembled as described by Gryglewski et al. (1986). Briefly, a column containing 1.5-3 x 10^7 BAEC on microcarrier beads was perfused (5ml/min) with Krebs' buffer gassed with 95%O_2/5%CO_2 at 37°C and containing indomethacin (5.6 μM) and superoxide dismutase (10U/ml). The column effluent superfused a cascade (Vane, 1964) of four de-endothelialized rabbit aortas (RbA). The delay between the BAEC in the column and the consecutive RbAs was 1, 4, 7 and 10 s, respectively. The bioassay tissues were contracted by either U46619 (30-60 nM) or noradrenaline (100-500 nM).

EDRF was released from the EC by several stimuli including bradykinin (10-100 pmoles), ADP (0.3-10 nmoles), arachidonic acid (30-150 nmoles), vasopressin (30-300 pmoles) and acetylcholine (3-30 nmoles). However, phospholipase A_2 (1-10U) from venom of viper Naja naja (n= 7) or Crotalus adamanteus (n=3) or bee venom (n=5) did not induce release of EDRF. Phospholipase C (0.5-2.0 U) from Clostridium perfringens (n=4) or Bacillus cereus (n=11) strongly released EDRF from BAEC.

Phorbol myristate acetate (PMA, 30-100 nM) inhibited the release of EDRF induced by bradykinin or ADP (n=3) as did gentamycin (10mM, n=3), a direct inhibitor of phospholipase C. Inhibitors of phospholipase A_2 such as dexamethasone (2 μg/ml, n=3) had no effect on the release of EDRF induced by the same stimuli.

These results suggest that activation of phospholipase C but not of phospholipase A_2 triggers the release of EDRF.

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Gryglewski,R.J.et al. (1986) Br. J. Pharmacol. 87, 685-694.
Ignarro,L.J.et al.(1987) Fed. Proc. 46, 644.
Khan,M.T. and Furchgott,R.F. (1987) Fed. Proc. 46, 385.
Palmer et al. (1987) Nature, 327, 524-526.
Vane,J.R. (1964) Br. J. Pharmacol. Chemother. 23, 360-373.

PHORBOL ESTER TREATMENT UNMASKS A PROSTAGLANDIN RECEPTOR INHIBITING CYCLIC AMP FORMATION IN T CELLS

B.B.Fredholm*, I. van der Ploeg and C. Nordstedt, Department of Pharmacology, Karolinska Institutet, Box 60 400, S-104 01 Stockholm, Sweden.

Cells from the human T-leukemia line Jurkat have several types of receptors, including CD2 and CD3 receptors, that are linked, via pertussis toxin (PTX)-sensitive G-proteins, to the formation of inositolphosphates, increase in intracellular Ca^{2+} and to activation of protein kinase C (PkC; Ng et al. 1987). In these cells both E-type prostaglandins (PG's) and adenosine increase cyclic AMP formation. We recently reported that activation of CD2 receptors or the addition of tumor promoting phorbol esters, both of which activate PkC, markedly potentiated the adenosine mediated cyclic AMP accumulation (Fredholm et al. 1987). By contrast, PG-mediated cyclic AMP accumulation was reduced by phorbol esters (Nordstedt et al. 1987). In the present study we have examined if the latter effect is due to phorbol ester induced inhibition of the PG-mediated cyclic AMP stimulation or to the stimulation of PG-mediated inhibition.

Jurkat cells were recultivated at a density of 0.5 10^{-6}cells /ml in RPMI 1640 supplemented with fetal calf serum 1 day before the experiment. The cells were incubated for 10 - 60 min with PG's and/or phorbol 12,13 dibutyrate (PDiBu) or the non-tumor promoting 4-α-phorbol-12,13-didecanoate (PDD) in the presence of 30 μM rolipram to block phosphodiesterase. PTX (List) was added at 0.1 -0.2 μg/ml for 3 - 5 hours.

PDiBu, but not PDD, potentiated NECA-induced cyclic AMP accumulation, and inhibited PGE_2-induced cyclic AMP accumulation (EC50 appr. 3 nM in both cases). Treatment of Jurkat cells with PTX blocked subsequent [^{32}P]-ADP ribosylation by PTX indicating that the treatment of the cell had been effective. By contrast PDiBu treatment did not affect [^{32}P]- ADP-ribosylation. PTX-treatment increased NECA (10 μM)-induced cyclic AMP accumulation by 43 %, and PGE_2 (1 μM)-induced cyclic AMP accumulation by 68 %. PDiBu (100 nM) increased NECA-induced cyclic AMP by 190 % in control cells and by 239 % in PTX treated cells (n.s). By contrast, PDiBu (100 nM) reduced PGE_2-induced cyclic AMP accumulation by 61 % in control cells while it was increased by 10 % in PTX treated cells.

The present results have confirmed that activation of PkC in a human T-cell line can simultaneously enhance cyclic AMP accumulation due to adenosine analogues and decrease that induced by PGE_2. The reason for the inhibition of the PG response appears to be that PkC activation unmasks a PG-receptor that is linked to inhibition of adenylate cyclase via a pertussis-toxin sensitive mechanism. The results also suggest that activation of receptors that are linked to the activation of PkC can in a receptor-selective manner alter the effect of agonists that regulate cellular cyclic AMP content.

Fredholm, B.B., Jondal, M & Nordstedt, C. (1987) Biochem. Biophys. Res. Commun. 145, 344 - 349.
Ng,J., Fredholm,B.B., Jondal, M. & Andersson, T. (1987) Int. J. Immunopharm.9, 17-22.
Nordstedt,C., Jondal, M. & Fredholm, B.B. (1987) FEBS lett. 220, 57 - 60.

METABOLISM OF EXOGENOUS LEUKOTRIENE C_4 IN BLOOD OF NORMAL AND ASTHMATIC SUBJECTS

Zakrzewski JT*, Sampson AP, Evans JM, Barnes NC, Piper PJ and Costello JF. Dept. of Thoracic Medicine, King's College School of Medicine & Dentistry, London and Dept. Pharmacology, Royal College of Surgeons, London.

It has been suggested that cysteinyl-containing leukotrienes (LTs), which account for the biological activity of slow-reacting substance of anaphylaxis (SRS-A), are important mediators in asthma. The detection of immunoreactive LTC_4 in blood of asthmatics during antigen challenge (Cromwell et al, 1984) or following a severe attack (Isono et al, 1985) is in accordance with this hypothesis. Interestingly, peripheral polymorphonuclear leukocytes (PMNs) obtained from patients with bronchial asthma have an increased capacity for SRS generation (Wang et al, 1986). Since the circulation may contribute to the overall regulation of LT bioavailability we have compared the in vitro metabolism of leukotriene C_4 in blood of six non-atopic, normal (N) and six asthmatic (A) subjects. The latter group was only studied if their lung function as assessed by the forced expiratory volume in one second (FEV_1), was <70% of their predicted value.

Heparinised venous blood samples (10 ml) were added to nine centrifuge tubes containing [^{3}H]-LTC_4 (10^{-8}M, 0.1 µCi, 40.2 Ci/mmol) and incubated in a shaking water bath at 37°C for different time periods (T_0-T_{120} min). The reaction was stopped at specific intervals (0, 1, 2, 4, 8, 15, 30, 60, 120 min) by immersion in ice. Furthermore, the conversion of LTC_4 to LTD_4 by γglutamyl transferase (γGT) and any protease attack was inhibited by the addition of L-serine Na_2 tetraborate (30mM) and phenyl methyl sulphonyl fluoride (0.1 mM) respectively. Blood samples were then centrifuged to obtain plasma. This was subsequently shaken with ethanol (80%), centrifuged (12000g for 20 min at 4°C) to remove insoluble material and partially purified by C_{18} Sep-Pak cartridges and millipore filters (0.45 µm) prior to reverse phase-high performance liquid chromatography (RP-HPLC) analysis using a degassed solvent system containing methanol/water/glacial acetic acid (69:31:0.01 v/v), pH 5.7 at 1ml/min (Mathews et al, 1981). Incubation of ^{3}H-LTC_4 (10^{-8}M) in blood of normal and asthmatic subjects produced a gradual exponential decline in substrate radioactivity over the reaction period (N: LTC_4 = 91.0±1.6% at T_0 min and 8.8±0.6% at T_{120} min, n = 6; A: LTC_4 = 92.1±0.7% at T_0 min and 13.0±2.3% at T_{120} min, n = 6). Furthermore, there was a corresponding increase in the amount of ^{3}H-label associated with two major peaks having an identical retention time on RP-HPLC as standard LTD_4 and LTE_4 (N: LTD_4 = 7.7±0.5% and LTE_4 = 79.1±1.9% at T_{120} min, n = 6; A: LTD_4 = 6.8±0.8% and LTE_4 = 76.4±3.0% at T_{120} min, n = 6). Values of $T_{1/2}$ for LTC_4 were 11.0 min in normal blood (Recovery = 33.5±0.6%, n = 54) and 13.0 min in asthmatic blood (Recovery = 34.3±0.8%, n = 53). Incubations of ^{3}H-LTC_4 (10^{-8}M) in normal venous plasma showed similar metabolic transformations ($T_{1/2}$ for LTC_4 = 14.0 min, Recovery = 37.6±1.2%, n = 27) which were totally suppressed by γGT inhibition. Metabolism did not occur in Hartmanns control (LTC_4 = 89.8±1.9% at T_0 and 90.6±1.1% at T_{120} min, n = 3, Recovery = 36.6±2.1%, n = 27).

Leukotriene C_4 is enzymatically degraded through the glutathione detoxification pathway in whole blood or plasma. Although no significant metabolic differences were observed between normal and asthmatic subjects, our results suggest that blood may play a role in regulating LT activity.

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Cromwell, O et al (1984). J Allergy Clin Immunol, 73, 147.
Isono, T et al (1985). Biochem Biophys Res Commun, 130, 486.
Wang, S et al (1986). J Allergy Clin Immunol, 77, 465.
Mathews, WR et al (1981). Analytical Biochemistry, 118, 96

EFFECTS OF THE SELECTIVE 5-LIPOXYGENASE INHIBITORS, BW A4C AND BW A137C, ON THE RAT GASTRIC MUCOSA

N.K. Boughton-Smith*, A.M. Deakin and B.J.R. Whittle. Department of Mediator Pharmacology, Wellcome Research Laboratories, Beckenham, Kent. BR3 3BS

A novel series of acetohydroxamic acids have been described (Jackson et al, 1987) which are potent and selective inhibitors of 5-lipoxygenase in vitro and in vivo, and which attenuate anaphylactic bronchospasm and leukocyte accumulation in experimental models (Bhattacherjee et al, 1987). Specific 5-lipoxygenase inhibitors may be devoid of the gastric toxicity associated with inhibition of protective prostanoid synthesis in the mucosa by non-steroid anti-inflammatory drugs. Furthermore, since leukotrienes have been implicated as mediators of gastric ulceration (Whittle et al 1985, Peskar et al 1986, Boughton-Smith & Whittle 1987), inhibition of their formation may prevent mucosal damage. The acute effects of the N-(3-phenoxycinnamyl)- and N-(4-benzyloxybenzyl)- acetohydroxamic acids (BW A4C and BW A137C), on the gastric mucosa have therefore been investigated.

BW A4C or BW A137C suspended in 1% methyl cellulose were orally administered to fasted rats (180-220g), and gastric mucosal damage compared after 3h to that induced by indomethacin. In further studies, fasted rats were pretreated with the acetohydroxamic acids 30 min prior to oral administration of absolute ethanol (1ml), and gastric damage measured 5 min later by computer-aided planimetry. Eicosanoid formation by chopped gastric mucosa (100mg), incubated (20 min, 37°C) in Tyrode's solution was determined by specific radioimmunoassay.

Neither acetohydroxamic acid (300 mg/kg p.o.) induced significant macroscopic damage in the rat gastric mucosa whereas indomethacin (10 mg/kg s.c.) induced linear or punctate erosions. Ethanol-induced gastric damage was accompanied by increases in mucosal leukotriene formation. Control formation of LTB_4 (4.5 ± 0.4 ng/g of tissue n = 25) and LTC_4 (68 ± 14 ng/g, n = 11) were increased 5.3 fold ($p<0.001$) and 2.2 fold ($p<0.05$) respectively by ethanol, whereas control of 6-keto-$PGF_{1\alpha}$ formation (1057 ± 88 ng/g, n = 24) was unchanged. Pretreatment with BW A4C or BW A137C (5-50mg/kg) dose-dependently inhibited ethanol-stimulated formation of LTB_4 and LTC_4 (ID_{50}, 5mg/kg for either compound). BW A4C (20mg/kg), near-maximally inhibited the formation of LTB_4 and LTC_4 (by 94 ± 1% and 97 ± 1%, n = 6; $p<0.001$). Likewise BW A137C (20mg/kg) substantially inhibited both LTB_4 and LTC_4 formation (67 ± 8% and 79 ± 10%, n = 6; $p<0.05$). However, neither compound at the doses used significantly affected 6-keto-$PGF_{1\alpha}$ formation nor ethanol-induced mucosal damage.

These findings indicate that BW A4C and BW A137C are potent inhibitors of rat gastric mucosal 5-lipoxygenase, which do not inhibit gastric prostanoid synthesis. The inability of these compounds to protect the gastric mucosa against ethanol-induced damage suggests that the leukotrienes are not primary mediators in this model. The failure of these acetohydroxamic acids to induce acute gastric mucosal damage, suggests that these compounds do not share the gastric irritancy displayed by cyclo-oxygenase inhibitors.

Bhattacherjee P. et al (1987) Ann. New York Acad. Sci. (in press).
Boughton-Smith, N.K. & Whittle, B.J.R. (1987) Gastroenterology 92, 1325.
Jackson W.P. et al (1987) J. Med. Chem. (in press)
Peskar, B.M. et al (1986) Prostaglandins 31, 283-293.
Whittle, B.J.R. et al (1985) Am. J. Physiol. 248, G580-588.

INHIBITION BY VASOACTIVE INTESTINAL PEPTIDE OF ACTION OF LEUKOTRIENE D_4 IN GUINEA-PIG PERFUSED LUNG *IN VITRO*

D.M. Conroy*, M.N. Samhoun and P.J. Piper, Department of Pharmacology, Hunterian Institute, Royal College of Surgeons, Lincolns Inn Fields, London WC2A 3PN

Vasoactive intestinal peptide (VIP), a potent relaxant of guinea-pig trachea and human bronchus, has little or no smooth muscle stimulating activity in peripheral airways of either species *in vitro* (Regal & Johnson, 1983; Barnes, 1987). VIP (10^{-8}M) appears to have a modulatory role, however, in strips of guinea-pig lung parenchyma, as it selectively inhibits contractions induced by leukotriene (LT) D_4 but not those due to histamine or acetylcholine (Samhoun et al, 1987). This modulatory mechanism of VIP is further investigated in this study, using guinea-pig isolated perfused lungs in a superfusion cascade system.

Lungs from male guinea pigs (Dunkin-Hartley, 600 g) were removed and perfused via the pulmonary artery with oxygenated Tyrode's solution (5 ml/min) at 37°C. Lung effluent superfused a spirally-cut strip of rabbit aorta (RbA). LTD_4 (3-30 pmol) was given either by i.a. bolus injection (3-30 ul) or directly (Dir) into effluent superfusing RbA. VIP (Peninsula Laboratories; 10^{-8}M) was given as a continous infusion over RbA during i.a. administration of LTD_4 and then into lungs, while doses of LTD_4 were repeated. In addition, effluent (30 ml) was collected during testing of various doses of LTD_4 alone and in the presence of VIP, and partially purified using C_{18} Sep-Paks (Waters). Levels of thromboxane (Tx) B_2 and 6-keto-prostaglandin (PG) $F_{1\alpha}$, stable metabolites of TxA_2 and prostacyclin respectively, and PGE_2 were quantitated using radioimmunoassay.

Following i.a. administration of LTD_4, RbA contracted in a dose-related manner, suggesting the release of mainly TxA_2 from lungs (Piper & Samhoun, 1981). Neither LTD_4 nor VIP administered Dir caused an effect on RbA. Further, as shown in Table 1, i.a. administration of LTD_4 caused a significant release of all cyclo-oxygenase products measured. In the presence of VIP, levels of TxB_2, 6-keto-$PGF_{1\alpha}$ and PGE_2 generated by 10 pmol of LTD_4 were inhibited by 97±2, 83±9 and 91±5% respectively.

Table 1 Generation of cyclo-oxygenase products by i.a. administration of LTD_4 in isolated, perfused lungs (control) and its inhibition by VIP (10^{-8}M) (treated)

	TxB_2		6-keto-$PGF_{1\alpha}$		PGE_2	
LTD_4	Control	Treated	Control	Treated	Control	Treated
3 pmol	15± 7	0	0.9±0.4	0.06±0.04	4.4± 2.0	1.0±0.4
10 pmol	346±113	6±4	5.9±2.8	0.5 ±0.2	9.8± 2.9	1.3±0.7
30 pmol	1088±341	15±8	14.3±4.3	1.4 ±0.5	51.2±10.6	5.2±2.9

Results expressed as mean ng total ± SEM. n = 5

These results strongly suggest that VIP plays a modulatory role by inhibiting LTD_4-induced generation of cyclo-oxygenase products in guinea-pig lung *in vitro*.

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Barnes PJ (1987) Experientia 43:832-839
Piper PJ & Samhoun MN (1981) Prostaglandins 21:793-803
Regal JF & Johnson DE (1983) Peptides 4:581-584
Samhoun MN et al (1987) In: Adv in Prostaglandins, Thromboxane and Leukotriene Res, Vol.17B, pp1023-1027. Raven Press, New York.

RELEASE OF LEUKOTRIENES IN CARDIAC ANAPHYLAXIS AND ITS INHIBITION BY A LIPOXYGENASE INHIBITOR, CGS 8515

P.J. Piper and H.B. Yaacob*, Department of Pharmacology, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN

Cardiac anaphylaxis in isolated sensitised guinea-pig hearts is characterised by coronary vasoconstriction, cardiac failure and arrhythmias (Cappuro and Levi, 1975). Since leukotrienes (SRS-A) are present in the effluent from anaphylactic hearts (Brocklehurst,1961) and leukotrienes C_4, D_4 or E_4 reduce coronary flow in guinea-pig hearts (Letts and Piper, 1982) we investigated the effect of methyl 2-(3,4-dihydro-3,4-dioxo-1-napthalenyl)amino benzoate (CGS 8515), a 5-lipoxygenase inhibitor, on the release of LTC_4-like material from guinea-pig anaphylactic hearts and on the associated increase in coronary perfusion pressure (Cpp).

Male guinea-pigs (200-250g) were sensitized with ovalbumin (100 mg ip:100 mg sc). 3-4 weeks later, they were killed and exsanguinated. The hearts were rapidly removed and perfused with oxygenated Tyrode solution containing indomethacin (2.8 μM) under constant flow of 10 ml/min at 37°C. Hearts were allowed to stabilize for 30 min prior to challenge with bolus injections of ovalbumin (100 μg). Cardiac anaphylaxis was also studied in untreated hearts. Increasing concentrations of CGS 8515 (0.03-0.3 μM) or BW755c (40 μM) were added to Tyrode solution. Cardiac effluent was collected at 1 min intervals before and for 15 min after challenge. Thromboxane B_2 (ir-TxB_2) was quantitated by radioimmunoassay and LTC_4 by bioassay on guinea-pig ileum smooth muscle.

Following ovalbumin challenge, Cpp immediately increased in a biphasic manner; this was accompanied by a decrease in cardiac developed tension (Cdt). Concomitantly, LTC_4 and ir-TxB_2 were released into the cardiac effluent. In the absence of indomethacin peak output of LTC_4 (6.2 pmol/min) occurred at 2-3 min. Indomethacin increased the duration of generation and the peak release of LTC_4 (9.0 ± 0.5 ml/min). The peak release of ir-TxB_2 (13.82 ± 1.02 pmol/min) was detected in the first min. During antigen challenge the peak release of ir-TxB_2 (9.24 ± 2.44 pmol/min) was not significantly inhibited by CGS 8515. Administration of CGS 8515 either alone or in the presence of indomethacin produced a dose-dependent inhibition of both the release of LTC_4-like material and the increase in Cpp. At concentrations of 0.1, 0.3 and 1.0 μM CGS 8515 inhibited release of LTC_4 by 40%, 66%, and 78%, respectively. Inhibition of LTC_4 release by BW755c (40 μM) was 67%. The inhibition of coronary vasoconstriction by doses of CGS 8515 (0.3-1.0 μM) was most marked in the early phase of cardiac anaphylaxis. The release of TxB_2 and 6-keto-$PGF_{1\alpha}$ following ovalbumin challenge was not inhibited by CGS 8515, but was markedly inhibited by indomethacin and BW755c. Like BW755c, CGS 8515 did not antagonise the coronary vasoconstriction elicited by exogenous LTC_4 or LTD_4.

These results show that CGS 8515 is a selective 5-lipoxygenase inhibitor which has a protective action against cardiac anaphylaxis and that LTs play a significant role in this response.

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Cappuro, N. and Levi, R. (1975). Circulation Res. 36: 521-528
Letts, L.G. and Piper, P.J. (1983). Br.J. Pharmac. 76: 169-176
Brocklehurst, W.E. (1961). J. Physiol. 151: 416

RELAXATIONS TO 15-HYDROXYEICOSATETRAENOIC ACID (15HETE) IN DOG SPLENIC ARTERY

M.J. Van Diest*, T.J.Verbeuren and A.G. Herman, Division of Pharmacology, Univ. of Antwerp (UIA), Universiteitsplein 1, B-2610 Wilrijk, Belgium.

In isolated canine blood vessels, the 15-lipoxygenase metabolites of arachidonic acid, 15HPETE and 15HETE, cause contractions that are inhibited by the thromboxane receptor antagonists BM13177 and BM13505 (Van Diest et al., 1986; 1987; Herman et al., 1986b). During contractions induced by prostaglandin $F_{2\alpha}$ or by the thromboxane mimetic U46619, 15HETE and 15HPETE evoked relaxations (Herman et al., 1986a; Van Diest et al., 1986; Takahashi et al., 1985). Cyclo-oxygenase inhibitors such as indomethacin, augmented the contractions to 15HPETE, 15HETE and also to $PGF_{2\alpha}$ and U46619 (Herman et al., 1986a; Van Diest et al., 1986). The present study was designed to investigate the influence of indomethacin on the relaxations to 15HETE in splenic arteries of the dog, contracted with $PGF_{2\alpha}$. The influence of the endothelium was also studied.

Segments of splenic arteries with or without endothelium were placed in organ chambers filled with Krebs-Ringer solution for isometric tension recording. Responses to 15HETE (10^{-8} - 10^{-5}M) were obtained during contractions caused by $PGF_{2\alpha}$ alone or in presence of increasing concentrations of indomethacin. $PGF_{2\alpha}$ was added in concentrations that resulted in comparable contractions in the different groups of tissues. The relaxations to 15HETE are reduced by removal of the endothelium and by indomethacin (Table 1); in segments without endothelium, indomethacin abolished the relaxations to 15HETE.

Table 1. Relaxations to 15HETE (n = 5)*.

15HETE	Control solution (+E)	Control solution (-E)	Indomethacin (+E) 3×10^{-7}M	Indomethacin (+E) 3×10^{-5}M
100 % :	9.66±2.15	8.44±2.06	9.94±2.56	9.58±2.62
10^{-7}M	94.1 ± 0.6	97.8 ± 1.1+	98.7 ± 1.3+	99.6 ± 0.3+
$3x10^{-7}$M	82.6 ± 3.2	90.8 ± 1.3+	92.5 ± 1.9+	94.2 ± 1.7+
10^{-6}M	60.8 ± 6.5	74.7 ± 3.2	65.5 ± 3.1	84.3 ± 6.8+
$3x10^{-6}$M	41.2 ± 6.3	61.0 ± 8.8	42.7 ± 7.8	89.9 ±15.4+

* : Results expressed as % of the contraction to $PGF_{2\alpha}$, Means ± SEM; +E, -E : segments with or without endothelium respectively.
\+ : Value different from control group (+E, $P < 0.05$).

These experiments show that endothelial cells contribute to the relaxing effects of 15HETE. Since indomethacin inhibits the relaxations to the metabolite, our results indicate that they occur via the formation of an intermediate product.

Herman, A., Van Diest M. & Verbeuren T.(1986a) Blood Vessels 23 : 74-75.
Herman, A., Van Diest M. & Verbeuren T.(1986b) Br.J.Pharmacol. 89 : 690P.
Takahashi, M. et al. (1985) Japan. J. Pharmacol. 37 : 325-334.
Van Diest, M., Verbeuren T. & Herman A.(1986) Prostaglandins 32 : 97-101.
Van Diest, M., Verbeuren T. & Herman A.(1987) Thromb. Haemostas. 58 : 272

DIFFERENT INHIBITION OF LEUKOTRIENE (LT) B_4 RELEASE FROM HUMAN POLYMORPHONUCLEAR CELLS (PMN) BY PROSTAGLANDINS

P. Ney*, B. Reupert, K. Schrör, Institut für Pharmakologie der Universität Düsseldorf, Moorenstr. 5, D-4000 Düsseldorf, F.R.G.

LTB_4 is an extremely potent chemoattractant for PMN and can amplify their biological effects. Since it has been suggested that PGE- but not PGI-type compounds attenuate O_2^--generation and lysosomal enzyme release from human PMN (Schrör & Hecker, 1987), we have studied the effect of these prostaglandins on LTB_4-formation by human PMN.

Washed human PMN (5×10^7/10 ml) were incubated with arachidonic acid (AA) (10 µM) and PGE_1, PGE_2, iloprost (10 µM) or the respective vehicle for 10 min at 37°C prior to stimulation with platelet activating factor (PAF) (3 µM), a chemotactic peptide (FMLP) (10 µM) or calimycin (A 23187) (10 µM) for another 10 min at 37°C. The generation of LTB_4 and its ω-oxidized metabolites was measured by high performance liquid chromatography (HPLC) using PGB_2 as an internal standard. All data are ± s.e.mean and refer to the sum of LTB_4 and its ω-oxidized metabolites.

Stimulation of human PMN with AA, PAF or FMLP alone caused minor generation of LTB_4 and its ω-oxidized metabolites: 23 + 8 ng, 21 + 8 ng and 15 + 6 ng, respectively per 5×10^7 cells (n = 5). Challenge with PAF or FMLP in the presence of exogenous AA increased this release about tenfold: 211 ± 32 ng and 170 ± 36 ng per 5×10^7 cells (n = 12). Treatment with PGE_2 or PGE_1 prior to PAF stimulation significantly reduced these amounts to 69 ± 33 ng and 81 ± 17 ng, respectively (n = 6-12; $P < 0.01$), whereas iloprost was ineffective at the same concentration: 192 + 29 ng; n = 11. Similar results were obtained using FMLP as agonist. Treatment with PGE_2 and PGE_1 reduced LT-release to 76 ± 27 ng and 65 ± 17 ng, respectively (n = 6-12; $P < 0.05$). Again iloprost was ineffective: 182 ± 39 ng; n = 11. There was no inhibition if calimycin was used as agonist (n = 3-4).

The data demonstrate that both, PGE_2 and PGE_1 inhibit PAF- and FMLP-induced generation of LTB_4 and its ω-oxidized metabolites while the synthetic prostacyclin analogue iloprost fails to do so. This is in accordance with our recent observations on inhibition of superoxide generation and lysosomal enzyme release from human PMN by these compounds (Schrör & Hecker, 1987) and suggests that the inhibitory prostaglandin receptor on human PMN belongs to the E-type.

Schrör, K., Hecker, G., VASA, Suppl. **17**, 11 - 16, 1987

THE EFFECT OF PROPRANOLOL AND ISOPRENALINE ON PORTAL VENOUS PRESSURE IN THE RAT

E.Burns*, T.M. Broadhead, M.Hoffig† and N.D.S. Bax, University Department of Therapeutics, The Royal Hallamshire Hospital, Sheffield S10 2JF;† Universitats Klinik, Kiel, FRG.

Propranolol (Prop) lowers the portal venous pressure in both man and animals (Lebrec et al 1981, Hillon et al 1982) although the mechanisms by which this occurs are not fully understood. It has been suggested that Prop may lower portal pressure predominantly by its effect on cardiac output thus decreasing hepatic blood flow. We have investigated the effect of Prop and isoprenaline (Isop) on portal venous pressure and vascular resistance in a constant flow isolated perfused rat liver (IPRL) system.

An IPRL system as previously described (Burns et al 1987) was established using male Wistar rats with a perfusate flow of 10 ml/min. Perfusate pressure was measured in the portal vein (Ppv) and hepatic vein (Phv). After a 30 min stabilization period, either Prop or Isop was added to give perfusate concentrations of 0.01, 0.1 or 1mM. The effects on pressure and resistance are shown in the table:

	Conc. (mM)	n	Pretreatment pressure (cm water ± sd) Ppv	Phv	Mean max.% change in Ppv with range			Mean max.% change in resistance with range		
Prop	0.01	6	10.9±1.2	4.7±0.4	+3;	−5 to	+19	+8;	−6 to	+34
	0.10	9	10.6±0.7	4.7±0.9	+11;	−5 to	+16	+15;	−4 to	+48
	1.00	15	8.9±1.5	4.0±0.7	+26;	−8 to	+116	+21;	−7 to	+83
Isop	0.01	6	10.3±0.8	4.8±0.4	−6;	−12 to	0	−6;	−12 to	0
	0.10	6	11.0±1.1	5.0±1.3	+16;	+9 to	+22	+10;	+3 to	+20
	1.00	6	12.2±2.2	4.4±0.8	+37	+15 to	+67	+14;	+1 to	+22

At all Prop concentrations the mean Ppv and resistance rose but there was great variability in response with some individual Ppv values falling. Isop also caused rises in mean Ppv except at the lowest concentration.

In a further experiment Isop and 15 min later Prop were added to the perfusate (1mM; n=6). This experiment was then repeated (with fresh preparations) with the drugs given in reversed order (n=6). When Isop was administered first, mean pretreatment Ppv (cm water ±sd) was 12.2±2.2; after Isop mean Ppv rose to 16.6±2.6 and after Prop fell to 13.4±4.9. When Prop was administered first, mean pretreatment Ppv was 9.1±2.1; after Prop mean Ppv rose to 10.6±1.3 and after Isop rose further to 14.8±1.5.

Prop appears to have a dual pharmacological effect: its partial reversal of the hypertensive action of Isop suggests a beta-receptor mediated effect. However, when given alone its vascular effect is in general opposite to when given in the presence of Isop. Thus it is possible Prop has a non beta-receptor effect that may result in a rise in Ppv; this requires investigation in view of the clinical use of Prop in the treatment of portal hypertension.

Burns et al (1987) Br J Pharmac; in press.
Hillon et al (1982) Clin Sci 63; 29-32.
Lebrec et al (1984) Hepatology 5; 355-358.

EXPERIMENTAL CONGESTIVE HEART FAILURE IN THE DOG: VASCULAR RESPONSIVENESS TO α-ADRENOCEPTOR AGONISTS

Christine Forster*, J. Main, & P. Armstrong, Division of Cardiology and Pharmacology, St. Michael's Hospital, University of Toronto, Toronto M5B 1W8, Ontario Canada.

A model of congestive heart failure (CHF) has been developed involving rapid ventricular pacing in the dog (Armstrong et al., 1986) showing a 25% increase in heart size with pulmonary congestion, a 10% increase in body weight, an increase in circulatory noradrenaline and renin activity. Haemodynamic parameters reveal decreased cardiac output, increased filling pressures and increased systemic vascular resistance. It was thus decided to investigate how dorsal pedal arteries (dpa), saphenous vein (sv), left anterior descending (LAD) and circumflex (cx) coronary arteries respond to α-agonists at CHF.

Dogs were anaesthetised for pacemaker implantation and removal of dpa and sv. Pacemakers were programmed to deliver 250 bpm and at peak CHF, animals were reanaesthetised under similar conditions for removal of contralateral dpa and sv. Following sacrifice the LAD and cx were dissected. Vascular rings (3mm) were prepared and mounted in organ baths. Isometric developed tension was recorded and cumulative concentration-effect curves were constructed to noradrenaline, adrenaline, phenylephrine, methoxamine, BHT 920 and BHT 933. Propranolol, (3×10^{-6}M), indomethacin (2.8×10^{-6}M), desipramine (10^{-6}M) and hydrocortisone (5×10^{-6}M) were incorporated in all experiments. Vascular responsiveness was compared in dogs with or without CHF.

Table 1 Mean EC_{50} and maximum responses to agonists on dpa before and at peak CHF

Agonist	EC_{50}(μM) before	EC_{50}(μM) peak CHF	maximum(g) before	maximum(g) peak CHF
noradrenaline	4.4(3.0 - 6.3)	1.5(0.8 - 2.8)*	1.5±0.1	2.7±0.3*
adrenaline	5.1(4.0 - 6.9)	2.9(1.2 - 3.6)*	2.3±0.2	3.8±0.4*
phenylephrine	5.6(4.1 - 8.1)	2.7(0.6 - 4.0)*	1.4±0.3	2.4±0.4*
methoxamine	45.7(36.7 - 57.3)	20.8(9.6 - 35.6)*	1.7±0.2	2.7±0.1*
BHT 920	111.4(72.7 - 171.0)	229.1(181.9 - 288.4)*	1.2±0.2	0.8±0.2
BHT 933	88.9(26.7 - 296.6)	140.3(68.5 - 287.1)	0.5±0.1	0.6±0.1

n=9 - 21 rings from 3-7 dogs: * $p<0.01$.

The table shows EC_{50} (geometric $\bar{x}$ with 95% confidence limits) and maximum responses ($\bar{x}\pm se$) for all agonists on dpa only. Similar results were seen on the sv although the EC_{50}'s to the BHT compounds were significantly lower than those on the dpa:-BHT 920 25.9(15.5 - 46.1) and 142.6(45.6-221.1); BHT 933 3.4(2.1 - 5.1) and 10.8(4.3 -26.9) before and at peak CHF respectively for both drugs. No contractile responses were seen with the BHT compounds on either of the coronary vessels. In addition, the LAD and cx showed decreased responsiveness to the α_1 and mixed agonists at CHF.

The results suggest a)an increased sensitivity α_1-agonists and mixed α-agonists and b)decreased sensitivity to α_2-agonists at peak CHF. No functional α_2 receptors could be detected in the coronary vessels both before and at peak CHF and in contrast to the peripheral vessels, the reactivity to α_1 and mixed agonists was apparently lower. These alterations may be important in mediating systemic vasoconstriction and myocardial abnormalities associated with CHF.

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Armstrong, P.W. et al. (1986) Circulation 74, 1075-1084.

β_2- ADRENORECEPTOR MEDIATED INOTROPIC RESPONSES OF HUMAN ATRIA: RECEPTOR SUBTYPE REGULATION BY ATENOLOL

J.A. Hall*, A.J. Kaumann, F.C. Wells[1], M.J. Brown, Clinical Pharmacology Unit, Addenbrooke's Hospital, Cambridge, CB2 2QQ. [1]Surgical Unit, Papworth Hospital, Papworth Everard, Cambridge. CB3 8RE.

Radioligand binding studies have shown human atria contain β-1 and β-2 adrenoreceptors (Stiles et al 1983, Brodde et al 1983). In human ventricle β-2 receptor stimulation contributes to inotropic responses to the endogenous catecholamines; adrenaline can cause up to half maximal increases in contractile force through β-2 receptors (Kaumann & Lemoine 1987). In atria from patients not treated with β-blockers the slightly β-2 selective (-)propranolol (Gille et al 1986) and (-)pindolol (Kaumann & Lobnig 1986) antagonise the effects of adrenaline more than noradrenaline suggesting a substantial contribution of β-2 receptors to the increase in contractile force. Using the specific β-1 and β-2 antagonists CGP20712A and ICI118551 we examined the contribution of β-1 and β-2 receptor stimulation to inotropic responses to adrenaline and noradrenaline. We also compared responses in atria from patients not pretreated with β-blockers with those from patients receiving atenolol (50mg or 100mg daily, >4 weeks pre-op, stopped >24h pre-op).

The right atrial appendage was dissected into several strips, incubated at 37°C with phenoxybenzamine 5μM for 2h and paced at 0.5Hz. A single concentration effect curve was constructed for each strip to either adrenaline or noradrenaline, with or without CGP20712A 300nM or ICI118551 50nM, preincubated for 2h. The effect of noradrenaline was significantly different in atria from treated and untreated patients. However adrenaline was more potent on atria from atenolol pretreated patients ($p<0.001$). In treated patients ICI118551 but not CGP20712A caused marked antagonism of the response to adrenaline. In untreated patients either ICI118551 or CGP20712A caused a small right shift of the adrenaline response whereas both drugs together produced marked antagonism. (-log EC50 approx 4M, n=3).

Table 1 Contractile force responses (-log EC50 M,+S.E.M., n=no of patients)

Patients' β-blocker	agonist / antagonist	norad none	adren none	adren CGP	adren ICI
Atenolol		6.79+0.16 n=7	7.59+0.08 n=11	7.22+0.18 n=7	6.24+0.20 n=6
None		6.61+0.20 n=5	6.66+0.21 n=7	6.10+0.39 n=4	6.44+0.43 n=4

CONCLUSIONS

(1) Both β-1 and β-2 adrenoreceptor stimulation contribute to the increase in contractile force in response to low adrenaline concentrations in atria from non-atenolol treated patients (2) in atria from atenolol treated patients low adrenaline concentrations acting at β-2 receptors cause a maximal increase in contractile force, high concentrations of adrenaline act also at β-1 receptors but without further increase in contractile force (3) for still unknown reasons chronic treatment with atenolol may selectively increase the density of β-2 receptors or their ability to mediate increases in contractile force.

Brodde OE et al 1983 Circ Res 53: 752-8
Gille E et al 1985 Naunyn-Schmiedebergs Arch Pharmacol 331: 60-70
Kaumann AJ Lemoine H 1987 Naunyn-Schmiedebergs Arch Pharmacol 335: 404-11
Kaumann AJ Lobnig BM 1986 Br J Pharmacol 89: 207-218
Stiles GL et al 1983 Life Sci 33: 467 73

DIFFERENCE IN CALCIUM-DEPENDENCY OF ANGIOTENSIN II-INDUCED CONTRACTIONS OF ISOLATED AORTIC PREPARATIONS OF RAT AND GUINEA-PIG

P.N.M. van Heiningen*& P.A. van Zwieten, Division of Pharmacotherapy/Pharmacology, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

Angiotensin II-amide (A II)-induced vasoconstriction has demonstrated to be dependent upon the influx of extracellular calcium ions (Ogawa et al., 1984; Cavero and Lefèvre-Borg, 1981). In the present study contractions of rat aorta induced by A II proved more sensitive to calcium entry blockade than those of guinea-pig aortic strips (Krebs-Henseleit solution; 1.25 mM Ca^{++}; 37°C; oxygenated with carbogen: 95% O_2 + 5% CO_2; aortic strips were pretreated with calcium entry blockers for 30 min, all responses to A II were expressed as a percentage of the preceding maximal response to noradrenaline (3.10^{-5} M)). Nifedipine (10^{-9} - 10^{-6} M), verapamil (10^{-8} - 10^{-5} M) and diltiazem (10^{-8} - 10^{-5} M) concentration-dependently diminished the constrictor effect to A II. The contractions were virtually abolished by the higher doses of calcium entry blockers (CEB) used, the maximal inhibition being in the range of 75-80%. The same pattern of inhibition was observed by stepwise lowering of the calcium concentration in the bath (Ca^{++}-"free": 88.9 ± 0.9% inhibition). In guinea-pig aorta preparations, however, complete suppression of the A II-induced contractions could not be achieved. Nifedipine (10^{-8} - 10^{-5} M) and verapamil (10^{-7} - 10^{-4} M) were less effective in inhibiting A II-induced vasoconstriction (40.3 ± 6.0% and 33.3 ± 9.1%, respectively) than diltiazem (10^{-7} - 10^{-4} M), which reduced the maximal response to A II by 65.9 ± 4.5%. Omission of calcium ions from the medium also resulted in a depression of the concentration-response curve to A II (66.8 ± 2.9%). The influence of calcium entry blockade on K^+-provoked contractions was quantified in both aortic preparations. The inhibitory potencies of the CEB on the vasoconstriction induced by A II and by potassium-triggered depolarization were calculated for comparison. The CEB were more potent in relaxing guinea-pig aortic strips contracted with potassium than those contracted with A II, whereas in rat aorta CEB were equipotent in inhibiting these two processes. These findings suggest the existence of a receptor-operated calcium channel in guinea-pig aorta which can be activated by A II, resulting in vasoconstriction.

Ogawa, N., Kushida, H. & Satoh, S. (1984). Arch. Int. Pharmaodyn. Ther. 268, 113.
Cavero, I. & Lefèvre-Borg, F. (1981). Br. J. Pharmac. 74, 839P.

ARE PRE-JUNCTIONAL β-ADRENOCEPTORS DESENSITISED BY IN VITRO EXPOSURE OF ISOLATED GUINEA-PIG ATRIA TO ISOPRENALINE?

K.J. Broadley*, H. Majewski, D.F. Story & M.J. Rand, Department of Pharmacology, University of Melbourne, Victoria 3052, Australia.

Postjunctional β-adrenoceptors in guinea-pig isolated atria are desensitized by prolonged exposure to isoprenaline (Kaumann & Birnbaumer, 1976; Broadley & Herepath, 1987). Prejunctional β-adrenoceptors located on sympathetic nerve endings facilitate release of noradrenaline induced by field stimulation (Majewski, 1983). The present study examines whether prejunctional β-adrenoceptors of guinea-pig atria are also desensitized by the same exposure to isoprenaline as used for the postjunctional receptor.

Guinea-pig paired atria were set up in Krebs-bicarbonate solution containing ascorbic acid (0.1mM) and EDTA (0.07mM) gassed with 5% CO_2 in oxygen at 37.5°C. They were incubated for 4h with (±)-isoprenaline (1μM). Time-matched controls received no isoprenaline. Atria were then washed (x4) for 10 min before loading with [^{3}H]-noradrenaline (0.28μM) for 20 min. The procedure was then essentially as described by Johnson & Majewski (1986) with phentolamine (1μM) present throughout. Drugs were added between two periods of field stimulation (S_1 and S_2) (1 min, 2Hz, 15V, 1ms) and their effect measured as the increase in radioactivity released expressed as a percentage (SI_2/SI_1).

In control experiments, where no drug was added between S_1 and S_2, SI_2/SI_1 was 91.6±2.4% (n=4) in tissues exposed to isoprenaline for 4h. This was not significantly different from the time-matched controls (91.3±1.5%, n=5). Isoprenaline (10nM) added between S_1 and S_2 in time-matched controls significantly elevated release of radioactive noradrenaline to 118.3±4.0% (n=6). After 4h exposure to isoprenaline, however, transmitter release was only raised to 107.9±1.9% (n=9). This was significantly less than in time-matched controls and indicates partial desensitization. The postjunctional atrial β-adrenoceptors of the same tissues displayed the anticipated desensitization. The positive chronotropic responses to isoprenaline (10nM) of control (92±4.9 beats min^{-1}) and isoprenaline-incubated atria (49.5±7.8) were significantly different.

The reduced sensitivity of the prejunctional β-adrenoceptor may have an explanation other than desensitization. For example, persistent stimulation at S_1 would mask the effect of added isoprenaline. This could occur through inadequate washout of isoprenaline or its neuronal uptake and corelease on stimulation (Brancho & Garrett, 1985). If persistent stimulation occurred, addition of the prejunctional β-adrenoceptor antagonist ICI 118551 (0.1μM) between S_1 and S_2 would reduce S_2/S_1. However, the value was the same in time-matched controls (86.5±1.5%) and after isoprenaline incubation (88.6±3.9%). Neuronal uptake was examined by incubating with [^{3}H]-NA before the 4h exposure to isoprenaline in the presence of DMI (1μM). Isoprenaline increased transmitter release in controls from 91.4±1.6 to 112.1±4.6%, while in the isoprenaline-incubated atria, the increase from 93.3±2.1 to 99.5±1.6% was again significantly reduced.

Thus neuronal uptake of isoprenaline or persistent stimulation could not explain the loss of sensitivity and these results suggest that the prejunctional β-adrenoceptor undergoes desensitization. Supported by the NH & MRC of Australia, Wellcome Trust and Ramaciotti Foundation.

Brancho, D. & Garrett, J. (1985) Arch.int.Pharmacodyn. 276, 50-59.
Broadley, K.J. & Herepath, M.L. (1987) Br.J.Pharmac. 90, 197P.
Johnson, H. & Majewski, H. (1986) Br.J.Pharmac. 87, 553-562.
Kaumann, A.J. & Birnbaumer, L. (1976) N-S.Arch.Pharmac. 293, 199-202.
Majewski, H. (1983) J.Auton.Pharmac. 3, 47-60.

NORMETANEPHRINE IS A HIGHLY SELECTIVE α_1-ADRENOCEPTOR AGONIST

J.J. Beckeringh, Biochem. Res. Lab., Div of Renal and Hypertensive Diseases, Med. Klin. & Poliklinik, University of Essen, Hufelandstrasse 55, 4300 Essen-1, F.R.G..

In contrast to noradrenaline, methoxamine is a highly selective α_1-adrenoceptor agonist albeit of a relatively low affinity (Van Meel et al. 1981, Wilffert et al. 1982). In normetanephrine, a major metabolite of noradrenaline formed by the activity of COMT, the phenolic group in the 3-position is methylated. By free rotation over the 1-phenyl-2-ethyl axis the 3-methoxygroup of normetanephrine might take up the position of the 5-methoxygroup of methoxamine. In the present study we have assessed the selectivity of normetanephrine for postjunctional α_1- and α_2-adrenoceptors in the cardiovascular system of the pithed rat using the selective α_1- and α_2-adrenergic antagonists prazosin and yohimbine, respectively.

Male Wistar rats (200-250g), anaesthetized with hexobarbitone (150 mg/kg, i.p.), were pithed and respired with room air. Catheters were introduced into the right jugular vein and the left common carotid artery for the administration of drugs and the measurement of arterial pressure, respectively. Dose-response curves to dl-normetanephrine were constructed 15 min after i.v. treatment with prazosin (0.1. mg/kg) or yohimbine (1 mg/kg). Maximal increases in diastolic pressure (mm Hg) were measured.

Normetanephrine dose-dependently induced an increase in diastolic pressure with a maximal vasopressor response of 105 ± 3 mm Hg. The ED_{50} value of the log-dose response curve was 1.9 ± 0.4 µmol/kg. In the presence of prazosin (0.1 mg/kg) the log-dose reponse curve of normetanephrine was displaced to the right in a parallel fashion (20.5-fold shift). In the presence of yohimbine (1 mg/kg) only a small 2.2-fold rightward shift of the log-dose response curve was observed.

The results show that in the pithed rat normetanephrine acts as a highly selective but partial α_1-adrenoceptor agonist; its selectivity for postjunctional α_1-adrenoceptors is similar to that of methoxamine (Van Meel et al. 1981). As compared to noradrenaline (Wilffert et al. 1982), O-methylation of noradrenaline also results in a dramatic, more than 500-fold, reduction of potency.

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Van Meel, J.C.A. et al., (1981), J. Pharmacol. Exp. Ther., 219, 760.
Wilffert, B. et al., (1982), J. Pharmacol. Exp. Ther., 221, 762.

ICS 205-930 INHIBITS CARDIO-PULMONARY REFLEXES THAT RESULT FROM I.V. INJECTION OF DEXTRAN IN THE RABBIT

L. Mazzoni and B.P. Richardson, Preclinical Research Department, Sandoz Ltd., CH-4002 Basle, Switzerland.

Rabbits (New Zealand White, 2.5-3.0Kg) were anaesthetised with pentobarbital (30 mg/kg, iv). Arterial blood pressure (MAP) and heart rate (HR) were recorded from the femoral artery using a Statham transducer , whilst airway resistance (R_l), dynamic compliance (C_{dyn}) and respiration rate (RR) in spontaneously breathing animals were monitored using a Buxco Respiratory Analyser (model 6). Drugs were dissolved in saline and injected (i.v.) 5 minutes prior to injection of dextran sulphate (20 mg/kg i.v. given as 1 ml/kg solution). Dextran caused a slow progressive decrease in MAP, HR, C_{dyn} and an increase in RR and R_l. Maximal effects were usually observed 80 sec after injection (see table). Pulmonary parameters returned to preinjection values within approximately 5 minutes, whereas the haemodynamic changes were protracted and only partly recovered within the 30 minute observation period. Four rabbits died acutely, whereas all animals pretreated with the 5-HT_3 receptor antagonist ICS 205-930 (1 mg/kg i.v.) or which had previously undergone vagotomy survived. Vagotomy or treatment with ICS 205-930 prior to dextran injection significantly reduced, but did not completely abolish these haemodynamic and pulmonary effects.

TREATMENT	n	MAP	HR	RR	C_{dyn}	R_l
SALINE	10	-35±6	-8±3	136±35	-44±7	121±25
ICS 205-930	11	-17±4*	-1±3*	33±19*	- 8±4*	31±12*
VAGOTOMY	10	-16±5*	-2±2*	24± 9*	-12±4*	14± 9*

*Responses are expressed as a mean percentage change (±s.e. mean) relative to values immediately prior to dextran injection. * Indicates a significance level of at least P<0.05*

Previous studies have shown that dextran sulphate elicits cardiopulmonary reflex effects by the release of 5-HT from platelets lodged in the pulmonary circulation (Wiggins et al. 1985).The effect of vagal section in the current study is consistent with this and the capacity of ICS 205-930 to inhibit these effects to a similar extent indicates an action mediated by 5-HT_3 receptors located on chemoafferent nerve endings.

Wiggins, R.G., Glatfelter, A., Campbell, A.M., Kunkel, R.G. and Ulevitch, R.J.(1985) Cir. Res. 57, 262-277.