ENKEPHALINASE IN THE GUINEA-PIG ILEUM

L.Miller & J.S.Shaw, ICI Pharmaceuticals Division, Alderley Park, Macclesfield Cheshire, SK10 4TJ.

We have previously reported that enkephalin is rapidly degraded by the myenteric plexus-longitudinal muscle of the guinea pig ileum (MPLM) and that aminopeptidase accounts for the major breakdown of the enkephalin (Miller & Shaw 1981).However, there appears to be a second enzyme involved, the nature of which has not yet been determined. In an attempt to elucidate the nature of this second enzyme we have examined the effect of thiorphan - a compound reported to inhibit the specific carboxydipeptidase 'enkephalinase' (Roques et.al.(1980)) - on the MPLM preparation and compared this with its effect on tissue homogenates.

In the MPLM preparation two types of experiments were performed. These involved either the addition of exogenous enkephalins to the bath, or the release of endogenous opiates from the tissue by high frequency stimulation for 1-3 minutes as described by Puig et al.(1977). In each case the maximum inhibitory effect, and the time taken for this response to decay ($t_{\frac{1}{2}}$), were determined. MPLM strips were prepared as described by Paton & Zar (1968) and mounted in 5ml organ baths. The tissues were stimulated through ring electrodes at supramaximal voltage with 1ms pulses at a frequency of 0.1Hz. In experiments involving high frequency stimulation, a frequency of 10Hz was used to release endogenous opiates. In the second series of experiments homogenates of guinea pig MPLM, brain or kidney were prepared in Kreb's buffer and incubated at 37°C with substrate and inhibitor for 1 hour. Samples were boiled to prevent any further enzyme activity,centrifuged and assayed on the field-stimulated mouse vas deferens preparation. From these results the degree of inactivation was calculated.

On the MPLM preparation thiorphan up to 5×10^{-5}M failed to increase the magnitude or the duration of the inhibitory response following high-frequency stimulation. In experiments involving the addition to the bath of exogenous met-enkephalin or the amino-peptidase-resistant analogue D-Ala_2-Leu_5-enkephalin, thiorphan had no effect on recovery time ($t_{\frac{1}{2}}$). However, the inhibitory potency of both met-enkephalin and D-Ala_2-Leu_5-enkephalin was increased (IC_{50} values 73.8 $\pm$ 13.1% and 73.6 $\pm$ 9.8% of control respectively). This effect is unlikely to be a consequence of enkephalinase inhibition since the stable analogue D-Ala_2-DLeu_5-enkephalin was potentiated to an even greater degree (IC_{50} 56.4 $\pm$ 9% of control).

This lack of activity in the MPLM preparation contrasts markedly with the reported inhibition by thiorphan of enkephalinase from a range of tissues (Llorens & Schwartz,1981),results which we have confirmed. Furthermore we have demonstrated that homogenates of MPLM are also able to inactivate D-Ala_2-Leu_5-enkephalin and that this inactivation is inhibited by thiorphan (80% inhibition at 10^{-5}M).

We have therefore been able to demonstrate that thiorphan is a potent inhibitor of the inactivation of D-Ala_2-Leu_5 in a range of homogenates including the MPLM. However the lack of activity of thiorphan in an intact system (MPLM) must pose the question as to whether enkephalinase is significantly involved in the degradation of endogenous enkephalin at the opiate receptor.

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THE INTERACTION OF ENDOGENOUS OPIOID PEPTIDES WITH THE μ-, δ- AND κ-BINDING SITES IN THE GUINEA-PIG

H.W. Kosterlitz, J. Magnan & S.J. Paterson, Unit for Research on Addictive Drugs, University of Aberdeen, Marischal College, Aberdeen, AB9 1AS

Evidence for the subdivision of the opiate receptors into μ-, δ- and κ-binding sites has been obtained by a comparison of the binding of tritiated opiates and opioid peptides and their differential inhibition by unlabelled compounds (Gillan et al, 1980; Kosterlitz et al, 1981). It was of interest to study the interaction of the endogenous opioid peptides with the three binding sites.

The potencies of the peptides to displace the binding of selective tritiated ligands were measured at 0°C. Binding at the μ-site was determined with [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAGO) (Kosterlitz & Paterson, 1981), at the δ-site with [^{3}H]-[D-Ala^2,D-Leu^5]enkephalin (DADL) and at the κ-site with [^{3}H]-bremazocine (Römer et al, 1980) in the presence of 100 nM unlabelled DAGO and 100 nM unlabelled DADL to suppress μ- and δ-binding.

The endogenous pentapeptides [Met]enkephalin and [Leu]enkephalin are more potent inhibitors of the binding of [^{3}H]-DADL (K_I = 0.72±0.02 nM, n = 3 and 1.01±0.17 nM, n = 3) than of [^{3}H]-DAGO (K_I = 9.6±2.0 nM, n = 4 and 18.5±2.7 nM, n = 4): their affinity at the κ-binding site is negligible (K_I = 2980±525 nM, n = 3 and 5540±702 nM, n = 3). The relative affinity of unlabelled [D-Ala^2,D-Leu^5]enkephalin to the δ-binding site is somewhat less than that of the parent compound, [Leu^5]enkephalin.

It is probably of physiological significance that of the endogenous opioid peptides, only β_H-endorphin, which is equipotent at the μ- and δ-binding sites (K_I = 1.88±0.27 nM, n = 3 and 2.08±0.16 nM, n = 3), approaches the high affinity to the μ-binding site found with the selective μ-ligand [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin. [Met^5]enkephalyl- Lys^6 , [Met^5]enkephalyl- Arg^6 and [Met^5]enkephalyl-Arg^6-Phe^7, precursors of [Met]enkephalin, have a binding pattern similar to that of β_H-endorphin in that they are equipotent at the μ- and δ-binding sites; however, their overall affinities are only about 10% of that of β_H-endorphin.

At the κ-binding site, only β_H-endorphin and [Met^5]enkephalyl-Arg^6-Phe^7 bind to a significant extent. β_H-Endorphin retains only 2.6% of its activity at the μ-binding site and 3.0% of that at the δ-binding site, whereas [Met^5]enkephalyl-Arg^6-Phe^7 retains 31% of its activity at the μ-binding site and 36% of its activity at the δ-binding site. Although the overall potency of the heptapeptide is low, it has to be considered as a possible ligand for the κ-binding site. None of the other endogenous peptides studied is a candidate in this respect.

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OSMOTICALLY EVOKED ACTIVITY OF VASOPRESSIN NEURONES CAN BE INHIBITED BY OPIATES

G. Clarke & Lynda Merrick, Department of Anatomy, Medical School, University Walk, Bristol, BS8 1TD.

In the rat a low dose of morphine injected intraventricularly inhibits the activity of vasopressin neurones (Clarke et al, 1980) and reduces plasma vasopressin levels (Aziz et al, 1981). We have now shown that opiates can also suppress the activation of vasopressin neurones and the associated release of hormone evoked by an osmotic stimulus.

Magnocellular neruones in the supraoptic nucleus of the lactating rat recorded with glass micropipettes, were identified antidromically by stimulating the neurohypophysis. Vasopressin neurones were distinguished from oxytocin neurones by their failure to respond during reflex milk-ejection and by their characteristic phasic firing pattern (Poulain et al, 1977). The intraventricular injection (i.c.v.) of 1-2µl of sodium chloride (3M) excited vasopressin neurones (3-30 fold excitation for 4-20 minutes) and a simultaneous rise in intramammary pressure and arterial blood pressure indicated an increase in the release of vasopressin. The rats were pretreated with the ganglion blocking drug mecamylamine (2mg. kg^{-1} i.v.)

The rise in intramammary pressure and blood pressure following an osmotic stimulus was totally abolished or reduced (>50%) in 9/10 rats given morphine (4µg i.c.v.), 7/9 rats given 2-D.Ala, Met, enkephalin (4µg i.c.v.), and 5/7 rats given β-endorphin (20ng i.c.v.). The osmotic response was restored in 15/19 rats given naloxone (1mg.kg^{-1} i.v.). The excitatory response of vasopressin neurones to hypertonic sodium chloride was reduced or abolished both by morphine (8/9 neurones) and enkephalin (4/5 neurones), but not by β-endorphin (5 neurones). The ongoing activity of the vasopressin neurones prior to osmotic stimulation was also inhibited by morphine and enkephalin. Naloxone restored all activity for every neurone tested. Non-magnocellular hypothalamic neurones were not affected by the osmotic stimulus nor by morphine or enkephalin.

Opiates can act directly at the nerve terminals to prevent the release of vasopressin (Iversen et al, 1980), but the present results show that morphine and enkephalin may also act to reduce electrical activity of the vasopressin neurones. Recent experiments using an isolated hypothalamic slice (Clarke et al, 1981) suggest this is not an effect upon the neurones directly but rather mediated through the osmotically sensitive periventricular organs which provide an afferent input to the vasopressin neurones.

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FURTHER EVIDENCE FOR V_1 VASOPRESSIN RECEPTORS ON HUMAN PLATELETS

M.E. Thomas, Katherine A. Clare & M.C. Scrutton, Department of Biochemistry, King's College, Strand, London, WC2R 2LS.

Michell, Kirk & Billah (1979) have proposed a sub-classification of tissue vasopressin receptors. This postulate suggests that the pressor response to vasopressin is mediated by V_1 receptors which act via an increase in cytosolic $[Ca^{2+}]$, whereas the anti-diuretic response to this hormone is mediated by V_2 receptors which act via an increase in tissue [cyclic-3',5'-AMP]. Structure activity analysis have revealed both agonists and antagonists having selectivity for the V_1 or the V_2 receptor respectively. Haslam and Rosson (1972) have shown that vasopressin causes aggregation of human blood platelets without any associated alteration in cellular [cyclic-3',5'-AMP]. We have confirmed this observation and have obtained further evidence in support of the proposal that human platelets carry a vasopressin receptor of the V_1 sub-type.

The selective pressor antagonist, (1-[β-mercapto-(β,β'-cyclopentamethylene-propionic acid)]-arginine-vasopressin ($d(CH_2)_5$ AVP), (Kruszynski et al, 1980) is a potent and specific antagonist for the response of human platelets to vasopressin (K_I = 1.3 ± 0.4 nM (3)). It has no effect on the response to other agonists, e.g. ADP, thrombin. In contrast the antidiuretic agonist, 1-desamino-8-D-arginine-vasopressin (dDAVP) (Manning et al, 1976) displays no agonist activity for human platelets and is a relatively weak antagonist of the response to vasopressin (K_I = 47 ± 4 nM (n = 3)). The loss of agonist activity in dDAVP depends primarily on the substitution at position 8 since 8-D-arginine-vasopressin is an even weaker antagonist (K_I = 600 ± 55 nM (n = 3)). In contrast 1-desamino-arginine-vasopressin is a partial agonist having a low efficacy, but a similar affinity, as compared with vasopressin itself.

Oxytocin is also a partial agonist at the platelet receptor having both a low affinity and a low efficacy as compared with vasopressin. Prior addition of a sub-threshold dose of the divalent cation ionophore, A-23187, induces a full aggregatory response to oxytocin which can be blocked by $d(CH_2)_5$ AVP or by the "calcium antagonist", D-600. These results suggest that the induced response to oxytocin results from enhanced mobilisation of Ca^{2+} to the cytosol in the presence of A-23187.

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INTERACTION OF RX 781094, A NEW SELECTIVE α_2-ADRENOCEPTOR ANTAGONIST, WITH HUMAN BLOOD PLATELETS

R. Kerry & M.C. Scrutton, Department of Biochemistry, King's College, Strand, London, WC2R 2LS.

Chapelo et al (1981) have described RX 781094 [2-(2(1,4-benzodioxanyl))-2-imidazoline HCl] as a new selective α_2-adrenoceptor antagonist. The properties of this compound have been assessed further by examination of its effect on aggregatory responses of, and binding of radioligands to, human blood platelets. RX 781094 is a potent antagonist of aggregation induced either by adrenaline (pA_2 = 7.3) or by the selective α_2-adrenoceptor agonist, UK-14304 (Grant & Scrutton, 1980). It has no effect on the aggregatory responses induced by ADP, thrombin, arginine-vasopressin or 5-hydroxytryptamine provided that these responses are not enhanced by the presence of endogenous catecholamines. RX 781094 is also more potent as an inhibitor of the pro-aggregatory response to clonidine (α_2) than of that to methoxamine (α_1). The selectivity ratio for the platelet α_2-adrenoceptor derived from these latter physiological response data is 11.4 as compared with greater than 16.7 for yohimbine and less than 0.04 for prazosin (Grant & Scrutton, 1980).

RX 781094 is as effective as yohimbine, and 200-fold more effective than prazosin, as an inhibitor of the binding of the non-selective α-adrenoceptor radioligand [^{3}H]-dihydroeryocryptine (Newman et al, 1978) to platelet lysates. In contrast binding of the α_1-adrenoceptor selective radioligand [^{3}H]-prazosin, (Greengrass & Bremner, 1979), to intact platelets obtained from methoxamine-sensitive donors is inhibited 400-fold more effectively by indoramin than by RX 781094 or yohimbine. These latter two drugs do not differ significantly in potency. The selectivity ratio of 781094 for the platelet α_2-adrenoceptor derived from these ligand binding data is therefore not significantly different from that exhibited by yohimbine.

Our studies demonstrate that RX 781094 acts as a very specific α-adrenoceptor antagonist in human platelets with an α_2 sub-type selectivity approaching or equal to that exhibited by yohimbine. The data also illustrate the potential of human blood platelets as a test system for assessment of the specificity and selectivity of putative α-adrenoceptor antagonists.

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MOUSE PLATELETS ARE UNRESPONSIVE TO PLATELET ACTIVATING FACTOR IN VITRO AND IN VIVO

Barbara Nunn, Beecham Pharmaceuticals Research Division, Great Burgh. Epsom, Surrey, KT18 5XQ

It has been suggested that 1-0-octadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (Platelet Activating Factor, PAF), acts as a mediator of platelet aggregation (Chignard et al, 1979). The possible role of PAF in mouse platelet aggregation has been examined.

Aggregation was measured in mouse and human platelet-rich plasma as previously described (Nunn, 1981a). PAF, stored at -20°C in ethanol, was dried under nitrogen and redissolved in Ca^{++}-free Tyrode solution containing 0.35% (w/v) bovine serum albumin (BSA). PAF (0.2 µM) caused biphasic aggregation in human platelet-rich plasma (Figure 1a). Mouse platelets either in plasma (Figure 1b) or when washed free of plasma and resuspended in Tyrode solution containing 0.35% BSA (Figure 1c) were unresponsive to high concentrations of PAF but aggregated normally to collagen added 4 min later.

PAF i.v. causes immediate thrombocytopenia in rabbits and guinea pigs (McManus et al, 1980; Vargaftig et al, 1980) at doses as low as 50 pmol/Kg. The effect of PAF on blood platelet count in mice was determined as described for collagen (Nunn, 1981b) except that mice were placed in CO_2 immediately after i.v. injection. PAF (50-1000 nmol/Kg) had no effect. For example, counts were 102 ± 2% (mean ± s.e. mean, n=7) of control counts after 1000 nmol PAF/Kg. Adenosine diphosphate (40nmol/Kg) given to alternate mice on the same occasion caused a 31 ± 7% fall. These results suggest that PAF does not mediate aggregation of mouse platelets in vitro or in vivo.

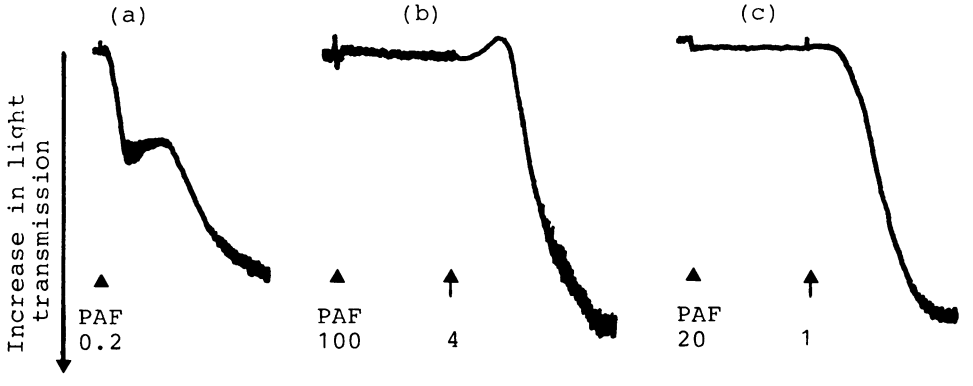

Figure 1 Responses of human (a) and mouse platelet-rich plasma (b) and mouse washed platelets (c) to PAF added as indicated. Collagen was added to the mouse preparations 4 min after PAF at the arrows. Figures are final concentrations of PAF (µM) or collagen (µg/ml).

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INHIBITION OF LYMPHOCYTE ACCUMULATION BY CYCLOSPORIN-A

A.T.M.M. Ali, J. Morley & Vivian M. Rumjanek,
Department of Clinical Pharmacology, Cardiothoracic Institute, Fulham Road, London SW3 6HP.

Cyclosporin-A is a potent inhibitor of cellular immunity in a number of mammalian species, including man. Its inhibitory action has been related to an effect upon lymphocytes, whose function can be shown to be impaired by cyclosporin-A in a range of test systems. In vitro proliferation of lymphocytes is highly susceptible to inhibition by cyclosporin-A, and it has been proposed that cyclosporin-A acts selectively during the initial phase of activation (Borel and Wiesinger, 1979) to prevent acquisition of reactivity to Interleukin 2 (Larsson, 1980).

In vitro lymphocyte proliferation can also be effectively inhibited by aspirin (Ali and Morley, 1981) and other NSAIDs (Panush, 1976); however in vivo, these drugs do not impair cellular immunity, so that it seems likely that cyclosporin-A will exhibit properties additional to those exhibited in vitro in order to achieve its effects in vivo. The accumulation of lymphocytes in a lymph node draining a site of antigen deposition is an early event during induction of an immune response. Using syngeneic cells labelled with 51-Cr, we have examined the action of therapeutic doses of aspirin, hydrocortisone and cyclosporin-A on the process of lymphocyte accumulation in lymph nodes of the rat, during the first 48 hrs. following innoculation by antigen (sheep red blood cells). Aspirin (60 mg/kg, 150 mg/kg) or hydrocortisone (10 mg/kg) were ineffective, whilst cyclosporin-A produced dose related (6.25-50 mg/kg) inhibition of cell accumulation. We suggest that this property of cyclosporin-A may have direct relevance to its actions on responses on cellular immunity and other aspects of lymphocyte function in vivo.

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OPIATE INDUCED REDUCTION OF PERIPHERAL WHITE BLOOD CELL AND PLATELET COUNTS IN MICE

D.J.M. WRIGHT (introduced by H.L. LEATHARD) Medical Microbiology, Charing Cross Hospital Medical School, London W6 8RF

The circulating white blood cells (WBC) and platelets fall to uncountable levels in mice injected i.p. with diamorphine 1-5 mg/kg. Counts approaching normal were seen after giving diamorphine 50 mg/kg or more (Wright, 1981). These results suggest an imbalance between an antagonist effect seen with larger opiate doses and agonist activity found when the dose was decreased.

A similar pattern of haematological response has now been observed with nalorphine and meptazinol, two long-acting opiates which are partial agonists/antagonists. All drugs tested, were dissolved and diluted to the appropriate concentration immediately before each experiment with sterile non-pyrogenic physiological saline, the dose being expressed in terms of free base. The drugs were given intraperitoneally to 6-week old inbred CBA/CA mice. The peripheral mean white blood cell and mean platelet counts were expressed as a percentage of the appropriate time-matched control count from groups given saline or left untreated. There were six mice in each group and each value of the mean and standard error represents the results of two experiments (n=12).

The peripheral count fell after giving nalorphine 10 mg/kg and 100 mg/kg. The respective counts at 24h were WBC 30.6 ± 4 and 38.5 ± 4.1, platelets 58 ± 9.1 and 70.6 ± 6. The peripheral WBC and platelets are uncountable 16h after nalorphine 1 mg/kg, 6h after nalorphine 100 µg/kg, and 4h after 10 µg/kg.

A fall in haematological values was also seen after injecting meptazinol 10 mg/kg and 50 mg/kg. The respective counts at 18h were, WBC 54.6 ± 6.5 and 46.3 ± 3.5, platelets 52.3 ± 6.6 and 70.5 ± 5. Normal counts were obtained before 8 and after 24h. Uncountable levels of WBC (< 8) and platelets (< 5.9) were observed 4h after meptazinol 1 mg/kg, 2h after meptazinol 1-100 µg/kg and again 4h after meptazinol 10-100 ng/kg. The counts had reverted to normal by 20h. Smaller doses of meptazinol did not alter the blood counts.

Naloxone injected 30 min. before giving meptazinol prevented the opiate-induced fall in counts. Naloxone 0.1 mg/kg and 1 mg/kg maintained the blood counts seen at 4h after injecting meptazinol, 10 µg/kg and 100 µg/kg respectively.

Meptazinol 10-50 mg/kg, nalorphine 1-100 mg/kg or diamorphine 10-100 mg/kg, like naloxone 1-10 mg/kg inhibited an Escherichia coli lipopolysaccharide induced reduction in circulating WBC and platelets, when given 30 min. before the i.v. challenge injection of endotoxin (for method: Wright & Weller, 1980).

The ability of opiates to depress the peripheral WBC and platelet counts may provide a new in vivo method of predicting and ranking the relevant agonist and antagonist potencies of these compounds.

I am grateful to Wyeth for meptazinol and to Endo for naloxone.

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A COMPARISON OF THE CARDIOVASCULAR EFFECTS OF MEPTAZINOL AND NALOXONE FOLLOWING ENDOTOXIC SHOCK IN ANAESTHETIZED RATS

P.M. Paciorek & M.H. Todd, (introduced by J.F. Waterfall), Department of Pharmacology, Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow, Maidenhead, Berks.

Naloxone reverses endotoxin-induced hypotension (Holaday and Faden, 1978) suggesting a role of endorphins in shock. Since opiate receptor antagonists may intensify pain (Levine et al, 1978) we have investigated the cardiovascular actions of meptazinol, an analgesic which also demonstrates opiate antagonist activity (Lien et al, 1979), in endotoxic shock.

Female normotensive rats were anaesthetised with a mixture of urethane (800 mg kg^{-1}) and chloralose (60mg kg^{-1}). B.P. and heart rate were monitored from a cannula inserted into the left common carotid artery and drugs were administered i.v. via the left jugular vein.

E. Coli endotoxin (10mg kg^{-1} i.v.) administration produced a decrease in mean arterial pressure (M.A.P.) of 50.4 ± 2.3 mmHg and no consistent effect on heart rate. Meptazinol (17mg kg^{-1} i.m.), naloxone (10mg kg^{-1} i.v.) or saline vehicle were administered 90 min after endotoxin. Both meptazinol and naloxone produced significant increases in M.A.P. above that of the control group without affecting heart rate (Table 1).

Table 1 The effects of meptazinol, naloxone and vehicle upon M.A.P. (mmHg)

Time (min)	Vehicle (1ml kg^{-1})		Meptazinol (17mg kg^{-1})		Naloxone (10mg kg^{-1})	
Pre-dose	135	(3.8)	123	(6.2)	127	(6.2)
Post endotoxin 90	76.7	(6.9)	75.4	(4.0)	81.7	(3.0)
Post-drug						
5	80.0	(6.8)	97.4***	(4.1)	92.5**	(3.9)
15	83.9	(6.4)	97.5***	(4.2)	97.3***	(4.7)
30	87.5	(7.3)	101***	(3.4)	104***	(4.7)

All values are mean (s.e. mean) n=4 for each group
** P < 0.01 *** P < 0.001 from control group

The effects of meptazinol and naloxone on b.p. were sustained for at least 30 min following administration and there was no statistically significant difference between their effects.

Our results with naloxone confirm those obtained by Holaday and Faden (1978) who demonstrated increases in b.p. of the same magnitude in conscious rats subjected to endotoxic shock. Meptazinol compares favourably with naloxone in its ability to raise b.p. during endotoxic shock but, unlike naloxone, is a clinically effective analgesic agent.

We are grateful to Endo Laboratories for supplying naloxone hydrochloride.

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PHARMACOLOGICAL RESPONSE TO VITAMIN K_1 IN ANTICOAGULATED RABBITS

J.B. Leck, S. Newby & B.K. Park, Department of Pharmacology and Therapeutics, The University, Liverpool L69 3BX.

4-Hydroxycoumarin anticoagulants, such as warfarin brodifacoum and difenacoum, are thought to inhibit the synthesis of clotting factors II, VII, IX and X by inhibiting the enzyme vitamin K epoxide reductase and thereby reducing vitamin K to ineffectual concentrations at its site of action (Park et al, 1979). However, brodifacoum and difenacoum are more powerful rodenticides than warfarin and are also equally effective in warfarin resistant and warfarin-susceptible rats (Hadler and Shadbolt, 1975; Leck and Park, 1981). In order to gain further insight into the mechanism of action of these compounds, we have studied the pharmacological response to vitamin K in anticoagulated male New Zealand White rabbits (2.5 - 3.0 kg). The pharmacological response to vitamin K was determined by measuring prothrombin complex activity (P.C.A.) in peripheral plasma (Park et al, 1979) in anticoagulated rabbits (P.C.A. < 30%) after i.v. administration of vitamin K_1 (Konakion®).

In animals pretreated with brodifacoum (1 mg/kg), the change in P.C.A. after vitamin K (0.5 mg/kg) administration appeared to be triphasic: a fast initial rise was followed by a slower increase and then at 4 hr there was a decline in P.C.A., at a rate which indicated a complete inhibition of clotting factor synthesis. The duration of action of vitamin K which we have arbitrarily defined as the time to reach maximum P.C.A. was not affected by increasing the dose of brodifacoum (10 mg/kg), indicating the effect of the anticoagulant (1 mg/kg) was maximal. Similar results were obtained with difenacoum (0.83 and 8.3 mg/kg): but with warfarin (63 and 189 mg/kg) a different type of response to vitamin K (0.5 mg/kg) was observed. After an initial rise, P.C.A. appeared to plateau for 11 hr and then fall at a rate which indicated incomplete inhibition of clotting factor synthesis. Therefore difenacoum and brodifacoum produce a greater maximum antagonism of vitamin K_1 *in vivo*, than warfarin, despite the fact that the dose of warfarin was, on a molar basis, 100 times greater.

The duration of action of brodifacoum and difenacoum was much longer than that of warfarin (Park et al, 1979). After administration of brodifacoum (1 mg/kg) animals were still anticoagulated (P.C.A. < 30%), at six weeks when the experiment was terminated. The response to vitamin K was found to be reproducible 1 or 8 days after administration of brodifacoum (1 mg/kg); we were therefore able to investigate several doses of vitamin K (0.5, 1, 2.5 and 5.0 mg/kg) in the same group of animals. There was a linear relationship between the duration of clotting factor synthesis and the log of the dose of the vitamin. The pharmacological half-life of vitamin K, defined as the increase in duration of clotting factor synthesis on doubling the dose, was only 1.71 ± 0.2 h. This is similar to its elimination half-life from plasma and the half-life of vitamin K metabolites in bile (Park et al, 1979; Leck & Park, 1981).

In conclusion, we have found that brodifacoum and difenacoum are both more potent and persistent antagonists of vitamin K than warfarin *in vivo*. In cases of poisoning with these compounds, it will be necessary to give repeated and frequent doses of vitamin K to maintain clotting factor synthesis.

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INCREASED RESPIRATORY STIMULANT AND PRESSOR RESPONSE TO OXOTREMORINE IN MORPHINE-TOLERANT RABBITS

M. Dalith, E. Shohami & M. Weinstock, Department of Pharmacology, Hebrew University Medical School, Jerusalem, Israel.

Narcotic analgesics depress respiration and lower blood pressure. Indirect evidence has indicated that these effects result from an inhibition by the drugs of acetylcholine release in the CNS (Jhamandas et al, 1971; Laubie et al, 1974; Weinstock et al, 1981a). On the other hand, activation of central cholinergic receptors by an agonist (oxotremorine) causes a rise in blood pressure and stimulation of the respiration (Weinstock, 1981b). Tolerance to the effects of narcotic drugs occurs on repeated administration. The purpose of the present study was to test the hypothesis that tolerance results from an increased response of the target cells to acetylcholine in response to prolonged inhibition of its release by the opiate (Collier, 1965).

Male rabbits, 2.5-3 kg, were treated twice daily with increasing doses of morphine or saline s.c. (5 $mg.kg^{-1}$, for 2 days; 10 $mg.kg^{-1}$, for 2 days; 20 $mg.kg^{-1}$, for 3 days). On day 8, control and morphine-treated rabbits were prepared for blood pressure recording and blood gas measurements as previously described (Weinstock et al, 1981b), and were injected into the ear vein with morphine, 4, 10 or 20 $mg.kg^{-1}$.

In control rabbits, morphine, 4 $mg.kg^{-1}$, reduced blood pressure by 10.9 ± 3.1, respiration rate by 38.4 ± 4.3, and increased PCO_2 by 22.1 ± 2.8%. It was necessary to give morphine, 20 $mg.kg^{-1}$, to obtain changes of this magnitude in treated animals, indicating that tolerance had developed.

To determine whether a change had occurred in the sensitivity of central cholinergic receptors, oxotremorine, 5, 10 or 33 $\mu g.kg^{-1}$, was injected i.v. into control and tolerant rabbits, on the 8th day after treatment as above. Rabbits were pretreated with atropine-methyl-nitrate, 1 $mg.kg^{-1}$, 30 min before oxotremorine to block peripheral muscarinic receptors. Oxotremorine caused a greater rise in blood pressure, and respiration rate, and fall in PCO_2 in morphine tolerant rabbits. This enhanced response was particularly evident at the highest dose of oxotremorine (See Table 1).

Table 1 Effect of oxotremorine on BP, respiration rate and arterial PCO_2 in morphine-tolerant rabbits

Dose of oxotremorine $\mu g.kg^{-1}$	n	% Change induced by oxotremorine s.e.mean					
		BP		Respiration rate		$PaCO_2$	
		Control	Tolerant	Control	Tolerant	Control	Tolerant
5	7	2 ± 4	15 ± 4***	16 ± 5	31 ± 7	16 ± 6	30 ± 6**
10	11	13 ± 4	20 ± 4	27 ± 7	53 ± 11*	20 ± 3	24 ± 2
33	9	15 ± 3	31 ± 4***	60 ± 12	100 ± 17*	14 ± 3	26 ± 4***

Differences from control: * $P < 0.05$; ** $P < 0.025$; *** $P < 0.01$. (Mann & Witney 'U' test)

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INHIBITION OF PERIPHERAL, BUT NOT CENTRAL, ANGIOTENSIN CONVERTING ENZYME BY CAPTOPRIL IN THE ANAESTHETISED RAT

T.C. Hamilton and J. Norton, Beecham Pharmaceuticals, Coldharbour Road, The Pinnacles, Harlow, Essex CM19 5AD

Reduction of the pressor responses to intravenous (i.v.) angiotensin I (AI), but not angiotensin II (AII), by captopril demonstrates the ability of this drug to inhibit angiotensin converting enzyme (ACE) in the periphery. Since the components of the renin-angiotensin system exist centrally (Ganten & Speck 1978), captopril may also inhibit ACE in the brain. In the present study using anaesthetised rats, this possibility has been investigated by comparing the effects of centrally and i.v. administered captopril on the pressor responses to AI and AII given by both routes.

Male Sprague Dawley rats were anaesthetised with pentobarbitone 65 mg/kg i.p. Drugs were administered intracerebroventricularly (i.c.v.) using the procedure of Hayden et al (1966) and i.v. into a jugular vein. Changes in diastolic blood pressure (DBP) (mmHg) were measured from a carotid artery and heart rate from the pulse in the pressure signal.

From the dose-related pressor responses to AI and AII (1-30 μg i.c.v. and 0.01-10 μg/kg i.v.), sub-maximal doses of each agonist were selected. Given centrally, captopril (10 μg-1 mg) caused dose-related falls in basal DBP with variable small changes in heart rate, whereas in doses up to 10 mg/kg i.v. captopril had little or no effect on basal DBP or heart rate.

Captopril (10-300 μg i.c.v.) had no effect on the pressor responses to AI or AII (10 μg i.c.v.); some apparent potentiation of these responses after captopril (1 mg i.c.v.) was probably a result of the lowered basal DBP. However captopril (10-100 μg i.c.v.) caused dose-related inhibition of the pressor response and bradycardia to i.v. AI (300 ng/kg), but not to AII (100 ng/kg), maximum inhibition occurring from 20 to 45 min after i.c.v. captopril. In contrast, inhibition of the pressor responses to i.v. AI, but not AII, by i.v. captopril (10-300 μg/kg) was maximal 5 min after captopril and, apart from the highest dose of captopril, had largely dissipated by 45 min. Captopril (300 μg/kg i.v.) had no effect on the responses to i.c.v. AI and AII.

I.v. captopril inhibited the renin-angiotensin system in the periphery but did not affect basal BP whilst i.c.v. captopril lowered basal DBP without affecting pressor responses to i.c.v. AI. The latter results indicate that in the anaesthetised rat captopril has a central hypotensive action independent of the brain renin-angiotensin system. Dependent on the limited penetration of captopril into the brain (Heald & Ita, 1977), the central hypotensive action of captopril may contribute to its anti-hypertensive activity; in our study captopril leaked from the brain to the periphery and therefore suggests that the reverse may occur to an appreciable extent. Failure of i.c.v. captopril to reduce pressor responses to i.c.v. AI confirms the suggestion of Solomon & Buckley (1974) that AI has pressor activity in the CNS independent of its conversion to AII. However in conscious SH-rats (as opposed to anaesthetised normotensive rats in our study) i.c.v. captopril reduces pressor responses to i.c.v. AI implicating the involvement of the brain renin-angiotensin in this model (Kondo et al, 1979; Unger et al, 1981).

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EVALUATION OF CAPTOPRIL AND SARALASIN IN CONSCIOUS RATS WITH PLASMA RENIN LEVELS ELEVATED BY FUROSEMIDE

V.C. Lewis & M.H. Todd (introduced by J.F. Waterfall), Department of Pharmacology, Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow, Maidenhead, Berks, SL6 OPH.

Current methods of producing high renin levels for investigating drugs active on the renin angiotensin system involve surgical manipulations to cause renal ischaemia; (Rubin et al, 1981). Furosemide also evokes marked increases in renin release (Keeton et al, 1980) and we have therefore investigated furosemide induced renin release in rats, and studied the effects on systolic BP (measured indirectly from the tail), of the orally acting converting enzyme inhibitor captopril in furosemide treated rats. We have also studied the effects of intravenous infusion of saralasin (measuring MAP directly from indwelling carotid cannulae). Furosemide was administered to normotensive rats at doses ranging from 0.1 to 50.0 mg/kg (s.c.). Systolic BP and HR did not alter from predose at 3 hours (t=3h) after dosing. Administration of captopril at this time point evoked decreases in BP (measured at t=5h) in all furosemide treated animals, the magnitude of which correlated with the dose of furosemide administered (Table 1). No changes occurred in control groups due to captopril or furosemide alone.

Table 1 Mean (s.e.mean) systolic BP (mmHg) (n=4 for each group)

Predose	114 (6.9)	115 (6.1)	121 (3.8)	123 (4.3)	111 (3.1)	118 (4.3)	128 (3.2)
Furosemide (mg/kg)	0.1	1.0	10.0	20.0	50.0	Vehicle	10.0
t=3h	115 (6.1)	120 (2.0)	119 (7.2)	124 (5.2)	114 (2.4)	119 (2.4)	116 (4.3)
Captopril (mg/kg)	20	20	20	20	20	20	Vehicle
t=5h	112 (2.7)	107*** (2.7)	99**** (4.3)	98**** (4.3)	91**** (6.3)	115 (2.0)	113 (5.2)

*** p<0.01 from t=3h **** p<0.001 from t=3h and from control groups

Rats prepared 18-24h previously, under halothane anaesthesia with indwelling right carotid and jugular cannulae were dosed with furosemide (10 mg/kg s.c.). At t=3h either i) an infusion of saralasin (i.v.) or ii) captopril (p.o.) was administered. Captopril (20mg/kg) caused a decrease in MAP of 29 ± 4.6 mmHg. The lowest mean recorded decrease in MAP during saralasin infusion (10µg/kg/min) was 14 ± 2 mmHg. Similar decreases were not observed in control groups. Measurement of plasma renin activity (PRA) in furosemide (10mg/kg s.c.) treated rats showed a change from 6.47 ± 1.42 to 27.8 ± 9.6 ng/ml/h (p<0.01) at the t=5h time point.

These results contrast with the findings of Ueno et al (1980), who observed no decrease in MAP in conscious dogs when furosemide and sar^1-ile^8 AII were given concurrently. In this study however, the experimental model was different and PRA levels were lower than those obtained in this rat model.

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MECHANISM OF THE HYPOTENSIVE ACTION OF CAPTOPRIL IN SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS

D.P. Clough, M.G. Collis, Karen L. Faulkner, R. Hatton , Helen Jennings and J.R. Keddie, Bioscience Dept. II, ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG.

Captopril lowers blood pressure in both man and animals although the mechanism of this action is unclear since it occurs when plasma renin activity is elevated, normal or low. This has led several groups to suggest that the antihypertensive action of captopril is unrelated to angiotensin converting enzyme inhibition. However this ignores the important interactions between angiotensin and the nervous system (Zimmerman, 1981), which are particularly relevent since an overactive sympathetic nervous system has been implicated in many forms of hypertension. Thus converting enzyme inhibitors could lower BP by interfering with neurogenic vasoconstriction.

We have recently reported in both the pithed rat (Clough et al, 1981) and the isolated mesenteric artery (Collis and Keddie, 1981) that captopril interferes with neurogenic constriction by an angiotensin dependent mechanism in normotensive Alderley Park Wistar rats (APW). In this study we have extended our observations on the effects of captopril and angiotensin on adrenergic neurotransmission to the spontaneously hypertensive rats (SHR) and the normotensive Wistar-Kyoto rat (WKY). We have also studied the effects of captopril on the blood pressure of these three strains of rat (SHR, WKY, APW).

In the pithed rat preparation previously described (Gillespie et al, 1970; Clough et al, 1981) vasoconstrictor responses to electrical stimulation of the spinal cord (1-30Hz, 0.5ms, 60V, 10s duration) were greater in SHR than WKY ($p < 0.01$). These responses were reduced by captopril and the reduction was greater in SHR than WKY ($p < 0.001$). After bilateral nephrectomy the responses to electrical stimulation were reduced and captopril was now without effect in either strain of rat. Isolated perfused mesenteric artery preparations (McGregor, 1965; Collis and Keddie, 1981) from SHR had a greater response to nerve stimulation than those from WKY in the absence of angiotensin II ($p < 0.05$). The responses of both strains of rat were potentiated by angiotensin II, however, this effect was more pronounced in SHR ($p < 0.002$). In conscious rats prepared with indwelling carotid artery cannulae, captopril (30mg/kg p.o.) lowered BP in SHR over a 5 day period but had no effect on the BP of WKY. In the normotensive APW, which we have previously shown to be very sensitive to the adrenergic facilitating actions of angiotensin II, captopril also lowered BP.

Captopril thus lowers BP in 2 strains of rat sensitive to the adrenergic facilitating actions of angiotensin II (SHR and APW) and does not lower BP in WKY which are relatively insensitive to this action of angiotensin II. The hypotensive actions of captopril may thus be related to an interference with the effect of angiotensin II to facilitate adrenergic neurotransmission.

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EFFECT OF CAPTOPRIL ON THE REGULATION OF NORADRENALINE RELEASE IN THE HEART AND VASCULAR SMOOTH MUSCLE OF THE PITHED RAT

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

As reported by Clough et al (1981) and by Antonaccio & Kerwin (1981), captopril antagonizes the vasoconstriction elicited by noradrenaline in the pithed rat before, but not after bilateral nephrectomy. The underlying mechanism of this interaction appears to be a selective impairment of vasoconstriction mediated by vascular postsynaptic α_2-adrenoceptors (Timmermans et al, 1981). The present study was designed to examine the effect of acute treatment of pithed normotensive rats with captopril on the vascular and cardiac sympathetic neurotransmission and on the regulation of noradrenaline release by presynaptic α_2-adrenoceptors.

Male Wistar normotensive rats (190-230 g) were pithed by the method of Gillespie et al (1970). Captopril (5 and 100 mg/kg, i.v.) had no effect on the increase in heart rate to continuous electrical stimulation of the sympathetic efferents to the heart (Th1-C7, 2 ms, 50V) over the total range of stimulation frequencies (0.1 -2 Hz). B-HT 920 (1, 10 and 100 µg/kg, i.v.) produced a dose-dependent inhibition of the cardiac response to electrical stimulation, which was more marked at low stimulation frequencies. Captopril did not affect the cardiac sympathetic inhibitory response to B-HT 920.

Bilateral adrenalectomy did not significantly alter the pressor response to electrical stimulation of the spinal cord (Th5-L4, 2 ms, 50 V, 60 s) at all frequencies (0.1-20 Hz). However, captopril (5 and 100 mg/kg) reduced the pressor response to sympathetic nerve stimulation more effectively after adrenalectomy than in intact pithed rats. After bilateral adrenalectomy, the pressor response to electrical stimulation was blocked by prazosin (0.1 and 1 mg/kg, i.v.),but less by rauwolscine (1 and 3 mg/kg, i.v.). B-HT 920 (100 µg/kg, i.v.)significantly diminished the vascular response to electrical stimulation at 0.1-5 Hz. After captopril (5 and 100 mg/kg, i.v.), B-HT 920 significantly inhibited the pressor response to nerve stimulation at 0.5-5 Hz.

The results confirm the observation of Antonaccio & Kerwin (1981) that captopril has no effect on sympathetic neurotransmission in the heart. Vasoconstriction elicited by sympathetic nerve stimulation is predominantly mediated by α_1-adrenoceptors. Because captopril does not interfere with pressor responses mediated by vascular postsynaptic α_1-adrenoceptors, the main effect of acute inhibition of angiotensin converting enzyme is the diminished release of noradrenaline from the sympathetic nerve terminals. Although captopril suppresses vasoconstriction mediated by vascular postsynaptic α_2-adrenoceptors, this drug is without effect on cardiac and vascular presynaptic α_2-adrenoceptors in the pithed rat.

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SELECTIVE INHIBITION OF α_2-ADRENOCEPTOR MEDIATED VASOCONSTRICTION IN VIVO BY CAPTOPRIL AND MK-421

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

Recently, the angiotensin converting enzyme inhibitor captopril has been demonstrated to antagonize noradrenaline-induced vascular smooth muscle contraction in pithed rats (Antonaccio & Kerwin, 1981; Clough et al, 1981). Following the discovery that vasoconstriction in vivo is governed by postsynaptic α_1- as well as α_2-adrenoceptors and that noradrenaline has no preference for either class (review by Timmermans & van Zwieten, 1981), the present study was undertaken to elucidate the type of α-adrenoceptor involved.

Artificially ventilated, male normotensive, pithed rats (190 - 230 g) were used throughout. In β-blocked (sotalol, 10 mg/kg, i.v.), pithed rats, captopril (5 and 100 mg/kg, i.v., -15 min) reduced the hypertensive responses to 10^{-10} - 10^{-9} mol/kg (-)-noradrenaline applied i.v. After suppression of the α_1-adrenoceptor-mediated component of the vasopressor effect of (-)-noradrenaline with the selective α_1-adrenoceptor antagonist prazosin (Cambridge et al, 1977) in a dose of 1 mg/kg (i.v., -15 min), captopril (5 and 100 mg/kg) impaired the resulting hypertensive effect elicited by 10^{-9} - 10^{-6} mol/kg (-)-noradrenaline. On the other hand, after selective blockade of that part of (-)-noradrenaline's vasoconstriction subserved by α_2-adrenoceptors with the preferential α_2-adrenoceptor antagonist rauwolscine (Weitzell et al, 1979) at a dose of 3 mg/kg (i.v., -15 min), captopril (5 and 100 mg/kg) was devoid of antagonistic activity against the residual vasoconstriction to (-)-noradrenaline.

The log dose-pressor response curve produced by stimulation of postsynaptic α_2-adrenoceptors with B-HT 920 (Van Meel et al, 1981) was shifted to the right by previous treatment with captopril (5 and 100 mg/kg). MK-421 (0.5 and 5 mg/kg) showed a similar inhibitory action as captopril when administered to intact rats 1 h before the pressor substance. Acute treatment of pithed rats with MK-421 was ineffective. In pithed animals which had been subjected to bilateral nephrectomy (- 20 h), the hypertensive responses to B-HT 920 were strongly reduced. In contrast, increases in diastolic pressure caused by cirazoline, a selective stimulant of α_1-adrenoceptors (Van Meel et al, 1981), were not or only slightly affected by captopril, MK-421 and bilateral nephrectomy. Infusion of angiotensin II (200 ng/kg/min) restored the vasoconstriction due to B-HT 920 in captopril--treated as well as in nephrectomized pithed rats.

The results show that the antagonism of vasoconstrictor noradrenaline by inhibiting angiotensin converting enzyme (Antonaccio & Kerwin, 1981; Clough et al, 1981) is limited to that part of the vasoconstriction which is mediated by postsynaptic α_2-adrenoceptors. It may be speculated upon a modulatory role of angiotensin II in postsynaptic α_2-adrenoceptor activation in vivo.

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ERGOMETRINE PREDOMINANTLY STIMULATES POSTSYNAPTIC α_2-ADRENOCEPTORS IN RAT VASCULATURE

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

In the pithed normotensive rat, stimulation of vascular postsynaptic α_1 - and α_2-adrenoceptors as well as 5-HT receptors gives rise to vasoconstriction. Under these in vivo circumstances, only the α_2-adrenoceptor-triggered hypertensive response appeared to be inhibited by the calcium antagonistic drug nifedipine (Van Meel et al, 1981; Kalkman et al, 1982). At present, nifedipine is used to treat Prinzmetal angina pectoris. On the other hand, the ergot alkaloid ergometrine is used for diagnostic purposes to provoke coronary spasms. Since ergometrine appears to stimulate both α-adrenoceptors and 5-HT receptors (Berde & Schild, 1978), an analysis of the agonistic activity at these receptors was performed.

Male Wistar, normotensive rats (weight 190 - 240 g) were pithed and subjected to artificial ventilation. Body temperature was kept at approximately 37°C. The right jugular vein and common carotid artery were cannulated to allow the administration of drugs and the continuous measurement of arterial pressure, respectively. Pressor responses to i.v. ergometrine were reduced by the α_1 - adrenoceptor antagonist prazosin (0.1 mg/kg, i.v., -15 min) and by the α_2-adrenoceptor blocking drug yohimbine (1 mg/kg, i.v., -15 min), but only moderately affected by the 5-HT receptor antagonist methysergide in a dose of 0.005 mg/kg (i.v., -15 min) which profoundly attenuated the pressor effects of 5-HT itself. Blockade experiments with a combination of both α-sympatholytic drugs showed that the 5-HT receptor-mediated vasopressor component is small and only significant at higher doses of ergometrine. Nifedipine (1 mg/kg, i.v., -15 min) shifted the dose-pressor response curve of ergometrine to the right in a dose-related non-parallel manner with a strong depression of the maximum. Since only vasoconstriction mediated via the α_2-adrenoceptor subtype is susceptible to inhibition by nifedipine (Van Meel et al, 1981), the outcome of the present experiments indicates that in ergometrine-provoked vasoconstriction the α_2-adrenoceptor component dominates.

Taking into account the clinical success of nifedipine in Prinzmetal angina pectoris and considering that this calcium antagonistic drug leaves the 5-HT--elicited pressor responses unaffected, one might speculate upon the role of postsynaptic α_2-adrenoceptors in coronary vasospasm. This hypothesis is at variance with that of Müller-Schweinitzer (1980) and Sakanashi & Yonemura (1980), who suggested a role of 5-HT receptors in coronary artery spasms based upon the finding that ergometrine contracted canine coronary artery by activating 5-HT receptors.

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α_2-ADRENERGIC PRESSOR RESPONSES AND CORTICOSTEROIDS IN THE PITHED RAT

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

In isolated vascular smooth muscle preparations, corticosteroids do not induce contractions. However, they potentiate the vascular tension induced by pressor agents, such as catecholamines (Fowler & Chou, 1961).Since catecholamines are non-selective α_1 / α_2-agonists, we have investigated which receptor type is involved in the potentiating effects of corticosteroids with respect to drug-induced vasoconstriction in vivo.

Male, normotensive Wistar rats (weight 200-250 g) were pithed under hexobarbitone-anaesthesia (150 mg/kg,i.p.).Subsequently, the animals were ventilated via a tracheal cannula connected to a positive pressure pump. Rectal temperature was maintained at approximately 37°C. A jugular vein was cannulated for the injection of drugs.Arterial blood pressure was recorded continuously via a cannulated common carotid artery. B-HT 920 was used to selectively stimulate α_2-adrenoceptors and cirazoline was administered to activate postsynaptic α_1-adrenoceptors (Van Meel et al.,1981).

Acute bilateral adrenalectomy had no effect on the vasopressor responses mediated by α_2-adrenergic receptors. However, bilateral adrenalectomy 18 h before the administration of B-HT 920, shifted the log dose-vasopressor response curve of this substance to the right and reduced the maximal increase in diastolic pressure evoked by the α_2-agonist. The pressor responses to cirazoline were not affected by the aforementioned surgical procedures.

Treatment of pithed rats with desoxycorticosterone (25 and 0.5 mg/kg,sc.), triamcinolone (10 and 0.5 mg/kg,sc.) and dexamethasone (10 and 0.5 mg/kg,sc.) followed by a 30 min period of stabilisation, had no influence on the vasopressor responses induced by B-HT 920 and cirazoline.

Substitution with desoxycorticosterone (25 mg/kg,sc.) and dexamethasone (10 and 0.5 mg/kg,sc.) restored the pressor responses to B-HT 920 in rats which had undergone bilateral adrenalectomy 18 h before. No complete recovery of the vasopressor responses to B-HT 920 was observed after substitution with triamcinolone (10 and 0.5 mg/kg,sc.) to adrenalectomized rats. Accordingly, mineralocorticosteroids are more effective than the glucocorticoids.

The results suggest a role for circulating mineralocorticoids in the regulation of vasoconstriction via postsynaptic α_2-adrenoceptor stimulation.

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DIFFERENTIAL ACTIVITY OF CLONIDINE AND α-METHYL NORADRENALINE ON α_2-ADRENOCEPTORS

D.R. Mottram, School of Pharmacy, Liverpool Polytechnic, Liverpool L3 3AF

Kapur and Mottram (1978) reported that a series of benzodioxanes which exhibited widely differing antagonist activity against postsynaptic α_1-adrenoceptors, had remarkably similar antagonist activity against presynaptic α_2-adrenoceptors. It was concluded that clonidine, the agonist used in the investigation, and the benzodioxanes may only partially overlap in their binding to α_2-adrenoceptors. In the present study it was intended to establish whether the same series of benzodioxanes exhibit similar equality of antagonistic activity against the phenylethylamine type of α_2-selective agonist, α-methyl noradrenaline (a-mNA), Ruffolo et al(1977) having reported that imidazolines and phenylethylamines may interact at different sites on the α-adrenoceptor.

Stripped vasa deferentia from male Wistar rats (175-225g) were set up in organ baths and bathed in Mg-free Krebs, maintained at 37°C and aerated with a mixture of 5% CO_2 in O_2. Field stimulation was produced by square wave pulses of 3 ms duration at a frequency of 0.1 Hz, using sub maximal voltages. Isometric contractions were recorded on Devices polygraph recorders. Clonidine ($3x10^{-10}$M to 10^{-8}M) and α-mNA (10^{-7}M to $3x10^{-5}$M) were added cumulatively to obtain dose-response curves. Prazosin ($2.5x10^{-8}$M) was included in the bathing fluid to prevent postsynaptically mediated contractions of the vasa at high concentrations of α-mNA (Brown et al, 1980).

Results proved to be somewhat unexpected. Yohimbine was used as a standard α_2-selective antagonist for comparative purposes. Against clonidine, increasing concentrations of yohimbine produced parallel shifts in the dose-response curve, from which the pA_2 value of 8.21±0.15 was calculated according to the method of Arunlakshana and Schild (1959). However against α-mNA, though 10^{-6}M and $2x10^{-6}$M yohimbine produced parallel shifts in the dose-response curve, further increase in the concentration of yohimbine (up to $5x10^{-5}$M) produced no further shift in the dose-response curve for α-mNA. A similar result was seen when phentolamine was used as the antagonist in that a shift was observed at $5x10^{-6}$M and $2x10^{-5}$M but no further significant shift was seen using $5x10^{-5}$M and 10^{-4}M phentolamine. Similarly with the benzodioxane WB 4101, only a partial shift was noted. Another interesting point observed was that following inhibition of the twitch response by clonidine, a very slow recovery of the response was observed even after repeated washing of the tissue with fresh Krebs solution. On the other hand, recovery from the inhibition of the twitch by α-mNA was very rapid requiring a single wash with fresh Krebs.

Results suggest that α-mNA and clonidine are both effective in inhibiting the twitch response of field stimulated rat vas deferens. However the results cast some doubt as to whether they are both acting through the same binding sites on the α_2-adrenoceptor. These results therefore add weight to the suggestion of Ruffolo et al(1977) that imidazolines and phenylethylamines may act through different sites. In addition, the inability of α_2-selective antagonists to produce an effective competitive blockade of α-mNA, coupled with the rapid reversibility of α-mNA's inhibitory activity suggests that, though clonidine exerts its effects through binding to both the discrete high and low affinity binding sites associated with the α_2-adrenoceptor (Rouot et al, 1980), α-mNA on the other hand may bind exclusively to the low affinity site.

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ANTAGONISM OF CLONIDINE-INDUCED HYPOTENSION AND BRADYCARDIA BY RX 781094 AND OTHER α-ADRENOCEPTOR ANTAGONISTS IN THE RAT

T.L.Berridge, J.C.Doxey and A.G.Roach, Pharmacology Department, Reckitt and Colman, Pharmaceutical Division, Hull, HU8 7DS.

Recent studies have implicated both α_1-adrenoceptors (Cavero and Roach, 1978; Hamilton et al., 1980) and α_2-adrenoceptors (Doxey et al., 1981) are involved in mediating clonidine's hypotensive and bradycardic effects in rats. This present study compares the effects of RX 781094 [2-(2-(1,4-benzodioxanyl))-2-imidazoline HCl], a new selective α_2-adrenoceptor antagonist (Chapleo et al., 1981), yohimbine, rauwolscine (two selective α_2-antagonists), corynanthine and prazosin (two selective α_1-antagonists) on the central cardiovascular effects of clonidine.

The antagonists or vehicle (saline or distilled water) were administered either i.v.(femoral vein) or into a lateral cerebral ventricle (i.c.v.;volume 10-20 µl) to pentobarbital (50 mg/kg, i.p.) anaesthetised male Sprague-Dawley rats (250-350g) 10 min before clonidine (10 µg/kg, i.v.). Prazosin was given 2 x 1.0 mg/kg, p.o. for 3 days and a single 1.0 mg/kg dose 2 hours before clonidine. In separate experiments increasing doses of RX 781094 (3-100 µg/kg,i.v.) were administered (at 5 min intervals) 10 min after either clonidine (10 µg/kg) or hydrallazine (1.0 mg/kg, i.v.).

Clonidine (10 µg/kg) reduced diastolic blood pressure (initial DBP= 103.8±1.8 mmHg; ΔDBP = -42.1±1.8 mmHg; mean of all control groups n=39) and heart rate (initial HR = 405.8±6.6 beats/min; ΔHR = -113.3±5.7 beats/min; n=39). Pretreatment with RX 781094 (0.1, 0.3 mg/kg, i.v. or 1-50 µg, i.c.v.) produced dose-related inhibitions of the clonidine-induced hypotension and bradycardia. RX 781094 (0.3 mg/kg, i.v. and 10 µg, i.c.v.) inhibited the clonidine hypotension by 83% and 73%, respectively. Yohimbine (0.1 - 1.0 mg/kg, i.v.) and rauwolscine (0.3 - 3.0 mg/kg, i.v.) reduced clonidine's bradycardia and hypotension in a dose-dependent fashion; the largest dose of each antagonist producing almost total inhibition of clonidine. Yohimbine (50 µg, i.c.v.) and rauwolscine (100 µg, i.c.v.) did not affect the clonidine hypotension and bradycardia. Corynanthine (1-3 mg/kg, i.v. or 100 µg, i.c.v.) did not alter the hypotensive and bradycardic responses to clonidine. Oral prazosin itself slightly reduced baseline blood pressure. Clonidine reduced diastolic blood pressure to a lower level in control animals (62.0±4.2 mmHg; n=6) than in prazosin treated animals (73.6±3.7 mmHg;n=5) but this difference was not statistically significant. Clonidine's bradycardia was unaffected by prazosin. In reversal experiments, RX 781094 (3-100 µg/kg, i.v.) induced dose-related reversal of clonidine's responses without influencing hydrallazine. The blood pressure and heart rate effects of clonidine were reversed by 94% and 45% after RX 781094 (30 µg/kg, i.v.).

In conclusion, RX 781094 was about 3-10 times more potent than yohimbine and rauwolscine in antagonising the central cardiovascular effects of clonidine. In contrast to the latter two compounds, RX 781094 was potent and soluble enough after central administration to inhibit clonidine. These results also indicate that clonidine's central cardiovascular effects are mediated, in rats, primarily via stimulation of α_2-type adrenoceptors.

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COMPARISON OF THE EFFECTS OF RX 781094 AND PRAZOSIN ON THE PRESSOR RESPONSES TO VARIOUS α-ADRENOCEPTOR AGONISTS

T.L. Berridge, J.C. Doxey, A.G. Roach and Diane A. Strachan, Pharmacology Department, Reckitt & Colman, Pharmaceutical Division, Hull, HU8 7DS.

Recent in vivo data suggest that at least two types of postsynaptic α-adrenoceptors may be present in blood vessels, one being the conventionally classified postsynaptic α_1-adrenoceptor and the other which resembles the presynaptic α_2- adrenoceptor on nerve terminals (Docherty et al., 1979) Experiments have been performed in which the effects of RX 781094 2-(2-(1,4-benzodioxanyl))-2-imidazoline. HCl , a new selective antagonist of presynaptic α_2-adrenoceptors (Chapleo et al., 1981) and prazosin, a selective α_1-adrenoceptor antagonist, on the pressor responses to noradrenaline (an agonist of both α_1- and α_2-adrenoceptors), UK 14304-18 (a selective α_2-adrenoceptor agonist; Cambridge, 1981) and cirazoline (a selective α_1-adrenoceptor agonist; Cavero et al., 1981) have been compared in pithed rats.

Male Sprague-Dawley rats (250 - 350 g) were pithed and blood pressure was measured from a carotid artery. Drugs were injected into a femoral vein. All animals were bivagotomised and received atropine (1.0 mg/kg,i.v.) and propranolol (1.0 mg/kg, i.v.) before starting the experiment. Agonist dose-diastolic pressor response curves were constructed in separate groups of animals (n=5-8) 5 min after i.v. pretreatment with either saline (1.0 ml/kg), RX 781094 (0.1,1.0 mg/kg), prazosin (0.1, 1.0 mg/kg) or RX 781094 (1.0 mg/kg) and prazosin (1.0 mg/kg) given together. The doses of each agonist producing a pressor response of 50 mmHg (ED_{50}) were calculated for each treatment.

Noradrenaline, cirazoline and UK 14304-18 control ED_{50} values were 0.4±0.1, 0.8±0.1 and 4.5±1.5 μg/kg, respectively. Prazosin competitively antagonised the cirazoline dose-response curve in a dose-dependent manner. The cirazoline ED_{50} value after 1.0 mg/kg prazosin was 244.3±32.4 μg/kg. RX 781094 (1.0 mg/kg) failed to antagonise cirazoline (ED_{50} value being 1.1±0.2 μg/kg). The antagonists given together antagonised cirazoline to the same extent as prazosin given alone. RX 781094 competitively antagonised the pressor responses to UK 14304-18. RX 781094 (0.1,1.0 mg/kg) produced 2.4 and 12.7 fold rightward shifts of the control UK 14304-18 dose-response curve. Prazosin was relatively inactive against UK 14304-18 (ED_{50} value after 1.0 mg/kg prazosin being 5.2±0.6 μg/kg) although prazosin depressed the maximum response to UK 14304-18. RX 781094 and prazosin (both 1.0 mg/kg) given together antagonised UK 14304-18 to a greater extent (21 fold shift) than observed with RX 781094 (1.0 mg/kg) given alone (13 fold shift). Prazosin (1.0 mg/kg) and RX 781094 (1.0 mg/kg) produced similar (4.9 fold and 6.0 fold) shifts of the noradrenaline dose-response curves, respectively. The antagonists given together produced a significantly larger antagonism of noradrenaline (26 fold shift) than observed with either antagonist given alone.

These results are consistent with the hypothesis that postsynaptic α_1- and α_2 -adrenoceptors exist in the rat vasculature. RX 781094 may therefore be a useful pharmacological tool to investigate the physiological relevance of postsynaptic α_2-adrenoceptors.

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ACTIONS OF CIRAZOLINE ON α-ADRENOCEPTORS IN THE RABBIT: IN VIVO AND IN VITRO STUDIES

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

Cirazoline 2(2'cyclopropylphenoxymethyl)imidazoline, exerts vascular postsynaptic α adrenoceptor stimulating properties both in vitro and in vivo (Lefevre et al, 1975). As cirazoline (CZ) does not produce sedation (Cavero & Roach, 1978) or stimulate cardiac presynaptic α adrenoceptors in rats and dogs, it was suggested that this compound might be a relatively selective agonist at vascular α_1 adrenoceptors (Roach et al, 1979). We have studied the pharmacological action of cirazoline on postsynaptic α_1 and α_2 adrenoceptors using a range of α adrenoceptor antagonists in vivo by measurement of mean arterial pressure and in vitro by radioligand binding studies (Hamilton & Reid, 1981).

In conscious rabbits pressor dose response curves to i.v. cirazoline (0.5-250 μg/kg) were constructed before and 20 min after α adrenoceptor blockade with prazosin (0.5 mg/kg), α yohimbine (1 mg/kg), phentolamine (1 mg/kg) or phenoxybenzamine (1 mg/kg). Pressor responses were compared to those using phenylephrine (PE 5-1000 μg/kg) as the agonist.

Studies in vivo indicated that cirazoline acted as a specific α_1 adrenoceptor agonist. Prazosin pretreatment shifted the pressor dose response curve to the right (CZ dose ratio 16.0 ± 5.4, PE 11.9 ± 0.7) whereas α yohimbine had little effect (CZ dose ratio 2.9 ± 2.8, PE 1.9 ± 0.9). The non-specific α adrenoceptor antagonist phentolamine had similar effects on pressor responses to cirazoline and phenylephrine (CZ dose ratio 6.2 ± 2.8, PE 5.2 ± 0.5). The pressor response to cirazoline (250 μg/kg) 30 min after phenoxybenzamine was greatly attenuated (6.4 ± 4.2 mm Hg as opposed to 30.8 ± 4.6 mm Hg produced by 25 μg/kg before phenoxybenzamine) and had not fully recovered 24 h later.

However radioligand binding studies with rabbit brain membranes using ^{3}H prazosin and ^{3}H clonidine as specific ligands for binding to α_1 and α_2 adrenoceptors respectively suggested that cirazoline had a higher affinity for α_2 than α_1 adrenoceptors (CZ IC_{50} vs prazosin 4×10^{-5}, IC_{50} vs clonidine 5×10^{-6}). In vivo studies supported an action on α_2 adrenoceptors as cirazoline demonstrated an inhibition of the centrally mediated α_2 adrenoceptor depressor response to clonidine. The fall in MAP of 11 ± 6 mm Hg after i.v. clonidine (15 μg/kg) was abolished by pretreatment 20 min before with cirazoline (12.5 μg/kg) 0.25 ± 8.6 mm Hg ($p < 0.05$). Further, the peripheral, postsynaptic, α_2 adrenoceptor pressor response to guanabenz (100 μg/kg) was attenuated by cirazoline (12.5 μg/kg), control guanabenz pressor response 26 ± 4, after cirazoline 4 ± 7. Unlike phenylephrine, no β adrenoceptor agonist activity of cirazoline could be demonstrated.

Thus while cirazoline is a specific α_1 agonist at peripheral α_1 adrenoceptors, devoid of β adrenoceptor activity, we believe that cirazoline may also have antagonist effects on both central and peripheral α_2 adrenoceptors.

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THE HYPOTENSIVE EFFECT OF KETANSERIN IN ANAESTHETIZED NORMOTENSIVE RATS

J.R. Fozard, Centre de Recherche Merrell International, 16, rue d'Ankara, 67084, Strasbourg, Cedex, France

Ketanserin is a potent inhibitor of ^{3}H-spiroperidol binding to 5-HT$_2$ receptors in rat frontal cortex (Leysen et al. 1981) and a selective antagonist of vascular responses to 5-HT in vitro (Van Nueten et al. 1981). The compound lowers blood pressure (BP) in both normotensive and spontaneously hypertensive rats (Van Nueten et al. 1981) and in hypertensive man (De Cree et al. 1981). Blockade of the 5-HT receptor(s) mediating vasoconstriction and/or vascular sensitization is the novel mechanism which has been suggested to be an important factor in the hypotensive response (De Cree et al. 1981; Van Nueten et al. 1981). However, ketanserin also interacts with α_1-adrenoceptors at relatively low concentrations in vitro (Leysen et al. 1981; Van Nueten et al. 1981), and it seemed important to define the extent to which α_1-adrenoceptor blockade contributes to the hypotensive effects of ketanserin in vivo.

Male Sprague-Dawley rats (Charles River, France) weighing 250-300 g were anaesthetized with sodium pentobarbitone, 60 mg/kg i.p. plus 15 mg/kg s.c. They were used either as anaesthetized preparations or they were pithed and respired artificially. Blood pressure and heart rate (HR) were recorded by standard techniques and injections were made into a femoral vein.

In anaesthetized, normotensive rats ketanserin, 0.25 and 1.0 mg/kg, produced dose-related falls in BP and HR which were immediate in onset and sustained for the period of observation (90 min). Over the same time period, in pithed preparations, BP responses to stimulation of the whole spinal sympathetic outflow (2Hz; 5s; 1ms; 80V) repeated every 5 min were reduced by 20-30% by the 0.25 mg/kg dose of ketanserin and by 40-50% by the higher dose. In similar experiments, responses to phenylephrine, 3 µg/kg, repeated every 8 min were reduced by 20-30% and 70-80% by the low and high doses of ketanserin respectively. By contrast, BP responses to noradrenaline, 0.2 µg/kg, or angiotensin II, 0.15 µg/kg, were affected less markedly by ketanserin. At 0.25 mg/kg, responses to angiotensin II were not modified and, although those to noradrenaline were reduced initially by 10-15%, this occurred only during the first 15-20 min after injection. Following ketanserin, 1 mg/kg, responses to noradrenaline were inhibited by 20-30% for the duration of the experiment but responses to angiotensin II were reduced by 20-30% only during the initial 25-30 min following the injection.

Administration of the vascular 5-HT antagonist, B.W. 501C67 (Mawson & Whittington, 1970), in a dose (0.2 mg/kg) chosen to give 5-HT receptor blockade equivalent to that produced by ketanserin, 0.25 mg/kg, neither lowered BP in the anaesthetized rat, nor inhibited BP responses to phenylephrine or to sympathetic stimulation in the pithed preparations.

Thus, doses of ketanserin which lower BP in anaesthetized rats cause sustained inhibition of BP responses to phenylephrine and to stimulation of the whole spinal sympathetic outflow in pithed preparations. Since essentially complete blockade of vascular 5-HT receptors by B.W. 501C67 did not result in a fall in BP, α_1-adrenoceptor blockade, rather than 5-HT receptor blockade, would appear to be a major factor contributing to the hypotensive action of ketanserin in normotensive anaesthetized rats. The relevance of these experiments to the antihypertensive action of ketanserin in humans remains to be established.

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PERGOLIDE DECREASES ARTERIAL PRESSURE IN PENTOBARBITONE ANAESTHETIZED DEBUFFERED AND CONSCIOUS RENAL HYPERTENSIVE DOGS

I. Cavero, Janine Lorrain and Josette Sabatier, Department of Biology SYNTHELABO (L.E.R.S.), 58 rue de la Glacière, 75013 Paris, France.

Pergolide is a dopamine receptor agonist which decreases arterial pressure in conscious spontaneously hypertensive (Yen et al., 1979) and pentobarbitone anaesthetized normotensive rats (Cavero et al., 1981). This effect is probably due to stimulation of dopamine receptors on peripheral sympathetic neurons leading to a reduction on sympathetic tone (Cavero et al., 1981). However, pergolide (30.0 µg/kg, i.v) did not lower blood pressure in pentobarbitone anaesthetized dogs (Cavero, 1981). In this study we investigated whether this lack of activity also occured in hypertensive dog models.

Mongrel dogs were anaesthetized with pentobarbitone sodium (35.0 mg/kg, i.v.), placed under artificial respiration and prepared for arterial pressure and heart rate measurement. The animals were debuffered by sectioning the vagi and carotid sinus nerves. Pergolide (30.0 µg/kg, i.v.) was given in control, sulpiride (0.5 mg/kg + 0.05 mg/kg/min, i.v.) or phenoxybenzamine (10.0 mg/kg, i.v.) plus yohimbine (2.0 mg/kg, i.v.) pretreated animals. A separate group of dogs was spinalized and the lumbar sympathetic chain to the right hind limb stimulated while femoral blood flow was measured. Pergolide was studied in control and sulpiride pretreated dogs. Finally, pergolide (150 µg/kg, p.o.) was administered to control and metoclopramide (1.0 mg/kg, p.o) pretreated renal hypertensive dogs which had a carotid loop for the measurement of systolic arterial presssure.

In debuffered dogs, pergolide significantly decreased mean arterial pressure by 38 $\pm$ 6 mmHg (initial value: 178 $\pm$ 6 mmHg, n=12) and heart rate by 27 $\pm$ 5 beats/min (initial value: 192 $\pm$ 7 beats/min). These effects lasted approximately 60 min and were blocked by sulpiride. In phenoxybenzamine plus yohimbine pretreated dogs pergolide failed to change arterial pressure. In spinal dogs pergolide inhibited the decrease in blood flow produced by stimulation of the lumbar sympathetic chain. This effect was blocked by sulpiride. In conscious, chronic renal hypertensive dogs, oral administration of pergolide was accompanied by intensive vomiting (11 times during the 1st hour) lasting approximately 2 h. During this period heart rate increased, reaching a peak of 31 $\pm$ 4 beats/min over the control value of 48 $\pm$ 2 beats/min (n=7). This effect lasted approximately 3 h. The systolic arterial pressure decreased maximally by 18 $\pm$ 2 mmHg (control value 192 $\pm$ 3 mmHg) by the end of the emetic phase and lasted for over 6 h. Metoclopramide blocked the effects of pergolide on arterial pressure and strongly reduced vomiting.

These results indicate that pergolide can lower arterial pressure in dogs with an experimental hypertension. This effect is proposed to result from stimulation of dopamine receptors on sympathetic terminals innervating the resitance vessels leading to a reduction on sympathetic tone.

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EFFECT OF THEOPHYLLINE ON THE PULMONARY VASCULAR RESPONSE TO MEDIATORS

Lesley.E. Boardman, J. Morley & S. Sanjar,
Department of Clinical Pharmacology
Cardiothoracic Institute, Fulham Road, London SW3 6HP.

Increased plasma protein extravasation (IPPE) in response to intradermal injection of histamine or bradykinin is inhibited by local administration of beta-adrenoceptor agonists in both animals (Beets & Paul, 1980) and man (Basran & Paul, 1981). We have only achieved insubstantial inhibition (< 25%) of skin responses in the guinea-pig using theophylline (10 ng-10 ug per site). However, theophylline has been reported to share with beta-adrenoceptor agonists the capacity to inhibit oedema in lung tissue following exposure to aerosolised histamine (Persson, Ekman & Erjefalt, 1979). In view of this discrepancy, we have sought to confirm their observation using a perfused guinea-pig lung preparation.

Guinea-pig lungs were perfused in situ without ventilation; gassed Krebs solution containing 4% (w/v) bovine serum albumen was infused via the pulmonary artery at constant flow (6 ml/min), with a perfusion pressure of circa 12 cm of water at an environmental temperature of 37 C (McDonald and Boardman, 1980). Extravasation of plasma protein (125 I-fibrinogen) was monitored by a gamma spectrometer and count rates logged by a micro computer system (Davies et al, 1981).

Histamine infusion (18 ug over 2 min) produced a transient (<10 min) increase in perfusion pressure and a progressive IPPE maximal at 5-10 min, whilst leukotriene D4 (300 ng over 2 min) produced a sustained plasma protein accumulation without noteworthy change in perfusion pressure. Both increased perfusion pressure and IPPE in response to histamine were inhibited by concomitant administration of theophylline (50 ug/ml), in agreement with the in vivo observations of Persson et al (1981). It is anticipated that this technique will allow analysis of the mechanism whereby theophylline achieves this effect in the lung.

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A PHARMACOLOGICAL COMPARISON OF DOPAMINE RECEPTORS IN THE CAT HEART AND THOSE MEDIATING EMESIS IN THE DOG

G.M. Drew, Alma J. Gower, A. Hilditch & A.S. Marriott, Department of Pharmacology, Glaxo Group Research Ltd., Ware, Hertfordshire, SG12 ODJ.

The potencies of several dopamine receptor agonists and antagonists were determined at presynaptic dopamine receptors in the cat heart (Ilhan & Long, 1975) and at dopamine receptors mediating emesis in the conscious dog (Long et al, 1979).

Dopamine and test compounds were injected, intravenously, into chloralose anaesthetised cats pretreated with DMI. Their potencies relative to dopamine, for inhibiting the tachycardia produced by continuous electrical stimulation (2Hz) of the decentralised cardiac accelerans nerve were determined. The same agonists were injected intravenously into conscious dogs and their relative potencies for inducing emesis were also determined.

Dopamine (0.3 - 10 μg/kg) decreased the stimulation-evoked tachycardia in anaesthetised cats. Higher doses (30 - 1000 μg/kg) were required to produce emesis in dogs. All agonists, with the exception of the 'selective postsynaptic' agonists SKF 38393 and SKF 82526 (Weinstock et al, 1980) mimicked dopamine in both tests. Results are shown in Table 1.

Table 1 Equipotent doses (Dopamine = 1) of agonists for reducing the tachycardia in cats or producing emesis in dogs.

Dopamine agonists	Reduction in tachycardia	Emesis
N,N-di-n-propyl 5,6-ADTN	0.005	0.001
N,N-di-n-propyl 6,7-ADTN	0.05	0.01
6,7-ADTN	0.15	0.08
Apomorphine	1.5	0.05
N,N-di-n-propyl dopamine (DPDA)	3.6	1.6
N,N-diethyl dopamine	6.3	2.3
5,6-ADTN	29.4	0.9
SKF 82526	> 100	20.9
SKF 38393	> 100	> 30

Cat heart results are geometric means from dose-response curves in 5 experiments: Emesis results were obtained from dose-response curves with at least 6 dogs per dose level.

Antagonist potency was determined 15 mins after intravenous administration, using DPDA as the agonist. In anaesthetised cats, DPDA (30 μg/kg) reduced the tachycardia to nerve stimulation by approximately 38%. In dogs, DPDA (600 μg/kg) was the lowest dose that consistently produced emesis. In both groups of experiments the dose of antagonist required to produce a 50% reduction (ED_{50}) in the response to DPDA (30 or 600 μg/kg) was determined. The ED_{50} values for fluphenazine, cis α-flupenthixol and sulpiride were 17, 19 and 33 μg/kg respectively in cats and 43, 50 and 72 μg/kg respectively in dogs.

The potencies of the agonists and antagonists in the two tests suggest that the receptors involved are similar in many respects. However, apomorphine and 5,6-ADTN were more potent at causing emesis than would be predicted from the cat heart results. Nevertheless, these receptors are clearly different from the vascular dopamine receptors in the dog mesentery (Drew & Hilditch, 1981).

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DOPAMINE RECEPTORS MEDIATING VASODILATION IN THE RABBIT ISOLATED PERFUSED MESENTERIC VASCULAR BED

R.A. Brown, S.E. O'Connor, G.W. Smith & Anne Verity, Department of Pharmacology, Fisons Ltd., Pharmaceutical Division, Research & Development Laboratories, Bakewell Road, Loughborough, Leicestershire, LE11 0QY.

We report a preliminary characterisation of postjunctional dopamine (DA) receptors in the rabbit mesenteric vascular bed. Dutch rabbits (1-2 kg) were stunned and exsanguinated. The superior mesenteric artery was catheterised and all other major vessels except for the mesenteric vein were ligated. The appropriate section of ileum was then removed into an organ bath and the vasculature perfused with Locke solution (6 ml/min, 37°C, 100% O_2). Phenoxybenzamine ($5x10^{-6}$M) was perfused for 30 minutes only, prior to inducing vasoconstriction with a high K+ (80 mM) Locke solution. Propranolol (10^{-6}M), cocaine ($5x10^{-5}$M) and metanephrine ($5x10^{-5}$M) were present throughout. Agonists were administered as bolus injections ($\leq$ .01 ml) into the arterial catheter and changes in perfusion pressure measured.

DA ($3x10^{-10}$ - $3x10^{-7}$ moles)-induced dilations (but not those to papaverine) were specifically antagonised by cis (Z)-flupenthixol (Lundbeck). Arunlakshana-Schild plots gave pA_2 8.5 ± 0.1, slope 1.51 ± 0.23, n=6. In some preparations cis (Z)-flupenthixol reduced resting perfusion pressure and on occasions maximum responses to DA were depressed. The relative potencies of a series of DA receptor agonists (antagonised by cis (Z)-flupenthixol) are shown in Table 1.

TABLE 1 - Relative potencies of agonists at vascular dopamine receptors in the rabbit isolated perfused mesenteric vascular bed.

	Relative Potency	
	Mean	Range
6,7-ADTN	0.6	(0.45 - 0.75)
Dopamine	1	
N,N-di-n-propyl 5,6-ADTN	2.9	(1.8 - 4.8)
Apomorphine	4.7	(3.2 - 7.0)
N,N-dimethyl 5,6-ADTN	6.7	(3.6 - 10.0)
N,N-di-n-propyldopamine	42	(30 - 50)
5,6-ADTN	51	(22 - 130)
SKF 38393	77	(32 - 123)

Results are means and ranges (n = 3-4). Reference agonist - DA.
5,6-ADTN = 2-amino-5,6,dihydroxy-1,2,3,4-tetrahydronaphthalene.
6,7-ADTN = 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene.

Comparison of the aminotetralin analogues indicates that the β-rotameric conformation (6,7-ADTN) is preferred, although increasing the N-alkyl substituent size of 5,6-ADTN progressively enhances potency.

Thus, vascular DA receptors in the isolated mesenteric vascular bed of the rabbit resemble those of the rabbit splenic artery (Hilditch and Drew, 1981), but differ in some respects (e.g. the potencies of apomorphine and N,N-dimethyl 5,6-ADTN relative to DA) from canine renal vascular DA receptors (Goldberg et al 1978).

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COMPUTER ASSISTED COMPARTMENTAL ANALYSIS OF ^{45}CALCIUM EFFLUX FROM VASCULAR SMOOTH MUSCLE

R.W. Foster, G.R. Warburton & A.H. Weston, Department of Pharmacology, Materia Medica & Therapeutics, Medical School, Manchester University, M13 9PT

The analysis of ^{45}Ca efflux from smooth muscles provides dynamic data on total calcium movement within a tissue. Although such analysis is not without its limitations (Daniel et al, 1979) it remains an important tool in the study of transmembrane calcium flux.

^{45}Ca efflux from almost all smooth muscles follows a multi-exponential time course which can usually be resolved into a minimum of three components (Brading, 1979). Analysis of these components is commonly attempted by "peeling" of the efflux curve and subsequent calculation of rate constants and ^{45}Ca compartment sizes for the individual components. This assumes the individual components are independent of each other with no back-efflux (re-uptake by one compartment from another) occurring. The method described here utilises computational techniques for determining the compartment parameters from the whole efflux curve.

The rat vascular tissues studied (mesenteric artery, renal artery, caudal artery and hepatic portal vein) were mounted for isometric tension recording in capillary organ baths (vol. approx 1ml) and superfused at 0.5 ml/min with a physiological salt solution (PSS) at 37^{0}C. Tissues were loaded with ^{45}Ca 250nCi/ml in a PSS containing 2.5 mmol/l Ca^{2+} for 2h (the time to load the tissues to equilibrium). ^{45}Ca was then allowed to efflux from the tissues by superfusing with a non-radioactive PSS. The amount of ^{45}Ca remaining in the tissue after 90 min efflux, and the amount effluxing in each sample was measured by liquid scintillation counting and used to construct a ^{45}Ca efflux curve.

When subjected to curve peeling analysis, efflux curves from all the tissues tested could be resolved into three components. Mathematical models consisting of three compartments were therefore designed. Different models allowed the possibility of ^{45}Ca efflux from the tissues through compartmental pathways in series, in parallel, or a combination of both, and could account for errors due to back-efflux. The equations describing the theoretical movement of ^{45}Ca in each model were supplied to a computer programme designed to produce a theoretical efflux curve when supplied with values for rate constants and compartment size parameters of a particular model. Using curve-fitting subroutines (Minuit function minimisation routines supplied by the CERN library) the computer programme then altered the various parameters to fit the theoretical efflux curve to an experimental curve provided using a least squares minimisation technique.

It was possible to find for each tissue one model which gave a better fit to the experimental data than any other model. Using these models to analyse efflux data obtained in the presence of agents such as hydralazine or D600 which interfere with calcium movement, it may be possible to clarify their locus and mechanism of action.

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DILTIAZEM PROTECTS THE RABBIT HEART AGAINST THE MECHANICAL DYSFUNCTION ASSOCIATED WITH THE "CALCIUM PARADOX" PHENOMENON

J.P. Boudot and I. Cavero, Department of Biology SYNTHELABO (L.E.R.S.), 58 rue de la Glacière, 75013 Paris, France.

The heart of several animal species when reperfused with Ca^{++} containing solutions after a brief exposure to Ca^{++}-free media exhibits severe mechanical, biochemical and structural dysfunctions (Zimmermans & Hulsmann, 1966). This phenomenon is known as "calcium paradox" and has been attributed to massive increase in cytoplasmic free Ca^{++} (Hearse et al., 1978). Recently, verapamil was shown to afford substantial protection against the biochemical effects associated with "calcium paradox". However, this observation was not supported by Ruigrok et al. (1980). We now report the effects of diltiazem on the mechanical effects characterizing the "calcium paradox".

Isolated rabbit hearts were prepared for Langendorff perfusion and paced, as previously described (Boudot & Cavero, 1979). After an appropriate equilibration period, they were exposed to a Ca^{++}-free medium for periods of 1, 2, 3, 4, 5, 7 and 10 min and, then, reperfused for 30 min with a Ca^{++} (2.2 mM) containing solution. Diltiazem (1.0-10.0 µM) was added to the perfusing medium at the beginning or during the last 2 of the 4 min Ca^{++}-free perfusion. In addition, diltiazem was given during the first 5 min of Ca^{++} readmission.

The rabbit heart ceased to contract within 1 min following the initiation of the perfusion with a Ca^{++}-free medium. When this procedure lasted more than 2 min, there was a significant rise in resting tension ("calcium paradox") upon readmission of Ca^{++}. The latter effect was maximal (5.5 $\pm$ 0.6 g, n=9, over the baseline value) after 4-5 min perfusion with a Ca^{++}-free medium and occurred within the first few min of Ca^{++} readmission. Diltiazem (10.0 µM) when present throughout the 4 min perfusion with a Ca^{++}-free medium significantly reduced the magnitude of the "calcium paradox" (0.9 $\pm$ 0.4, n=5). At the end of 30 min reperfusion with a Ca^{++} containing medium, hearts treated with diltiazem recuperated 47% of their initial contractile force, in contrast to the 17% recovery of the control preparations. Diltiazem, 1.0, 3.0 and 10.0 µM, given during the last 2 min of the 4 min Ca^{++}-free perfusion reduced by 20, 60 and 90%, respectively, the extent of "calcium paradox". The corresponding values for the recuperation of contractile force at the end of 30 min reperfusion were 23, 40 and 59%. However, diltiazem (10.0 µM) failed to modify "calcium paradox" when given during the first 5 min of Ca^{++} readmission.

These results indicate that addition of diltiazem to a Ca^{++}-free medium used to perfuse the isolated rabbit heart inhibits markedly the increase in resting tension occuring upon readmission of Ca^{++} to the perfusate. It is suggested that diltiazem prevents either myocyte ultrastructure disruption and/or cytoplasmic overloading of Ca^{++} which are probably responsible for the "calcium paradox" phenomenon.

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CALCIUM ENTRY BLOCKING PROPERTIES OF TANSHINONE II-A SULPHONATE, AN ACTIVE PRINCIPAL OF THE ANTIANGINAL EXTRACT, DAN SHEN

L. Patmore & R.L. Whiting, Department of Pharmacology, Syntex Research Centre, Heriot-Watt University, Edinburgh EH14 4AS.

Dan Shen, from the roots of *Salvia miltiorrhiza* Bunge, is a well known traditional Chinese remedy used for the treatment of coronary disorders. Recently an alcoholic extract, tanshinone II-A sulphonate (tanshinone) has been found to be an effective antianginal (Ming-Kun et al, 1978). An explanation for the antianginal properties of tanshinone could be calcium entry block (CEB) since CEB compounds such as verapamil and diltiazem have been used clinically to treat angina (Nayler, 1981). The possible CEB activity of tanshinone was evaluated on isolated guinea-pig papillary muscles using three methods:

1. Investigation of negative inotropic effects.
2. Effects on the extracellular calcium ion concentration ($(Ca^{2+})_o$) vs generated tension relationship.
3. Recording slow action potentials from depolarised myocardial cells.

Papillary muscles (1-5mg) were dissected from the right ventricle of 200-300g female guinea-pigs. The muscles were perfused with a Ringer solution of composition (mmol.litre^{-1}): 145 NaCl; 6 KCl; 2 $CaCl_2$; 1 $MgCl_2$; 5.5 glucose and 10 Hepes buffer, oxygenated and adjusted to pH 7.4 at 30^{0}C. The preparation was stimulated through field electrodes (2V, 0.5ms) at a frequency of 0.5Hz. After 1h equilibration, solution changes were made.

In experiments on the inotropic effects of tanshinone, the drug was added to the perfusate at concentrations in the range 1 x 10^{-6} to 5 x 10^{-5}mol.litre^{-1}. An ID_{50} value for tanshinone was calculated as 1 x 10^{-5}mol.litre^{-1}. The twitch tension generated by isolated papillary muscles is sigmoidally related to $(Ca^{2+})_o$. This relationship was mapped out at calcium concentrations in the range 2 to 20mmol.litre^{-1}. 1 x 10^{-6} and 1 x 10^{-5}mol.litre^{-1} tanshinone depressed the relationship in a dose-dependent manner. The negative inotropic effects of tanshinone were directly reversible by increasing $(Ca^{2+})_o$. A method of studying the calcium current in the myocardium is to record calcium dependent slow action potentials. These can be induced when the sodium current is inactivated by perfusing with 20mmol.litre^{-1} potassium. Increasing the stimulus intensity to 8V induced slow action potentials which were dependent on $(Ca^{2+})_o$ (Patmore, 1981).

Tanshinone at concentrations in the range 1 x 10^{-5} to 5 x 10^{-5}mol.litre^{-1} inhibited slow action potentials over a period of 15min. The magnitude, duration and area under the waveform were reduced in a dose-dependent manner. These effects were reversed by increasing $(Ca^{2+})_o$. 5 x 10^{-5}mol.litre^{-1} tanshinone completely inhibited slow action potentials. Similar results have been reported for verapamil and diltiazem (Harman & Poole-Wilson, 1981).

The data show that tanshinone is a negative inotropic agent which is due to block of the slow inward calcium current. The CEB properties of tanshinone are a possible explanation of its clinical efficacy.

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A NEW METHOD FOR INVESTIGATING THE ISOMETRIC CONTRACTILE RESPONSES OF SMALL BLOOD VESSELS

R. Towart, Institute of Pharmacology, Bayer A.G., D5600 Wuppertal 1, F.R.G. (present address: Miles Laboratories Ltd., Stoke Court, Slough SL2 4LY, England).

Present methods for investigating the contractile behaviour of isolated small blood vessels involve either spirally-cut strips (which may be technically difficult, and produce much damage to small vessels) or ring preparations suspended between two wires (e.g. Edvinsson et al., 1974). The latter method is elegant,but requires much care in positioning the rings, and is not suitable for testing many vessels simultaneously. Our new method uses two open, triangular metal holders (see Figure 1). The vessel (lumen 0.3mm or more) is threaded carefully on to the two straight ends (see Figure 1, No. 1-3), moved to the middle portion (No. 4-5), and one of the wires is then lifted out of the plane of the page to give the configuration shown (Figure 1, No. 6). This assembly of the vessel between the two wires may then be suspended in the organ bath between a fixed hook and the strain gauge transducer. This method has been used to investigate the pharmacology of rabbit saphenous and basilar arteries (Towart, 1981), and to compare arterial and venous reactivity (Berndt et al., 1981) The method is simple and inexpensive, and allows the investigation of many vessels simultaneously.

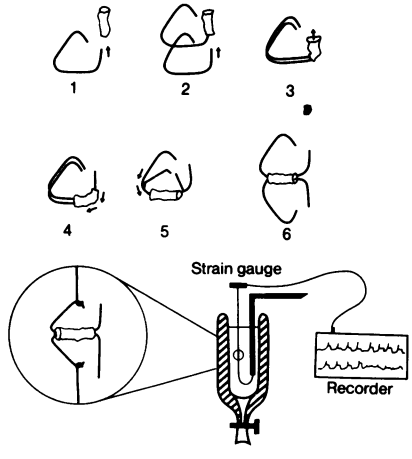

Figure 1 Procedure for suspending vessel segments between the holders.

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AGONIST INTERACTION WITH α_2-ADRENOCEPTOR BINDING SITES ON HUMAN PLATELET LYSATES: EFFECTS OF Mg++ AND TEMPERATURE

D.B. Barnett, Y.-D. Cheung & S.R. Nahorski, Department of Pharmacology, University of Leicester, Leicester, LE2 7LX.

Agonist interaction with catecholamine receptors has been shown to induce high and low affinity states of the receptor. These states, which are interconvertible under the influence of GTP and are a prerequisite of agonist activation of the receptor, have been demonstrated for systems positively (beta) and negatively (alpha) coupled to adenyl cyclase (Kent et al, 1980; Michel et al, 1980). ^{3}H-yohimbine, a potent and selective alpha$_2$-adrenoceptor antagonist, specifically labels the alpha$_2$-adrenoceptor of human platelet membranes (Daiguji et al, 1981). Using this ligand we have examined adrenaline displacement of specific binding with reference to the effects of Mg++, G.T.P. and temperature.

Platelet rich plasma was obtained from 5 healthy male volunteers. After centrifugation at 27,000 g. the platelet pellet was resuspended in lysing buffer (5mM Tris. HCl pH 7.5 with 5mM EDTA). The lysates were gently homogenised, washed twice and resuspended in assay buffer (50mM Tris. HCl pH 7.5 with 0.5mM EDTA). Assays were performed on freshly prepared membranes with approximately 250 µg membrane protein, 2nM ^{3}H-yohimbine (84 Ci/mmol, NEN) and variable concentrations of (-)-adrenaline in a final volume of 250 µl. Incubations were for 30 minutes at room temperature (RT) or 37°C as appropriate, and were terminated by addition of 1 ml. of ice cold assay buffer and rapid vacuum filtration. Bound radioactivity on the filters was assessed by liquid scintillation counting. Non-specific binding was calculated in parallel incubations using 5 µM phentolamine. In this system ^{3}H-yohimbine Kd = 1.6nM, B.max = 180 fmol/mg. protein.

Results are the means ± SE of the 5 separate experiments. At RT in the presence of 8mM $MgCl_2$ (Mg++) adrenaline displacement of ^{3}H-yohimbine produced curves of low Hill slope (nH = 0.66 ± 0.03) and overall IC_{50} = 7.4 ± 1.0 x 10^{-8}. Addition of 0.1mM GTP caused a 10 fold shift of these curves to lower affinity (IC_{50} = 7.5 ± 1.8 x 10^{-7}) and steepening of the Hill slope (nH = 1.07 ± 0.04). Parallel experiments at 37°C showed adrenaline was significantly less potent at displacing ^{3}H-yohimbine binding in the presence of 8mM Mg++; overall IC_{50} = 3.3 ± 0.2 x 10^{-7} ($P < 0.001$). This was associated with a significant steepening of the Hill slope (nH = 0.72 ± 0.02, $P < 0.05$). Addition of 0.1mM GTP at 37°C produced curves little different from RT (IC_{50} = 1.9 ± 0.14 x 10^{-6}, nH = 1.05 ± 0.06).

Computer iterative curve fitting of the mean data from displacement curves in the presence of Mg++ indicated the presence of two binding sites with very similar affinities at the two temperatures; low affinity (L) 1.1 x 10^{-6} and 6.9 x 10^{-7} (RT and 37°C respectively), high affinity (H) 2.4 x 10^{-8} (RT and 37°C). However, the proportion of these two sites was different at the two temperatures. Thus, H 66%, L 34% at RT and H 24%, L 76% at 37°C accounting for the increase in Hill slope towards unity at the higher temperature.

These results confirm, using the specific radioligand ^{3}H-yohimbine, the presence of high and low affinity agonist states of the alpha$_2$-adrenoceptor on platelet membranes (Michel et al, 1980). The effect of temperature on overall agonist affinity for these binding sites is a novel observation. The indication in preliminary experiments that this effect is due to alterations in the proportion of high and low affinity states of the receptor requires further evaluation. The clinical relevance of these observations with regards to the effects of temperature on adrenaline induced platelet aggregation is being investigated.

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SELECTIVE POST-JUNCTIONAL SUPERSENSITIVITY TO α_2- ADRENOCEPTOR AGONISTS AFTER RESERPINE PRETREATMENT IN RATS

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

Postjunctional receptor supersensitivity frequently occurs after sympathectomy (Trendelenburg 1963), although after reserpine pretreatment it is unclear whether supersensitivity to agonists is due to receptor denervation or as a result of non-specific post-receptor mechanisms (Carrier 1975). Postjunctional α_1 and α_2-adrenoceptors are now thought to mediate vasoconstriction in the rat (see review Timmermans & Van Zwieten 1981). A small degree of supersensitivity has been demonstrated at postjunctional α_2-adrenoceptors after treatment with 6-hydroxydopamine in the pithed rat (Baker & Drew 1981), furthermore α-adrenoceptor binding characteristics in rat salivary gland (Bylund & Martinez 1980) and mesentery (Colucci et al 1981) are altered after reserpine pretreatment. The effects of reserpine, at a dose which caused functional sympathectomy, have been studied on the vascular responses induced by α_1 and α_2-adrenoceptor agonists in vivo.

Male Wistar rats (250-350g n = 6-8) were pithed under pentobarbitone anaesthesia (60mg/kg i.p.) and respired with room air. Vasoconstrictor dose-response curves (diastolic blood pressure) were induced by i.v. injections of the following agonists, noradrenaline (NA), phenylephrine (PE), methoxamine, the α_2-agonists, TL99 or UK14.304 or 5-hydroxytryptamine (5HT) in reserpinised rats (2mg/kg/day, 3 days), or matched ascorbic acid (20%, 1 ml/kg/day, 3 days) controls. Only two agonist response curves were studied in each pair of rats. Sympathectomy was assessed by the lack of vasoconstriction to stimulation of the sympathetic chain (30V, 1 ms, 1-10Hz). A further group of rats (n = 8) were adrenal demedullated 2 weeks prior to pretreatment with 6-hydroxydopamine (2x50mg/kg, day 1, 2x100mg/kg, day 6, used on day 8)

In comparison to untreated controls reserpine induced a mean 8.1, 4.4, 4.2 ($p<0.01$) and 1.84 (NS) fold-shift to the left in the dose response curves to UK14.304, TL99, NA, and PE respectively at an EC50 level; reserpine did not affect the maximum responses obtained for these agonists. A rightward 1.2 or 1.4 ($p<0.05$) fold-shift was obtained for methoxamine or 5HT respectively, the maximum responses to these agonists were significantly ($p<0.05$) decreased. In contrast no supersensitivity to TL99 or PE was shown in 6-hydroxydopamine treated adrenal-demedullated rats, but a 3.9 ($p<0.01$) fold-shift to the left of the noradrenaline response curve was obtained, possibly as a result of removing neuronal uptake. The dose of rauwolscine (α_2-antagonist) required to cause a 50% inhibition (ED50) of the vasconstriction induced by TL99 (5µg/kg i.v.) was 0.78(0.47-1.30)mg/kg for controls and did not differ 1.11(0.81-1.52)mg/kg in reserpinised rats; for prazosin against PE, antagonist ED50 values of 0.002(0.0014-0.0035)mg/kg for controls and 0.0019(0.0016-0.0036)mg/kg for reserpinised rats were obtained.

Reserpine pretreatment induced selective supersensitive pressor responses to α_2-adrenoceptor agonists, which were not demonstrated in 6-hydroxydopamine, adrenal-demedullated rats. Antagonist potency at postjunctional α_1 or α_2-adrenoceptors was not changed. Increased sensitivity to α_2-adrenoceptor agonists may therefore result from post-receptor changes, rather than post-junctional denervation supersensitivity.

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CIRAZOLINE POSSESSES α_2-ADRENOCEPTOR BLOCKING PROPERTIES IN ADDITION TO ITS α_1-ADRENOCEPTOR AGONIST ACTIVITY

I. Cavero, S.Z. Langer, F. Lefèvre-Borg & N. Shepperson Department of Biology SYNTHELABO (L.E.R.S.), 58 rue de la Glacière, 75013 Paris, France.

Cirazoline, an imidazoline derivative being developed as a nasal decongestant, is a potent α_1-adrenoceptor agonist (Roach et al, 1979; Massingham et al, 1979). Cavero et al (1981) found that cirazoline did not stimulate presynaptic α_2-adrenoceptors in the dog and rat heart. However, this property was suggested by studies with the cat spleen preloaded with ^{3}H-noradrenaline where cirazoline inhibited the overflow of tritium elicited by postganglionic stimulation (Dubocovich et al, 1980). We now report that cirazoline possesses α_2-adrenoceptor antagonist properties in several preparations.

Rats were pithed and prepared to study cardiac presynaptic α_2-adrenoceptors (Cavero et al, 1981). Heart rate was increased by 75-85 beats/min by stimulation of the thoracic spinal cord. Dose-response curves to clonidine (requiring 20 min to be performed) were generated in control and cirazoline pretreated rats. Cirazoline (1.0 µg/kg/min, i.v.) was infused for 5 min and 10 min later clonidine was administered. In a further study the same dose of cirazoline was infused continuously 10 min before and during the construction of the dose-response curve to clonidine. Cirazoline itself did not significantly modify the experimental tachycardia. In control experiments the dose of clonidine required to inhibit by 50% (ED_{50}) the submaximal sympathetic tachycardia was 0.8±0.1 µg/kg, i.v. (mean±s.d.). After 5 min infusion of cirazoline this value was significantly increased to 2.1±0.1 µg/kg. In the continuos infusion experiments the ED_{50} was further increased to 5.4 ± 0.3 µg/kg.

In the field stimulated rat isolated vas deferens (Massingham et al, 1979) concentration-response curves to cirazoline were generated before and after prazosin. In preparations pretreated with prazosin (30 nM), the twitch response was inhibited with clonidine (30.0 nM) and increasing concentrations of cirazoline and yohimbine added. Finally, a concentration-response curve was generated in control and cirazoline (1.0 and 5.0 µM) pretreated preparations. As previously reported (Massingham et al 1979) cirazoline (10 nM to 30 µM) enhanced the twitch responses of the field stimulated vas deferens. An increase of 100% in the twitch response was observed with 1.0 µM cirazoline. After pretreating the preparation with prazosin (10nM), the concentration-response curve to cirazoline was slightly shifted to the right and its maximum was increased by approximately 50%. In vas deferens pretreated with prazosin, yohimbine (EC_{50} = 0.32 ± 0.03 µM) and cirazoline (EC_{50} = 4.9 ± 1.5 µM) antagonized in a concentration-related manner the inhibitory effects of clonidine (30 nM) on the twitch response. Furthermore, pretreatment with cirazoline (1.0 and 5.0 µM) displaced the concentration-response curve to clonidine to the right, the calculated pA_2 value being 7.3. ± 0.1 (n=7).

These results indicate that cirazoline behaves as a functional antagonist of presynaptic α_2-adrenoceptors in rat heart and vas deferens. This property of cirazoline (confirmed by: Hannah et al., P62, this meeting; R. Ruffolo; A.G. Roach and C.F.C. Smith: personal communications) might be favourable for a nasal decongestant since it could prevent or reduce the "rebound" congestion often reported with other imidazoline derivatives.

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SELECTIVE α_2-ADRENOCEPTOR ANTAGONIST ACTIVITY OF NOVEL SUBSTITUTED BENZOQUINOLIZINES

N. Lattimer, K.F. Rhodes, T.J. Ward, J.F. Waterfall & J.F. White, Departments of Pharmacology and Chemistry, Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow, Maidenhead, Berkshire.

Selective α_2-adrenoceptor antagonist activity has been reported amongst the yohimbine stereoisomers (Weitzell et al, 1979) and the benzodioxans (Michel & Whiting, 1981). We describe here novel substituted benzoquinolizines which also possess selective α_2-adrenoceptor antagonist actions. Comparisons have been made with the α_2-selective antagonist yohimbine and the α_1-antagonist indoramin (Rhodes & Waterfall, 1978).

α_1-adrenoceptor antagonism was assessed using the rat isolated anococcygeus muscle (Doxey, 1979). Cumulative dosing with methoxamine hydrochloride was used and contractions were recorded isotonically. The rat isolated vas deferens was used for determining α_2-adrenoceptor antagonism (Rhodes & Waterfall, 1978). Cumulative dosing with clonidine hydrochloride was used and contractions were recorded isometrically. Each tissue was exposed to 3 increasing concentrations of a single antagonist (30 min equilibration). Agonist dose-response curves were analysed by the method of Arunlakshana and Schild (1959). Indoramin was tested only at 10^{-5}M in the α_2-estimation, but was treated like the other agents for the α_1-estimation.

Table 1 α_1/α_2 selectivity of adrenoceptor antagonists

Compound	Vas deferens (α_2)		Anococcygeus (α_1)		α_1/α_2*
	pA_2 (95% limits)	Slope	pA_2 (95% limits)	Slope	ratio
(1) Wy 24965	6.27 (5.98-6.98)	0.68	5.33 (5.20-5.48)	1.12	9
(2) Wy 25309	7.81 (7.52-8.32)	1.17	6.04 (5.98-6.10	1.14	59
(3) Wy 26392	8.08 (7.84-8.43)	1.18	6.34 (6.23-6.46)	1.08	55
(4) Wy 26703	8.46 (8.17-8.94)	0.89	6.49 (6.37-6.63)	0.96	93
Yohimbine	7.58 (7.44-7.70)	0.83	6.58 (6.42-6.76)	1.02	10
Indoramin	<5.0	-	8.15 (7.98-8.35)	1.14	<0.0007

* Antilog (α_2 pA_2 - α_1 pA_2)

General structural formula: $R_1\text{-}N\begin{smallmatrix}R_2\\R_3\end{smallmatrix}$

R_1=1,3,4,6,7,11bα-hexahydro-2H-benzo-{a}-quinolizin-2β-yl. (1) R_2=H;R_3=$MeSO_2$. (2) R_2=Me; R_3=$MeSO_2$. (3) R_2=Me; R_3=n-$PrSO_2$.(4) R_2=Me; R_3=i-$BuSO_2$.

The results (Table 1) show that the benzoquinolizines Wy 25309, 26392 and 26703 have greater selectivity for the α_2-adrenoceptor than does yohimbine. Wy 26392 and 26703 are also more potent antagonists than yohimbine at the α_2-adrenoceptor. The Schild plot slopes are close to the theoretical value of unity for competitive antagonism in all cases with the exception of the value for Wy 24965 at the α_2-adrenoceptor.

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THE RELEASE OF (^{3}H)-NORADRENALINE FROM THE MOUSE VAS DEFERENS BY TRAINS OF 1-100 ELECTRICAL PULSES

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

The hypothesis of α_2-adrenoceptor mediated regulation of noradrenaline release has recently been questioned (Kalsner, 1979; 1980). However, the interpretation of these experiments is difficult because tritium release (after preloading with ^{3}H-noradrenaline) was measured and this may not reflect accurately changes in noradrenaline. In the present experiments ^{3}H-noradrenaline released by 1 - 100 pulses (in the presence and absence of the α_2-adrenoceptor agonist clonidine and antagonist yohimbine) was separated from its metabolites.

Vasa deferentia were removed from T.O. mice (Marshall et al., 1978) and incubated for 30 mins in 0.59 µM 1-7,8-^{3}H-noradrenaline (39 Ci/mmol, Radiochemical Centre, Amersham). Subsequently 4 vasa were tied together in an organ bath filled with magnesium-free Krebs solution (2.5 mM calcium, no neuronal or extra-neuronal uptake blockers present) at 37°C. The vasa were stimulated using parallel platinum electrodes with pulses of 2.0 ms and 64 V. The tritium released into the Krebs solution was separated into noradrenaline and its metabolites using alumina and Dowex column chromatography (Graefe et al., 1973).

About 6% of the basal release of tritium was noradrenaline, the rest being dihydroxyphenylethyleneglycol (DOPEG, 34%) and o-methylated metabolites about 60%. Stimulation with a single pulse did not alter this pattern. Trains of 10 and 100 pulses at 1.0 Hz increased noradrenaline overflow above the basal level by about 60 and 900% resepctively while total tritium only increased by about 10 and 70%. The large increase in ^{3}H-noradrenaline was not accompanied by similar rises in its ^{3}H-metabolites. The fractional release per pulse of ^{3}H-noradrenaline did not differ with trains of 1, 10 or 100 pulses ($6.6 \pm 1.0 \times 10^{-6}$, $5.2 \pm 0.3 \times 10^{-6}$ and $5.6 \pm 0.4 \times 10^{-6}$ respectively, mean ± s.e. mean).

Clonidine (0.3 - 30 nM) decreased the fractional release per pulse of ^{3}H-noradrenaline following 10 pulses at 1.0 Hz in a concentration-dependent manner (84% inhibition at 30 nM) in parallel with decreases in maximum twitch tension.

The effect of yohimbine was investigated to see if noradrenaline released by 1, 10 or 100 pulses inhibited its own release via α_2-adrenoceptors. Yohimbine (10, 30 and 100 nM) did not alter the amount or composition of the basal release of tritium or the fractional release of ^{3}H-noradrenaline and twitch tension elicited by a single pulse ($P > 0.05$, paired t test). However, with 10 or 100 pulses at 1.0 Hz the fractional release of ^{3}H-noradrenaline and the maximum twitch tension was greater after yohimbine 30 or 100 nM compared with 1 or 3 nM ($P < 0.05$).

Under the present experimental conditions the fractional release per pulse of noradrenaline was independent of the number of pulses and could be reduced by the α_2-adrenoceptor agonist clonidine. The results with yohimbine suggest that noradrenaline regulates its own release via α_2-adrenoceptors with trains of 10 and 100 pulses at 1.0 Hz but no evidence for this regulation was found with a single pulse.

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ON THE SITES OF APOMORPHINE'S ACTION TO RELAX AND CONTRACT CIRCULAR SMOOTH MUSCLE FROM THE BODY OF GUINEA-PIG STOMACH

Brenda Costall, S.J. Gunning & R.J. Naylor, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP.

Dopamine, dependent on concentration, can cause both a relaxation and a contraction of circular smooth muscle strips taken from the body of guinea pig stomach, and both responses can be antagonised by α- and/or β-adrenoceptor antagonists. Whilst neuroleptic agents can also antagonise the relaxation responses it has been hypothesised that this antagonism also reflects an α_1-adrenoceptor antagonism (Costall et al, 1981). In an attempt to clarify the specificity of the dopamine-neuroleptic α-adrenoceptor site, the present experiments employ apomorphine as a reference dopamine agonist to determine whether the dopamine sites are also responsive to other dopamine agonists.

Male Dunkin-Hartley guinea pigs, 350-400 g, were killed by cervical trans-section and smooth muscle strips (15 mm x 5 mm) isolated from the body region of the stomach. The strips were dissected in a plane allowing investigation of tension changes in the circular muscle layer. The mucosal layer was removed and the tissue bathed in 15 ml oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution at 37°C containing 100 mg/l ascorbic acid. Tension changes were detected by Grass tension transducers and the response areas integrated (Illingworth & Naylor, 1980) in addition to display on a multichannel Grass recorder. One gram of tension was applied to the tissue which was allowed to equilibrate for 30-45 min before the addition of drugs. Antagonists were allowed a 45 min pretreatment.

A contraction and relaxation to dopamine (3.3×10^{-7} - 2.6×10^{-6} M) was initially established in each tissue to ensure tissue viability. The responses to the first addition of apomorphine (7.25×10^{-7} M) was invariably one of contraction, but this diminished on a second administration and was absent on the third and fourth when a relaxation phase became apparent. Relaxation responses to subsequent additions of apomorphine were repeatable and concentration-dependent (7.0×10^{-7} - 3.0×10^{-6} M). The relaxations were resistant to yohimbine (10^{-7} - 10^{-6} M), prazosin (10^{-8} - 10^{-6} M) and haloperidol (10^{-7} - 10^{-5} M) although dl-propranolol (10^{-7} - 10^{-6} M) effected a competitive antagonism. Subsequently, dl-propranolol (5×10^{-7} M) was routinely included in the Krebs-Henseleit solution to preclude action on β-adrenoceptors, an effectiveness of the antagonism being assessed against both the apomorphine and isoprenaline (2.5×10^{-9} - 10^{-8} M) relaxations. In the presence of dl-propranolol the contractile response to apomorphine was notably enhanced although, again, response size diminished on repeated administrations. The inclusion of yohimbine (10^{-6} M) in the propranolol-Krebs-Henseleit solution prevented the contraction response to apomorphine; prazosin (10^{-5} M) and haloperidol (10^{-5} M) were ineffective. That apomorphine interacts at the same contractile initiating site as dopamine was shown by the ability of repeated apomorphine administration to abolish a dopamine contraction.

Thus, similarly to dopamine, apomorphine can cause both contraction and relaxation of the circular smooth muscle of the body of guinea pig stomach. The contraction response to apomorphine is mediated at an α_2-type adrenoceptor, common with the α_2-type receptor activated by dopamine, but whose stimulation is rapidly followed by a 'desensitisation', perhaps reflecting a partial agonist-antagonist potential. The apomorphine induced relaxation is mediated at a β-adrenoceptor.

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EVIDENCE FOR AN Ra PURINE RECEPTOR IN THE GUINEA-PIG TRACHEALIS MUSCLE

C.M.Brown and M.G.Collis, Imperial Chemical Industries PLC, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4LF.

Burnstock (1978) has proposed that there are two major types of extracellular purine receptor. The P_1 purinoceptor is more responsive to adenosine and A.M.P. than to A.T.P. and is antagonised by the methylxanthines while the P_2 purinoceptor is activated by A.T.P. and A.D.P. and is antagonised by 2, 2^1 pyridylisatogen tosylate and quinidine. The effects of adenosine on adenylate cyclase from a variety of tissues has led to the further sub-classification of the adenosine (P_1) receptor into Ra receptors which activate and R_i receptors which inhibit adenylate cyclase (Londos et.al. 1980). The order of potency of certain adenosine analogues is different on the two receptor sub-types. However, they are both antagonised by methylxanthines. The purpose of the present study was to determine which of these sub-types of adenosine receptor is present in the guinea pig trachealis muscle.

Tracheal strips were prepared by the method of Emmerson & Mackay (1979) and mounted in Krebs solution at 37°C for isometric recording. The tissues were contracted with carbachol (5×10^{-7}M) and the effects of purines studied by the construction of cumulative concentration/effect curves. The response was expressed as a percentage of the maximal relaxation evoked by noradrenaline.

Adenosine, 2-chloroadenosine, adenosine 5^1 N·ethylcarboxamide (NECA) and L - N^6 phenylisopropyladenosine(PIA) all exhibited concentration dependent relaxations of the trachealis muscle, the order of potency being NECA>2-chloroadenosine>PIA>adenosine. 2-chloroadenosine elicited a greater maximum response than did the other three agonists, although a full dose response curve to PIA could not be constructed due to poor solubility.

Treatment of the tissues with dipyridamole (5×10^{-6}M), which blocks purine transport, potentiated responses to adenosine (n=4) did not effect those evoked by NECA, (n=9) or PIA, (n=7) but reduced the maximum response to 2-chloroadenosine (control 74 ± 3% (n=9); dipyridamole, 53 ± 3% (n=8)). In the presence of dipyridamole the agonist order of potency became NECA>2 -chloroadenosine> adenosine>PIA.

In tissues treated with dipyridamole (5×10^{-6}M), theophylline (1×10^{-4}M) antagonised the responses to adenosine, NECA and PIA (n>6). The responses evoked by 2-chloroadenosine were slightly antagonised but this effect was not significant.

These findings indicate that adenosine, NECA, 2-chloroadenosine and PIA are all potent relaxants of tracheal muscle. 2-chloroadenosine appears to have both an extracellular action , and an intracellular action which is apparent at higher concentrations and can be reduced by blocking purine transport. The relative potencies of adenosine, PIA and NECA in relaxing the trachealis muscle suggests that the extracellular adenosine receptor belongs to the Ra sub-type.

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THE EFFECTS OF PENTOBARBITONE SODIUM ON THE RESPONSES OF GUINEA-PIG TRACHEA TO ACETYLCHOLINE AND FIELD STIMULATION

N.S. Clennett, D.M. Jackson & I.M. Richards, Department of Pharmacology, Fisons Limited, Pharmaceutical Division, Research & Development Laboratories, Bakewell Road, Loughborough, Leicestershire, LE11 0QY.

Pentobarbitone sodium is commonly used as an anaesthetic in experimental animal work and the guinea pig is frequently used to measure the bronchoconstrictor or bronchodilator actions of drugs. It was of interest therefore to investigate the actions of pentobarbitone on tracheal smooth muscle in this species. In the present experiments the effects of pentobarbitone on acetylcholine or field stimulation-induced responses of guinea pig tracheal tubes were investigated. The isolated tracheal tubes were prepared to record changes in intraluminal pressure following application of drugs or field stimulation according to the method of Farmer & Coleman (1970).

Electrical field stimulation of the tracheal tube caused an initial increase in intraluminal pressure (the excitatory response) followed by a rapid fall in pressure (the inhibitory response). Neither response was affected by hexamethonium (3×10^{-7}M) but the excitatory response was blocked by atropine (2.8×10^{-7}M) and the inhibitory response was reduced (by 64%) by propranolol (3.4×10^{-6}M).

Increases in intraluminal pressure induced by field stimulation but not those induced by acetylcholine were inhibited by pentobarbitone (5×10^{-4}M). Pentobarbitone (5×10^{-4}M) also reduced the inhibitory response to field stimulation of the isolated trachea.

These findings may explain the differing reports regarding the effects of pentobarbitone on histamine-induced bronchoconstriction in the guinea pig *in vivo*. (Dennis & Douglas, 1970; Advenier et.al.1978; Drazen & Austen, 1975). In experiments where high doses of histamine cause a direct contraction of airways smooth muscle, the modulating effect of adrenergic and non adrenergic inhibitory neurones may be inhibited by pentobarbitone, thus potentiating the actions of histamine. In experiments were low doses of histamine cause a reflex bronchoconstriction the influence of pentobarbitone on the parasympathetic nerves would reduce the effects of histamine. The results of these experiments also demonstrate the care that is needed when selecting an anaesthetic for the assessment of bronchoactive substances in guinea pigs.

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DOPAMINERGIC MODULATION OF EVOKED VASOPRESSIN RELEASE FROM THE ISOLATED NEUROHYPOPHYSIS AND INVOLVEMENT OF ENDOGENOUS OPIOIDS

E. Muscholl, K. Racké, H. Ritzel & B. Trapp, Department of Pharmacology, University of Mainz, D-6500 Mainz

Neurohypophyses were isolated from adult female Sprague-Dawley rats. The effects of dopamine agonists and antagonists on the evoked vasopressin (VP) overflow from isolated neurointermediate lobes incubated in either modified Locke or Krebs medium were studied. The pituitary stalks were fixed by a fine platinum wire clip electrode. The VP content of the incubation media was determined either on the BP of the pithed rat or by a radioimmunological method. Two kinds of electrical stimulation were carried out either during one(S1) or two (S1 and S2) periods (Douglas & Poisner, 1964; Iversen et al, 1980).

Procedure A (1 ms, 50 V, 10 Hz, 5times for 1 min with 1 min intervals, total of 10 min; during stimulation stalk raised above fluid surface): S1 induced a VP overflow of 5.0 + 0.3 mu (n=14, mean + s.e.mean) and S2, 50 min after onset of S1, released VP at a S2/S1 ratio of 0.65 ± 0.05 (n=8). The evoked VP release was greatly (80%) Ca-dependent but not significantly (21%) sensitive to tetrodotoxin (TTX). Apomorphine (Apo) 10 µM given before S1 reduced the evoked VP output by 50% (or by 65% if added before S2). The dopamine antagonists sulpiride (Sul) (1 - 100 nM) and flupenthixol (Flu) 10 µM also inhibited the evoked VP output at S1 by 35% and 45%, respectively. Nevertheless, the combination of Apo 10 µM with Sul (1 nM at S1) or Flu (10 µM at S2) resulted in a significant increase of the VP overflow if compared with either Apo alone or Sul (or Flu) alone, indicating agonist-antagonist interactions rather than additive drug effects. Naloxone 1 µM alone had no effect on the evoked VP release but completely antagonized the inhibitory action of Sul 100 nM.

Procedure B (0.2 ms, 30 V, stalk elevated (B1) or 80 V, stalk in fluid (B2), 15 Hz, 10 s trains with 10 s intervals for a total of 10 min): S1 induced a VP output of 3.0 + 0.2 mu (B1, n=39) or 3.2 + 0.1 mu (B2, n=100). S2, 60 min after onset of S1, released VP at a S2/S1 ratio of 0.64 + 0.02 (B1, n=8) or 0.71 + 0.03 (B2, n=9). Under these conditions the evoked VP release was completely Ca-dependent and TTX-sensitive. Series B1: Apo (100 nM or 1 µM, 15 min before S2) caused an inhibition of the evoked VP release by 20%. Sul 100 nM increased the evoked VP output by 25%. Sul dose-dependently antagonized the inhibitory action of Apo. Bromocriptine (Br) 1 nM increased the evoked VP release by 25% and at higher concentrations (1 - 10 µM) inhibited the evoked VP output by 20%. Sul 100 nM had no effect on the facilitatory action of Br 1 nM, antagonized the inhibitory effect of Br 1 µM but not that of Br 10 µM. Series B2: Sul 1 µM caused an increase by 15% and Flu 100 nM an inhibition by 30% of the evoked VP release.

It is concluded that the VP release from the neurohypophysis is under complex dopaminergic regulation. There exist probably two dopamine receptors, one mediating inhibition (activated by Apo and inhibited by Sul) and one mediating facilitation (inhibited by Flu) of the evoked VP release. Both receptors may be activated by endogenous dopamine. At present it can not be decided if Br activates both receptors or if it acts as a partial agonist at the inhibitory receptor. Furthermore, the secretion of opioids in the intermediate lobe is known to be inhibited by a dopamine receptor. Under the condition of field-stimulation (procedure A) dopamine antagonists may remove the suppression by endogenous dopamine of secretion of opioids which, in turn, cause inhibition of evoked VP release.

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THE EFFECTS OF PROSTAGLANDIN E_1 AND THEOPHYLLINE ON INTESTINAL SECRETION OF FLUID AND ELECTROLYTE IN THE RAT

K.T. Bunce & C.F. Spraggs, Department of Pharmacology, Glaxo Group Research Ltd., Ware, Hertfordshire. SG12 0DJ.

Prostaglandin E_1 (PGE_1) stimulates fluid secretion in the entire small intestine of the rat (Robert et al, 1979). However, Coupar (1978), using one dose of PGE_1 only (2 μg/min i.a.), found that the compound inhibited net water absorption in the ileum, but did not stimulate net water secretion. In addition little information is available about the effect of prostanoids on colonic function in the rat in vivo. We have investigated the dose-response relationship for PGE_1 on both water and electrolyte fluxes in the ileum and colon of the anaesthetised rat. The effect of intraluminal theophylline on this relationship was also examined.

Female rats were anaesthetised with an allobarbital/urethane mixture, and predosed with indomethacin, 10 mg/kg s.c. Segments of ileum and colon were ligated and filled with 1.5 ml of Tyrode's solution which contained [^{14}C]-polyethylene glycol-4000 ([^{14}C]-PEG, 5 g/l;20 μCi/l) as a volume marker. In some experiments 25 mM theophylline was added to this solution. PGE_1 was given at doses of 1, 3, 10 and 30 nmol/kg/min by continuous retrograde infusion into the left carotid artery. After 1h the contents of the intestinal segments were collected and analysed for concentrations of [^{14}C]-PEG, Na^+ and Cl^-.

In the absence of theophylline, the rate of water absorption under control conditions was -36 ± 3.9 μl/cm/h in the ileum, and -34.4 ± 9.4 μl/cm/h in the colon. Infusion of the highest dose of PGE_1 (30 nmol/kg/min i.a.) did not significantly change the rate of fluid absorption in the ileum (-32.1 ± 4.7 μl/cm/h), but it caused a small net secretion of fluid in the colon (+1.6 ± 10.0 μl/cm/h). Addition of 25 mM theophylline to the intraluminal solution markedly potentiated the effects of PGE_1, which stimulated dose-related changes in the net fluxes of water, Na^+ and Cl^- both in ileum and colon; the effects of the highest dose of PGE_1 are shown in Table 1.

Table 1 The effect of intra-arterial PGE_1 (30 nmol/kg/min) plus intraluminal theophylline (25 mM) on net water and electrolyte fluxes in the ileum and colon of the rat.

	Ileum		Colon	
	Control	PGE_1	Control	PGE_1
	(mean ± s.e. mean, in all cases n = 6)			
net water flux[a]	- 30.0 ± 2.8	+ 20.5 ± 8.1*	- 2.2 ± 10.8	+ 95.6 ± 10.5*
net Na^+ flux[b]	- 5.3 ± 0.3	+ 1.3 ± 1.4*	- 8.5 ± 0.9	+ 2.7 ± 1.0*
net Cl^- flux[b]	- 7.9 ± 0.4	- 2.8 ± 1.0*	- 9.7 ± 1.7	- 1.1 ± 0.7*

minus (-) sign indicates a net loss (absorption) from the lumen; positive (+) sign indicates a net gain (secretion). *$P < 0.01$ compared with control.
[a] net water flux, μl/cm/h. [b] net electrolyte flux, μeq/cm/h.

Thus, intra-arterial infusion of PGE_1 only stimulated a substantial net secretion of water in the ileum and the colon of the rat in the presence of intraluminal theophylline. This change was accompanied by a small net secretion of Na^+ in both segments of intestine, and although the net absorption of Cl^- was reduced, a net secretion of this ion was not observed.

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PROSTAGLANDIN E_2 AND INDOMETHACIN MODIFY IN VITRO IMMUNOGLOBULIN PRODUCTION BY HUMAN B-LYMPHOCYTES

G.S.Panayi & N.D.Staite, Department of Rheumatology and Medicine, Guy's Hospital Medical School, London SE1 9RT.

Prostaglandins (PG) clearly play a significant role in the inflammatory response and it is widely accepted that non-steroidal anti-inflammatory drugs (NSAID) act primarily by inhibition of PG synthesis. The involvement of PG and the influence of NSAID on the regulation of the human immune response has however received little attention. We have therefore investigated the effect of indomethacin and PGE_2 on immunoglobulin production (IgP) from human peripheral blood lymphocytes (HPBL) of healthy individuals in vitro. IgG and IgM production, were measured using an enzyme linked immunosorbent assay (ELISA) at 11 days of microwell culture, following polyclonal activation of HPBL with pokeweed mitogen (PWM). Indomethacin 10^{-6} - 10^{-8}M, on addition with PWM, produced consistent, concentration-related inhibition of both IgG and IgM production. Optimal responses to mitogen were inhibited by 69.4% $\pm$ 9.9 (IgG mean $\pm$ s.e.mean n=5) and 76.2% $\pm$ 10.1 (IgM mean $\pm$ s.e.mean n=5) using 10^{-7}M indomethacin. Inhibition of fixed mitogen responses was 50.7% $\pm$ 5.1 n=10 and 48.5% $\pm$ 6.5 n=10 respectively also using 10^{-7}M indomethacin. Exogenous PGE_2 10^{-6}-10^{-8}M, added with PWM, produced variable effects on IgP. IgG production was enhanced 41.3% $\pm$ 11.4 n=5, whilst little effect on IgM production was observed (inhibition 4.3% $\pm$ 11.3 n=7). The inhibitory effects of 10^{-7}M indomethacin on Ig production were fully reversed with approximately 10^{-7}M PGE_2. However inhibition of IgM production could not be antagonised, paralleling the apparent lack of effect of PGE_2 on IgM production. Time course experiments suggest that indomethacin exerts its effect at an early stage in culture following mitogen activation. However no significant effects of PGE_2 or indomethacin were found on PWM induced T and B lymphocyte proliferation. Similarly indomethacin did not decrease HPBL viability in culture. These results suggest that indomethacin, at physiological concentrations can inhibit human B-lymphocyte immunoglobulin production in vitro. This is similar to the findings in the rabbit (Gerblich et al 1979) but not of those in mouse (Webb et al 1976) and rabbit (Mattingly et al 1979) and supports the concept that PGE_2 and other arachidonic acid products are involved in immunoregulatory processes.

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ENHANCEMENT OF PHA-STIMULATED LYMPHOCYTE ACTIVATION BY BW 755C

A.T.M. Mobarok Ali & J. Morley,
Department of Clinical Pharmacology, Cardiothoracic Institute, Fulham Road, LONDON, SW3 6HP.

It has been proposed that during lymphocyte activation PGE2 is formed as an inhibitory agency (Goodwin & Webb, 1980), whilst generation of thromboxanes and lipoxygenase products provides a stimulating effect (Kelly, Johnson & Parker, 1979). Therefore, we have been interested to ascertain the effect of a range of non-steroidal anti-inflammatory drugs upon lymphocyte activation with guinea pig lymph node lymphocytes. These drugs are usually either ineffective at therapeutic concentrations or exert inhibiting effects, that may be of relevance to their use in rheumatoid arthritis, since increased lymphocyte activation is a feature of this disease (Carter, Bacon & Hall, 1981).

BW755C (3-amino-1-[m-(trifluoromethyl)-phenyl] 2-pyrazoline) was included in this study as this drug inhibits both cycloxygenase and lipoxygenase enzymes. The effect of BW755C was unusual in that it enhanced phytohaemagglutinin (PHA) induced tritiated thymidine incorporation at lower concentration (ED50 0.3 ug/ml) but inhibited lymphocyte activation at higher concentration (ED50 8-16 ug/ml). The stimulatory effect of BW755C was only evident when lymphocytes were exposed to an activation stimulus and only occured when the drug was in contact with cells during the initial period of cell activation.

Since the concentration of BW755C which stimulate lymphocyte activation is lower than those reported to affect arachidonic acid (AA) metabolism (Higgs, Flower & Vane, 1979), and since 5,8,11,14-eicosatetraynoic (ETYA), nordihydroguaiaretic acid (NDGA) & benoxaprofen all exhibit only inhibitory effects, we suggest that this property of BW755C may be unrelated to metabolism of AA. These effects of BW755C on lymphocyte function may contribute to its actions in vivo.

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THE PRODUCTION BY HUMAN LEUCOCYTES OF A TRIHYDROXYEICOSATETRAENOIC ACID WITH NEUTROPHIL AGGREGATING AND CHEMOKINETIC PROPERTIES

R.D.R. Camp, N.J. Fincham, A.W. Ford-Hutchinson[1], A.I. Mallet & P.M. Woollard, Institute of Dermatology, Homerton Grove, London E9 6BX and [1]Department of Chemical Pathology, King's College Hospital Medical School, Denmark Hill, London SE5 8RX

Several hydroxylated metabolites of arachidonic acid have been shown to be synthesised by leucocytes of various species including those of man (Borgeat & Samuelsson, 1979). In the present study the formation of a trihydroxy metabolite is described, and its biological activity is reported.

Suspensions of mixed human leucocytes were prepared from fresh venous blood by dextran sedimentation and centrifugation (Cunningham et al, 1978). The cells were suspended in modified essential medium (Eagle) buffered to pH 7.4 with HEPES, at a concentration of 3.5×10^7 cells per ml. This suspension was incubated with 0.11 mM arachidonic acid and 20 uM calcium ionophore A23187 for 4 min at 37°C. The reaction mixture was extracted and purified by silicic acid chromatography (Borgeat and Samuelsson, 1979). Following this the ethyl acetate fraction was subjected to high performance liquid chromatography (HPLC). The solvent mixture contained methanol/water/acetic acid (72:28:0.01) and the effluent was monitored for ultraviolet (UV) absorbance at 280 nm. The presence of the UV absorbing compounds described by Borgeat & Samuelsson (1979), including leukotriene B_4, was demonstrated. In addition a major polar UV absorbing compound was seen to elute 5 min after injection, in several experiments. This peak was collected and purified by reversed phase HPLC using a solvent mixture containing methanol/water/acetic acid (60:40:0.01). The major peak eluted at 12 min was re-collected. The compound had a UV absorbance spectrum identical to that of leukotriene B_4, with absorbance maxima at 260, 270 and 281. The mass spectrum of the methyl ester trimethylsilyl ether derivative showed ions at m/z 551, 492, 461, 402, 383, 293, 267, 229, 217,203 and 129. This indicated that the compound was a C-20 tetraunsaturated fatty acid with hydroxyl groups at C-5 and C-12, and with a third hydroxyl group, the position of which could not be clearly assigned. After catalytic reduction of the compound, the methyl ester trimethylsilyl ether derivative revealed ions at m/z 389, 303, 299, 203, 147, 129 and 103, indicating that the third hydroxyl group was at C-20. The structure of the compound was thus determined to be 5,12,20-trihydroxy-6,8,10,14-eicosatetraenoic acid.

The effects of the compound on the chemokinesis of human polymorphonuclear leucocytes (PMNs) and the aggregation of rat peritoneal PMNs were determined as described (Bray et al, 1981; Smith & Walker, 1980; Cunningham et al, 1980) and were compared with those of leukotriene B_4. Its activity was less than that of leukotriene B_4 but greater than that reported for 5-hydroxy-6,8,11,14-eicosatetraenoic acid, which has greater chemotactic activity *in vitro* than any other monohydroxy derivative of arachidonic acid (Goetzl et al, 1980). It is concluded that the 5,12,20-trihydroxyeicosatetraenoic acid may play a **role** as a mediator of inflammation.

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ESTIMATION OF SMALL QUANTITIES OF PROSTACYCLIN USING RABBIT PLATELET RICH PLASMA TREATED WITH PAPAVERINE

D.S. Bamford, M.P. Seed[1] & K.I. Williams[1], Department of Obstetrics, Royal United Hospital, Bath and [1]Department of Pharmacology, School of Pharmacy & Pharmacology, University of Bath, Bath, BA2 7AY.

Prostacyclin (PGI_2) release from the rat pregnant myometrium can be assayed by inhibition of induced platelet aggregation (El Tahir and Williams, 1980). However the human myometrium produces smaller quantities of PGI_2 (Bamford, et al 1980) which are often too low to estimate using rabbit platelet rich plasma (PRP). Human PRP is more sensitive than rabbit PRP to PGI_2 (Whittle et al 1978) but is not routinely available in our laboratory. We have attempted to increase the sensitivity of the assay using rabbit PRP with papaverine, as PGI_2 acts by increasing platelet cAMP (Gorman et al 1977) which is potentiated by phosphodiesterase inhibitors (Whittle, et al 1978).

Rabbit blood was collected into sodium citrate (1ml of 3.8% w/v citrate: 9ml blood) and centrifuged at 200g for ten mins. Samples of PRP (0.5ml) were maintained at 37°c in an EEL aggregometer. A 3 min schedule of drug addition was used. In test samples after 1 min preincubation, papaverine, PGI_2, and ADP (2.5-10μM) were added at 1 min intervals. Papaverine was omitted in the control group leaving a 2 min preincubation period. Aggregation responses in the presence of PGI_2 were expressed as % of the control ADP responses. ID50 values were calculated, and expressed as ng PGI_2/ml PRP±s.e. mean. PGI_2 gave a dose related inhibition of aggregation (ID50 7.16±0.68 ng/ml n=7) and was decreased by papaverine: ID50 3.88±0.39 (50μM) $p< 0.05$ (n=4): 2.18±0.62 (100μM) $p< 0.05$ (n=4): 1.45±0.41 (200μM) $p< 0.02$ (n=4) (unpaired 't' test). The highest dose affected the ADP-induced responses and thus 100μM papaverine was adopted for routine assays. The assay coefficients of variation in the presence and absence of papaverine were then calculated.

Human pregnant myometrial tissue was suspended in Tris buffered saline (50% w/v, pH8.0). The tissue was chopped and incubated for 30 mins at 20°c, the incubation fluid aspirated, adjusted to pH6.5 and heated for 60 mins at 37°c to destroy generated PGI_2. The pH was readjusted to 8.0 and authentic PGI_2 was added to an aliquot of incubation medium to give a concentration of 250 ng/ml. The PGI_2 content was then determined using 2+2 doses assay in the presence or absence of papaverine (100μM). The concentration of PGI_2 in the absence of papaverine was found to be 242.4±4.0 ng/ml and in the presence of papaverine 262.2±4.0 ng/ml. The intra and inter-assay coefficients of variation for control PRP were 9.9±2.2% and 10.3±1.8 respectively and in the presence of papaverine 12.6±3.4% and 10.1±1.5% respectively. The values for treated and control PRP are not significantly different (paired 't' test, n=4).

It is concluded that pretreatment of rabbit PRP with papaverine considerably increased the sensitivity of the platelets to PGI_2. This property allows estimation of smaller amounts of PGI_2 without affecting the accuracy or reproducibility of the assay procedure.

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THE USE OF PROSTACYCLIN FOR THE SEPARATION AND WASHING OF HUMAN PLATELETS FROM PLASMA

S. Moncada, M. Radomski and J.R. Vargas, Dept. of Prostaglandin Research, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.

Several methods for washing and separating platelets from blood have been described previously. Most of these methods are based on the preparation of platelet suspensions by differential centrifugation, gel filtration or centrifugation through an albumin gradient. In addition, solutions without calcium and magnesium or with chelating agents (EDTA, EGTA or citrate) have been used. Moreover, low temperature, low pH solutions, apyrase and prostaglandin E_1 have also been used to prevent platelet activation during the procedure or the subsequent action of the substances released by the platelets (Ardlie, 1968; Ardlie et al, 1970; Rotman and Heldman, 1980). The problems encountered with these methods include the production of platelets with low sensitivity to ADP; spontaneous platelet aggregation after warming and stirring the suspension; short survival of platelets after washing; low recovery of platelets from blood; changes in the capacity of the platelets to undergo release reaction, and decreased ability to show shape change after the addition of pro-aggregating agents. Because of this, none of the existing methods has been totally satisfactory for the study of metabolic, physiological and pharmacological functions of isolated platelets (Mustard et al, 1971).

We now describe a method for isolation and washing human platelets from plasma using prostacyclin, a powerful inhibitor of platelet aggregation (Moncada et al, 1976). Blood was obtained from the antecubital vein of human volunteers who had not taken aspirin for two weeks previously. The blood was immediately centrifuged at 220 g for 20 min at 22°C. The resulting platelet rich plasma (PRP) was removed with a plastic syringe and after adding PGI_2 (2 ng/ml) recentrifuged at 110 g for 10 min to sediment red and white cells still present in the PRP. The resulting PRP was separated, prostacyclin added (50-300 ng/ml) and centrifuged at 800 g for 10 min. Tyrode's solution containing heparin (25 U/ml) and bovine albumin (0.3 mg/ml) was then used to resuspend the platelets by gently sucking and blowing with the plastic syringe (within 10 min of centrifugation). After further addition of PGI_2 (50-300 ng/ml) the suspension was centrifuged at 600 g for 10 min and the process repeated. The final suspension was prepared in prostacyclin-free Tyrode's solution by adding half of the volume used in the previous steps and then an additional volume to obtain a platelet count of 1.9-2.1 x 10^8 platelets/ml. The final suspension was kept at 4°C in a double-walled siliconised glass container to avoid abrupt changes in temperature. Platelet aggregation was then measured photometrically in a Paton Dual Channel aggregometer according to the method of Born (1963).

This method produced a 60-70% yield of platelets from the PRP. The platelet sensitivity to tested agonists (ADP, collagen, arachidonic acid and thrombin) was similar to that obtained in human PRP. However, for immediate and full recovery of ADP induced aggregation, addition of fibrinogen (0.04-0.40 mg/ml) was necessary. The platelet sensitivity to tested agonists remained unchanged for periods up to 48 h if platelets were stored at 4°C. We conclude that this technique used for separation and washing of human platelets from plasma has advantages over commonly applied methods.

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ACTIONS OF HUMAN PLATELETS ON THE DURATION OF THE ANTI-AGGREGATING ACTIVITY OF PROSTACYCLIN

Susan Doe, S. Moncada, J.R. Vane and B.J.R. Whittle, Dept. of Prostaglandin Research, Wellcome Research Laboratories, Langley Court, Beckenham, Kent.

Prostacyclin is a potent inhibitor of platelet aggregation which readily decomposes to 6-oxo-$PGF_{1\alpha}$ in aqueous media at physiological temperatures and pH (Moncada et al. 1976). The rate of hydrolysis of prostacyclin is, however, significantly decreased in human plasma (Blasko et al, 1980; Borda et al, 1980), which may reflect its binding to serum albumin (Wynalda and Fitzpatrick, 1980; Pifer, Cayen and Chesney, 1981). It has also been proposed that prostacyclin can be metabolised by a platelet enzyme to a more stable anti-aggregating product, 6-oxo-PGE_1 (Wong et al, 1980). We have now investigated the actions of human platelets on the stability and duration of the anti-aggregating activity of prostacyclin.

Platelet aggregation was measured with a Born-type optical aggregometer using platelet-rich plamsa (PRP) prepared from freshly collected citrated (0.315% w/v trisodium citrate) human blood. The near-maximal inhibition of ADP (5 µM)-induced platelet aggregation by prostacyclin (2-10 nM) following 1 min incubation at 37°C, was still apparent in aliquots (0.5 ml) of PRP incubated at 37°C for up to 2 h. The time taken for this anti-aggregating action of prostacyclin on PRP to decrease by 50% was 53 ± 6 min (mean ± s.e. mean, n=7). In contrast, when the prostacyclin concentration in platelet-free human plasma at 37°C was determined by transferring aliquots (50 µl) to untreated PRP for bioassay, the rate of hydrolysis of prostacyclin was significantly ($P < 0.001$) less, with a $t_{\frac{1}{2}}$ (time taken for 50% disappearance) of 15 ± 2 min (n=4). However, when prostacyclin breakdown in PRP at 37°C was likewise determined by transferring aliquots (50 µl) to untreated PRP for bioassay, the $t_{\frac{1}{2}}$ was 17 ± 3 min (n=4). Thus, the presence of platelets *per se* does not significantly increase the stabilising actions of plasma on prostacyclin breakdown. Furthermore, the rate of breakdown of prostacyclin at 37°C in suspensions of human platelets (2×10^8 ml^{-1}) washed and re-suspended in albumin-free Tyrode's solution (pH 7.7) was comparable to that in Tyrode's solution alone ($t_{\frac{1}{2}}$ was 6 + 1 min and 8 ± 1 min respectively, n=3). The formation of the putative metabolite 6-oxo-PGE_1 would have little influence on the prostacyclin assay procedure or contribute to the duration of anti-aggregating action, since it was 0.06 times as potent as prostacyclin. The dose causing 50% inhibition of platelet aggregation in PRP was 17 ± 2 nM for 6-oxo-PGE_1 compared with 1.1 ± 0.3 nM (n=4) for prostacyclin.

These studies confirm that prostacyclin is more stable in plasma than in buffer solutions of comparable pH, but show that the presence of human platelets has no significant action on its rate of breakdown. The long-lasting anti-aggregating action of prostacyclin in PRP *in vitro*, which extends further than the measured stability of prostacyclin in plasma, could suggest that the prostacyclin which interacts with the platelet binding sites and which is responsible for the anti-aggregating actions, is protected from hydrolysis.

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THE ROLE OF THROMBOXANE A_2 (TxA_2) AND ADP IN COLLAGEN-INDUCED PLATELET AGGREGATION

H. Emms, G.P. Lewis & I.S. Watts, Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons, Lincoln's Inn Fields, London WC2A3PN

Collagen-induced platelet aggregation is accompanied by the release of adenosine diphosphate (ADP) from platelet granules (Hovig, 1963) although its essential role in the mechanism of aggregation has been questioned (Nunn, 1979). In addition to ADP it is evident that collagen gives rise to formation of prostaglandin (PG) endoperoxides PGG_2/PGH_2 and thromboxane A_2 (TxA_2) which are platelet aggregating and release-inducing agents in their own right (Malmsten et al, 1975).

We have modified a previously described bioassay procedure (Ladd & Lewis, 1980) to enable simultaneous monitoring of TxA_2 and PGs released during aggregation of rabbit platelets induced by collagen fibrils (Horm) or arachidonic acid (AA). Inhibitors of platelet cyclo-oxygenase (CO), Indomethacin (Indo) and Tx synthetase (1-(7-carboxyheptyl)imidazole) (Carb) were used alone and in combination with the substrate/enzyme complex creatine phosphate/creatine phosphokinase (CP/CPK) (which converts ADP to ATP) to investigate the relative importance and inter-relationship of ADP release and the formation of PGG_2/PGH_2 and TxA_2 during collagen and AA-induced aggregation.

At low dose collagen (5 to 10 µg/ml) Indo and Carb completely inhibited platelet aggregation in a dose-related manner. In contrast, at high dose collagen (50 ug/ml) these compounds gave only a partial inhibition (30%) of aggregation at a dose which abolished CO and Tx synthetase activity. CP/CPK (5mM.10 $u.ml^{-1}$) (but not CP or CPK alone) completely inhibited low dose collagen but only partially inhibited (20%) that induced by high dose collagen. A mixture of both CP/CPK and Indo or Carb abolished high dose collagen-induced platelet aggregation. Collagen-induced aggregation is therefore dependent on two mechanisms. At low collagen concentration sub-threshold levels of released ADP and TxA_2 act synergistically in inducing platelet aggregation since either CP/CPK, Indo or Carb abolished aggregation. At high concentration sufficient ADP and TxA_2 are released to induce aggregation independently of each other since CP/CPK, Indo or Carb produced only partial inhibition of aggregation, whilst in combination aggregation was abolished.

Arachidonic acid (60 to 600 µM) produced platelet aggregation with accompanying release of TxA_2 and PGs. Carb completely inhibited low-dose AA-induced platelet aggregation, but when the concentration of AA was increased the inhibition of aggregation was surmounted, but was reversible in nature, in contrast to the irreversible AA-induced aggregation in the absence of inhibitor. Indo (28 µM), however, abolished aggregation at all concentrations of AA. CP/CPK did not significantly inhibit AA-induced aggregation.

AA-induced aggregation is therefore partially dependent on TxA_2 formation since Carb was able to abolish aggregation induced by low AA concentrations. It seems likely that the remaining activity of AA is attributable to endoperoxide formation since Indo causes complete inhibition of aggregation at any concentration of AA. Furthermore, PGH_2 (1 - 10 µg/ml) added to platelet-rich plasma produced only reversible platelet aggregation.

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RESPONSES OF HUMAN SKIN TO INTRADERMAL INJECTION OF LEUKOTRIENES C_4, D_4 AND B_4

R.D.R. Camp, A.A. Coutts, M.W. Greaves, A.B. Kay[1] & M.J. Walport[1], Institute of Dermatology, Homerton Grove, London E9 6BX and [1]Department of Clinical Immunology, Cardiothoracic Institute, Fulham Road, London SW3 6HP.

Although the vascular responses of skin to leukotrienes C_4, D_4 and B_4 (LTC_4, LTD_4 and LTB_4) have been studied in the guinea pig, rat and rabbit (Peck et al, 1981; Ueno et al, 1981; Wedmore & Williams, 1981), little is known about their effects in human skin.

LTC_4 and LTD_4 were chemically synthesised by the method of Rokach et al (1980), and were supplied by Dr. Rokach, Merck Frosst Labs., Quebec. LTB_4 was biosynthesised from human leucocytes, and was purified, identified and quantified as described by Borgeat & Samuelsson (1979). Up to 6 healthy volunteer subjects received intradermal injections of a range of doses of the leukotrienes. The following measurements were made: area of erythema at 1 min, 5 min and 15 min, measured planimetrically; weal diameter measured as the mean of two perpendicular diameters; and subjective sensation. Histamine (1.63 nmol), prostaglandin E_2 (PGE_2) (0.14 or 0.18 nmol) and phosphate buffered isotonic saline diluent were injected as controls. Skin punch biopsies, obtained from leukotriene injection sites 1.5 or 4 h after injection, were examined histologically.

Both LTC_4 and LTD_4 caused immediate erythema and wealing but no delayed erythema or induration. The areas of erythema were maximal at doses of 0.19 nmol (LTC_4) and 0.38 nmol (LTD_4) and at 5 min after injection. Increase of the doses of both leukotrienes up to 7.5 nmol caused no further increase in the areas of erythema, which were significantly less than that due to intradermal injection of 1.63 nmol histamine, and approximately equal to that due to 0.18 nmol PGE_2. Mean diameters of the weals were maximal at 0.19 nmol for both LTC_4 and LTD_4 at 15 min, and approximately equal to those due to histamine (1.63 nmol) and PGE_2 (0.18 nmol). The responses to injections of LTC_4 (0.38 nmol) combined with PGE_2 (0.18 nmol), or LTD_4 (0.75 nmol) combined with PGE_2 (0.18 nmol) did not suggest any synergistic action between PGE_2 and either leukotriene. Both leukotrienes caused a variable degree of pain or discomfort but no itching. Histological examination of the injection sites biopsied 1½ h after injection showed no significant leucocytic infiltration.

LTB_4 (0.15 to 1.5 nmol) caused immediate erythema and wealing in one of the two subjects studied, followed by a delayed reaction in both. At the site of each LTB_4 injection each subject developed a delayed, ill-defined area of erythema and raised induration with mean diameters of 3-8 mm and which were maximal at 60-120 min after injection. These lesions persisted for at least 4 h, were tender, and in one subject were associated with itching. Neither the saline diluent, PGE_2 (0.14 nmol) or histamine (1.63 nmol) caused any response detectable at 4 h. Histological examination of skin biopsies removed from the indurated sites 4 h after injection of 0.3 nmol LTB_4 in both subjects showed a neutrophil-rich leucocytic infiltrate, with a few eosinophils, mainly located perivascularly in the upper and mid-dermis.

These results support the possibility that leukotrienes C_4, D_4 and B_4 are mediators of cutaneous inflammation.

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INCREASED CONCENTRATIONS OF ARACHIDONIC ACID, PROSTAGLANDINS AND HISTAMINE IN HUMAN SKIN AFTER UV-A IRRADIATION

R.M.Barr[1], A.K.Black[1], B.A.Gilchrest[2], M.W.Greaves[1], J.L.M.Hawk[2], C.N.Hensby[3], K.Jaenicke[4], J.A.Parrish[4], N.Soter[2], [1]Institute of Dermatology, Homerton Grove, London E9 6BX; [2]Department of Dermatology, Harvard Medical School and Beth Israel Hospital, Boston, MA 02215, USA; [3]Centre International de Recherches Dermatologie, Sophia Antipolis, 06580 Valbonne, France; [4]Department of Dermatology, Harvard Medical School and Massachusetts General Hospital, Boston, MA 02114, USA.

Elevated concentrations of arachidonic acid and prostaglandins $F_{2\alpha}$, E_2, D_2 and 6-oxo-$F_{1\alpha}$ have been found in human skin exudates following UV-B (290-320 nm) and UV-C (100-290 nm) irradiation (Black et al, 1980; Black et al, 1981; Camp et al, 1978). The increase in prostaglandin levels was coincident with the development of erythema. Although biologically active on human skin, the effect of long wavelength ultraviolet (UV-A, 320-400 nm) on arachidonic acid and prostaglandin levels has not previously been investigated.

Volunteers were irradiated using an Oriel 2.5 kw Xenon arc lamp with filters transmitting the UV-A spectrum between 320-420 nm. A circular field 9 cm in diameter was irradiated at a dose rate of 40 mW/cm^2 with 2.5 times the minimal erythema dose. Exudates were obtained from control and irradiated skin as previously described (Camp et al, 1978). Arachidonic acid and prostaglandins were assayed by gas chromatography-mass spectrometry after purification by high pressure liquid chromatography. Histamine was measured by a radioenzyme assay method.

Erythema, assessed visually, was present immediately after irradiation and declined slightly before increasing to a maximum between 9 and 15 h. It was still present at 48 h. Arachidonic acid and prostaglandin concentrations are shown in Table 1.

Table 1 Effect of UV-A irradiation on the concentrations of arachidonic acid, PGE_2 and PGD_2 in human skin exudates. Values represent means ± s.e.means; n = number of samples; p values (Student's 't' test) represent significance relative to controls

		Time After Irradiation				
	Control	3	8	15	24	48 h
Arachidonic Acid ng/ml^{-1}	2120±340 n=7	3600±800 n=6 .1>p>.05	3560±1040 n=5 .2>p>.05	2580±370 n=7 p>.3	2390±440 n=7 p>.6	1920±550 n=4 p>.7
PGE_2 ng/ml^{-1}	37±12 n=8	102±32 n=7 .1>p>.05	94±23 n=7 .05>p>.025	60±20 n=7 p>.3	62±16 n=7 p>.2	25±10 n=4 p>.5
PGD_2 ng/ml^{-1}	46±10 n=8	161±42 n=7 .02>p>.01	180±40 n=7 .005>p>.001	128±61 n=7 .2>p>.1	56±10 n=6 p>.5	31±15 n=4 p>.3

The average histamine concentration in control exudates was 3.3 ng ml^{-1}. It increased to maximum levels of up to 69 ng ml^{-1} between 9 and 15 h after irradiation.

These results show that UV-A irradiation caused increase of concentrations of histamine, arachidonic acid and prostaglandins E_2 and D_2 in human skin. The role of these agents in the observed inflammatory changes remains to be established.

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