Br. J. Pharmacol. (1993), **110**, 633–638

Adenylate cyclase-mediated vascular responses of rabbit aorta, mesenteric artery and skin microcirculation

A.J. Wilson & [1]J.B. Warren

Department of Applied Pharmacology, National Heart & Lung Institute, London SW3 6LY

1 The importance of adenylate cyclase-mediated vascular relaxation in the macro and microcirculation was assessed in rabbit aortic and coeliac artery bioassay rings *in vitro* and skin microvessels *in vivo*.

2 The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP38), the β-agonist, isoprenaline, and the prostaglandins, PGE_1 and PGE_2, were compared with the activity of nitroprusside, which acts by stimulating guanylate cyclase.

3 In aortic tissue the relative relaxant potencies were ($-\log$ M EC_{50}, 100% = response to nitroprusside 10^{-6} M): nitroprusside 7.0, PACAP38 6.8, isoprenaline 6.3; PGE_1 and PGE_2 were weak constrictors. In coeliac artery rings relative potencies were ($-\log$ M EC_{50}, 100% = response to nitroprusside 10^{-5} M): PACAP38 6.6, PGE_1 6.6, nitroprusside 6.5, PGE_2 4.9, and isoprenaline 4.3.

4 Comparative potencies when injected into anaesthetized rabbit skin *in vivo* were ($-\log$ mol/site required to increase blood red cell flux by 75%): PACAP38 13.0, PGE_2 10.7, isoprenaline 9.7, PGE_1 9.1, nitroprusside <7.

5 Nitroprusside, the most effective relaxant tested in the aorta, was 10^7 fold less potent than PACAP in its effect on skin blood flow. PGE_1 and PGE_2 were constrictors of the aorta, of intermediate effect in the coeliac artery, but potent vasodilators of the microcirculation.

6 In this model, the importance of adenylate cyclase-mediated vascular relaxation increases with decreasing vessel size.

Keywords: Prostaglandins; pituitary adenylate cyclase activating polypeptide; cyclic AMP; cyclic GMP; β-adrenoceptors; nitric oxide

Introduction

The cyclic nucleotides adenosine and guanosine 3′:5′-cyclic monophosphates (cyclic AMP and cyclic GMP, respectively) have both been implicated as intracellular second messengers which mediate vascular smooth muscle relaxation. Adenylate cyclase can be stimulated to generate cyclic AMP by several first messengers, including prostaglandins, β-adrenoceptor agonists and some of the neuropeptides (Andersson, 1973; Scheid *et al.*, 1979; Schoeffter *et al.*, 1987; Sata *et al.*, 1988; Warren *et al.*, 1991; Wood & Owen, 1992). The elevation in concentration of cyclic AMP activates cyclic AMP-dependent protein kinases, leading to actin myosin relaxation. The stimulation of soluble guanylate cyclase to generate cyclic GMP is by nitric oxide, the endogenous nitrovasodilator released by several cell types, which is a key component of endothelium-derived relaxing factor (EDRF) (Moncada & Higgs, 1991).

Many of the studies of EDRF and nitric oxide have involved bioassay systems using rabbit aorta or large vessel endothelial cells in culture. In these systems the inhibition of nitric oxide synthase abolishes the relaxant activity derived from endothelial cells (Gryglewski *et al.*, 1986; Moncada & Higgs, 1991). Agonists such as bradykinin which cause the release of EDRF also stimulate endothelial cells in culture to increase substantially their release of prostaglandins (Gryglewski *et al.*, 1986). However, preventing the synthesis of prostaglandins with cyclo-oxygenase inhibitors does not affect relaxant activity (Gryglewski *et al.*, 1986). Furthermore, pre-contracted rabbit aortic rings do not relax in response to vasodilator prostaglandins (Bunting *et al.*, 1976) suggesting that the prostaglandin-adenylate cyclase vasodilator pathway is not important in these systems when compared to the nitric oxide-guanylate cyclase pathway.

The nitric oxide-guanylate cyclase vasodilator pathway does not predominate to the same extent in smaller blood vessels. For example, the peptide bradykinin is a potent stimulus to nitric oxide release from endothelial cells from large vessels, yet in rabbit coeliac artery bradykinin relaxes this vessel by a cyclo-oxygenase dependent mechanism (Aiken, 1974; Cherry *et al.*, 1982; Ritter *et al.*, 1989). Another stimulus to endothelium-derived nitric oxide in large arteries, acetylcholine, acts by a nitric oxide independent mechanism in the rat small mesenteric artery (Garland & McPherson, 1992) and is only a weak vasodilator in skin (Williams, 1982).

In the microcirculation, prostaglandins E_1, E_2, and I_2, as well as vasoactive intestinal polypeptide (VIP) and calcitonin gene-related peptide are potent vasodilators in man and experimental animals and all stimulate adenylate cyclase (Williams & Peck, 1977; Joyner *et al.*, 1979; Messina *et al.*, 1975; 1980; Messina & Kaley, 1980; Williams, 1982). Pituitary adenylate cyclase activating polypeptide (PACAP) is approximately 100 fold more potent than VIP at stimulating adenylate cyclase in several cell lines and has similar potency as a vasodilator of large vessels and the microcirculation (Warren *et al.*, 1991; 1992c).

The aim of the present study was to define the relative sensitivity of rabbit aorta, coeliac artery and the skin microcirculation to four agonists which stimulate adenylate cyclase. This has been compared with the sensitivity of these tissues to nitroprusside, given as a nitric oxide donor. Nitroprusside was chosen as it readily releases nitric oxide in tissue (Newman *et al.*, 1990). Both aortic and coeliac artery rings were studied without endothelium and in the presence of indomethacin to remove any complicating effects of endogenous nitric oxide or prostaglandin release.

Methods

Aortic and coeliac arteries

Aortic and coeliac arteries were dissected from male New Zealand White rabbits which had been killed with an over-

[1] Author for correspondence.

dose of sodium pentobarbitone. They were placed in Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4.7H_2O$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 1.5, which was bubbled with 5% CO_2 in air (pH 7.4). The blood vessels were cleaned of connective tissue and cut into 3 mm rings. The endothelium was removed by rotating gently around the closed tips of a pair of fine forceps.

Superfusion bioassay system

Rings of aorta or coeliac artery were mounted on hooks attached to a force transducer (Dynameter UF1, Pioden Controls Ltd, Canterbury, Kent) and amplifier (Transbridge TBM4, World Precision Instruments, Sarasota, Florida, U.S.A.) and the output recorded on a Maclab analogue to digital conversion system and Macintosh Apple computer (Apple Computer Inc, Cupertino, Ca, U.S.A.). They were maintained at 37°C and perfused continuously at 2 ml min^{-1} with Krebs-Henseleit buffer bubbled with 5% CO_2 in air. The bioassay rings were adjusted over a minimum period of 100 min to maintain a resting tension of 2–3 g for the aorta and 1–1.5 g for the coeliac artery rings. Rings were then perfused with buffer containing potassium 100 mM for 2 min and allowed a further 45 min to return to baseline. The rings were contracted by perfusing with buffer containing 1 μM phenylephrine and the absence of endothelium confirmed by the lack of response to 1 μM acetylcholine given for 1 min. Albumin (0.3% w/v) was added to the buffer for peptide dilutions and also to the bioassay perfusate to prevent peptide sticking to plastic tubing.

All doses of the agonists were perfused over the bioassay tissue for 1 min, the concentration of phenylephrine being maintained at 1 μM and indomethacin 10 μM added to all solutions. The relaxation response of each bioassay ring was determined by giving 1 μM nitroprusside for 1 min and results are expressed as a percentage of this response. Drugs were given in the concentration range 10^{-10}–10^{-4} M and 6–10 rings were used at each drug concentration.

Measurement of skin blood flow

The microcirculation vasodilator response to intradermal injections was assessed by measuring skin blood flow *in vivo* with a laser Doppler flow probe (Perimed II, Stockholm, Sweden; Warren *et al.*, 1992a,b,c; 1993). Male New Zealand White rabbits, 2.5–3.5 kg, were anaesthetized with sodium pentobarbitone 30 mg kg^{-1}, i.v. The dorsal skin was shaved with electric clippers and depilated with a commercial depilating cream (Immac). The dorsal skin was then rinsed thoroughly with warm water and the animal left for 1 h before measurements were made. The animal remained anaesthetized throughout the procedure in an air-conditioned room at 24 ± 1°C and was not allowed to recover consciousness.

Up to 10 sites were marked out in each quadrant of dorsal skin and baseline blood flow was measured in each site with the laser-Doppler flow probe. The probe was held at right angles to the skin by a plastic guide. Each reading took 15 s with a 10 s interval between readings and the mean of three readings per site were taken. Results were recorded as red blood cell flux (the number of moving red cells detected by the laser beam × mean cell velocity) and expressed as a percentage of a standardized signal. The laser Doppler was set at 4 Hz, gain 10 and a time constant of 3 s. The output was recorded on a Maclab and Macintosh Apple Computer (Apple Computer Inc, Cupertino, Ca, U.S.A.) set at an input of 10 V and chart speed of 2 mm s^{-1}.

Skin blood flow protocols

Test agents dissolved in phosphate buffered saline or control buffer were injected intradermally with a 27 wire gauge needle in 100 μl volumes and the change in red cell flux measured at 30 min. Experiments were repeated four times in each rabbit so that each datum point per rabbit is the mean of four sites. Test compounds were injected in a randomized site pattern. Results are expressed as the percentage change from basal.

Cell culture

Vascular smooth muscle cells were prepared by non-enzymatic methods from rabbit aorta (Warren *et al.*, 1990). The endothelium and adventitia were removed from a length of lower thoracic aorta resected from a New Zealand White rabbit that had been killed with an overdose of sodium pentobarbitone. The medial layer was cut into 1 mm squares and seeded into a culture flask. Smooth muscle cells grew out from these explants and, when confluent, were passaged using a rubber policeman. Contaminating cells were identified by microscopy and removed by selective scraping when passaging. Flasks were passaged 1:3 approximately every 6 weeks and cells used between passages 3 and 7. The identity of the smooth muscle cells was confirmed by their spindle shaped aligned appearance, by electron microscopy and by positive immuno-staining for alpha smooth muscle actin. Smooth muscle cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 20% foetal calf serum, glutamine 480 mg l^{-1}, penicillin 60,000 u l^{-1}, streptomycin 60 mg l^{-1}, thymidine 2 mg l^{-1}, insulin 5 mg l^{-1}, transferrin 5 mg l^{-1} and selenium 5 μg l^{-1}; this medium was changed every 3–4 days.

For the incubation experiments cells were passaged into 24 well plates (Falcon, Becton Dickinson, London UK) and used when confluent at 4–5 days.

Measurement of cyclic AMP

Aortic smooth muscle cells in multi-well plates were washed three times with Krebs-Henseleit buffer and left in 270 μl of buffer, containing phenylephrine 1 μM and indomethacin 10 μM for 45 min. The plates were kept throughout the experiment in an incubator at 37°C in an atmosphere of 5% CO_2 in air. Test compounds were added in 30 μl volumes to the wells, to give a final volume of 300 μl, and incubated for a further 15 min. The reaction was then stopped by adding 300 μl tri-chloroacetic acid 1 M at 4°C and the plates left overnight at 4°C. The test agents used (final concentration ranges) were: PACAP38 10^{-13}–10^{-7} M, prostaglandin E_1 (PGE_1) 10^{-10}–10^{-6} M, PGE_2 10^{-10}–10^{-5} M, isoprenaline 10^{-10}–10^{-6} M and nitroprusside 10^{-6} M. All incubations were in the presence of the phosphodiesterase inhibitor rolipram 100 μM, other than the control wells.

To extract the cyclic nucleotides, 500 μl was removed from each well, centrifuged, and the supernatant added to 50 μl of 25 mM EDTA and vortexed. Freon:tri-n-octylamine, 1: v/v, 100 μl was added and vortex mixed. The mixture was centrifuged and 450 μl of the upper aqueous phase removed and neutralized with 50 μl $NaHCO_3$ (120 mM).

For the radio-immunoassay of cyclic AMP, 100 μl samples were taken in duplicate. Goat cyclic AMP antibody, 100 μl, was added together with 50 μl of adenosine 3′:5′-cyclic phosphoric acid 2′-O-succinyl-3 [^{125}I]-iodotyrosine methyl ester, 2,000 Ci $mmol^{-1}$, to give total c.p.m. in the range 10,000–15,000. The mixture was left overnight at 4°C. Charcoal suspension in potassium phosphate buffer was added at 4°C, vortex mixed, left for 15 min, centrifuged, and 800 μl of supernatant removed and radioactivity measured in a gamma counter.

For the protein assay, cells were removed from the plate wells with 0.1 M NaOH and protein measured with a spectrophotometric dye method using Bradford reagent.

Drugs and chemicals

PACAP38 was obtained from Penninsula Laboratories, St Helen's, UK. PGE_1 and PGE_2 were from Cascade Biochem

Ltd, Reading, UK and adenosine 3':5'-cyclic phosphoric acid 2'-O-succinyl-3 [^{125}I]-iodotyrosine methyl ester from Amersham International Ltd, Amersham, UK. Rolipram was the generous gift of Schering AG, Berlin, Germany. Freon:tri-n-octylamine was from the Aldrich Chemical Co, Gillingham, UK. [1R-[1α(Z),2β,3β,5α]]-(+)-7-[5-([1,1'-biphenyl]-4-ylmethoxy)-3-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptenoic acid, hydrochloride (GR32191B) was the generous gift of Glaxo Group Research, Greenford, UK.

Foetal calf serum, DMEM, penicillin and streptomycin were obtained from Gibco (Paisley, UK). Gas mixtures were obtained from BOC Medical Gases (Middlesex, UK). Other drugs and chemicals were obtained from Sigma (Poole, UK).

Statistical analysis

Results are expressed as mean ± s.e.mean. In the bioassay experiments of vascular rings, 6–10 rings were used for each drug concentration tested. For the measurement of skin blood flow, all data points are the mean of 4 animals, each experiment performed 4 times in each rabbit. In experiments measuring cyclic AMP generation, all data points are the mean of 6 experiments and each assay was performed in duplicate. Statistical comparisons were made by analysis of variance and taken as significantly different if $P<0.05$.

Results

Vasodilator effects in aorta

The mean resting tension of the aortic rings was 2.44 ± 0.08 g and this increased to 10.55 ± 0.66 g with phenylephrine 1 μM. There was no vasodilatation in response to acetylcholine, confirming that the endothelium had been removed. Nitroprusside 1 μM caused a mean maximal reduction in contraction of − 64%. The maximal response to 10 μM nitroprusside was not significantly different from the response to 1 μM. The response to nitroprusside 1 μM was taken as − 100% and results expressed as a percentage of this response.

Nitroprusside, PACAP38 and isoprenaline caused dose-dependent relaxation of pre-contracted aortic rings (Figure 1). The following potencies (− log M EC_{50}, 100% = response to nitroprusside 1 μM) were observed: nitroprusside, 7.0; PACAP38, 6.8 and isoprenaline, 6.3. Both of the prostaglandins tested had no effect up to 10^{-7} M but caused some contraction of aortic rings at higher concentrations. The isoprenaline dose-response was significantly different from the PACAP38, nitroprusside and prostaglandin responses: $P<0.05$, ANOVA.

To determine if high doses of PGE_2 could stimulate constrictor thromboxane receptors, the thromboxane receptor antagonist, GR32191B, was tested on aortic rings without endothelium. At a concentration of 100 μM it reversed the constrictor effects of thromboxane-mimetic, U46619 at 10 nM, but did not affect the contraction produced by phenylephrine 1 μM. Rings contracted with phenylephrine 1 μM contracted a further 44 ± 3% with PGE_2 10 μM in the absence, and 42 ± 2% in the presence, of 100 μM GR32191B (results are mean ± s.e., $n=4$; − 100% = fall in tension in response to nitroprusside 1 μM).

Vasodilator effects in coeliac artery

Phenylephrine was a less effective constrictor of coeliac artery than of aorta and therefore used at a higher concentration of 10 μM which elevated resting tension from a mean of 1.37 ± 0.05 g to 5.53 ± 0.73 g (with 1 μM phenylephrine the corresponding figure was 2.45 ± 0.80 g). A higher dose of nitroprusside was used (10 μM) and results expressed as a percentage of this response. Nitroprusside 10 μM caused a − 72% relaxation of the phenylephrine-induced contraction and only a − 20% relaxation at 1 μM.

Figure 2 shows that PACAP38 was of similar potency to nitroprusside as a relaxant of pre-contracted coeliac artery. In contrast to their vasoconstrictor effects in aortic tissue, both prostaglandins were vasodilators in the coeliac artery with PGE_1 being of equivalent potency to PACAP38. The vasodilator potency (− log M EC_{50}, − 100% = response to nitroprusside 10 μM) was PACAP38, 6.6, PGE_1, 6.6; nitroprusside, 6.5; PGE_2, 4.9; isoprenaline, 4.3. PGE_2 and isoprenaline were each significantly different from the other three, $P<0.05$ in each case.

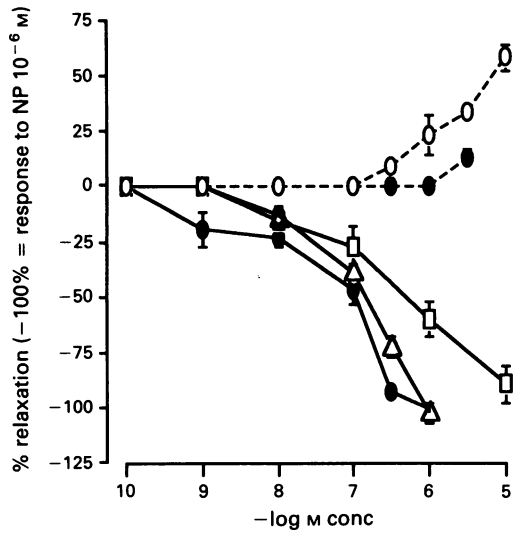

Figure 1 Graph showing the concentration-dependent effect of five agonists on rabbit aorta. Aortic rings were precontracted with phenylephrine 1 μM in the presence of indomethacin 10 μM. Agonists were given for 1 min in the superfusate of the bioassay: prostaglandin E_1 (●– – –●); E_2 (○– – –○); isoprenaline (□——□) and PACAP38 (Δ——Δ). Results are expressed as a percentage of the relaxation to nitroprusside 1 μM (●——●). Results are the mean ± s.e.mean of 6–10 rings.

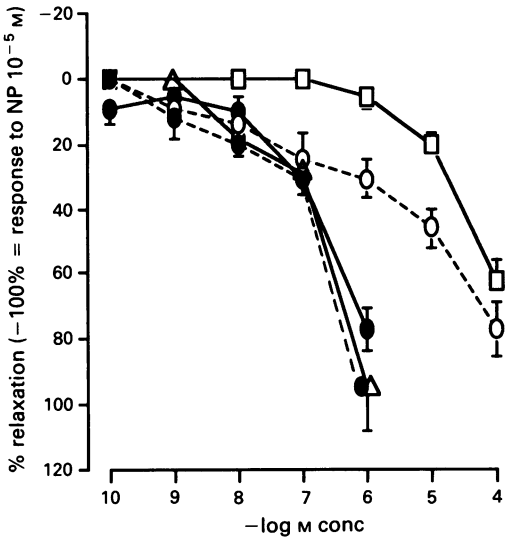

Figure 2 Graph showing the concentration-dependent effect of five agonists on rabbit coeliac artery rings. Rings were precontracted with phenylephrine 10 μM in the presence of indomethacin 10 μM. Agonists were given for 1 min in the superfusate of the bioassay: prostaglandin E_1 (●– – –●); E_2 (○– – –○); isoprenaline (□——□) and PACAP38 (Δ——Δ). Results are expressed as a percentage of the relaxation to nitroprusside 10 μM (●——●). Results are the mean ± s.e.mean of 6–10 rings.

Effects on skin blood flow

Of the agonists tested on skin blood flow *in vivo*, PACAP38 was the most potent and nitroprusside the least potent, there being a 10^7 fold shift in the dose response between them (Figure 3). Both PACAP38 and nitroprusside were significantly different from the other three agonists and from each other ($P<0.05$ in each case, ANOVA). Comparative potencies (− log mol/site of the dose required to increase basal flow by 75%) were: PACAP38 13.0, PGE_2 10.7, isoprenaline 9.7, PGE_1 9.1, nitroprusside <7. Sodium nitroprusside was not given in doses higher than 10^{-7} mol per site, as a preliminary experiment measuring blood pressure in rabbits with an intra-arterial cannula suggested that at 10^{-6} mol per site, or above, nitroprusside injected intradermally can affect systemic blood pressure.

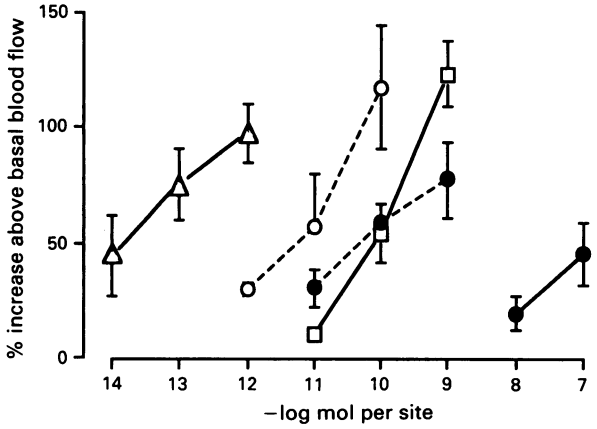

Figure 3 A comparison of the vasodilator effects of five agonists on skin blood flow in the shaved dorsal skin of the anaesthetized rabbit. Each agonist was injected in 100 µl aliquots and blood flow measured at 30 min with a laser Doppler flow probe: prostaglandin E_1 (●- - -●); E_2 (O- - -O); isoprenaline (□——□); PACAP38 (Δ——Δ) and nitroprusside (●——●). Each point represents the mean ± s.e.mean of 4 rabbits and each experiment was repeated four times in each rabbit. Results are expressed as the percentage increase in basal blood flow.

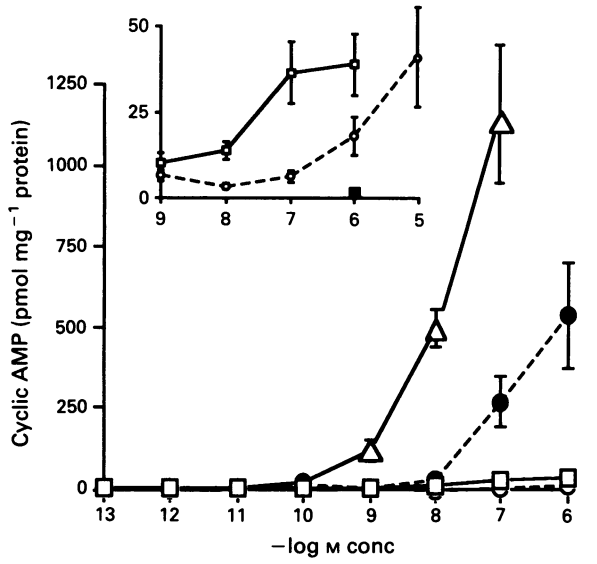

Figure 4 Graph showing the concentration-dependent effect of five agonists on cyclic AMP production by cultured rabbit aortic smooth muscle cells. Intracellular cyclic AMP was measured by radioimmunoassay after a 15 min incubation with each dose of agonist in the presence of a phosphodiesterase inhibitor. Nitroprusside (■) had no effect whereas the other four agonists tested increased cyclic AMP concentrations significantly and were significantly different from each other: prostaglandin E_1 (●- - -●); E_2 (O- - -O); isoprenaline (□——□) and PACAP38 (Δ——Δ). Results are expressed as the concentration of cyclic AMP per mg of protein. Each point is the mean ± s.e.mean of six experiments.

Stimulation of adenylate cyclase

Nitroprusside had no effect on cyclic AMP generation in cultured aortic vascular smooth muscle cells whereas all four of the remaining agonists caused dose-dependent increases (Figure 4). The order of potency was PACAP38 > PGE_1 > isoprenaline > PGE_2 and the agonists were significantly different from each other ($P<0.05$ in each case).

Discussion

The present study shows that within a single species, the adenylate cyclase-mediated responses to the four agonists tested were more prominent in the microcirculation than the macrocirculation. PGE_1 and PGE_2 were constrictors of the aorta, of intermediate effect in the coeliac artery, but potent vasodilators in skin, consistent with the concept that prostaglandins are important vasodilators of the microcirculation (Messina *et al.*, 1975; 1980; Messina & Kaley, 1980).

The results confirm PACAP38 as a vasorelaxant (Warren *et al.*, 1991; 1992c) and in addition show it to be particularly effective at increasing blood flow in the skin microcirculation. PACAP is a powerful stimulus to adenylate cyclase in many tissues and it is likely that PACAP causes vasorelaxation via the generation of cyclic AMP in vascular smooth muscle (Warren *et al.*, 1991). It stimulated adenylate cyclase in aortic smooth muscle cells in culture in the present experiments at much lower concentrations than the other three agonists tested.

The prostaglandins did not relax precontracted aortic rings and this is well described (Bunting *et al.*, 1976). The sensitivity of the aortic rings to PACAP suggests intracellular adenylate cyclase is coupled to the vasodilator mechanism in this tissue and that the insensitivity to prostaglandins may be at the receptor level. The contraction observed with higher concentrations of prostaglandins could be caused by cross reactivity with vasoconstrictor receptors. These are unlikely to be thromboxane receptors as the constrictor effects of higher concentrations of PGE_2 were not inhibited by GR32191, a compound known to be a specific thromboxane receptor antagonist (Humphrey *et al.*, 1990).

The radio-immunoassay data show both prostaglandins stimulated cyclic AMP generation in aortic smooth muscle cells in culture indicating that the receptors are linked to adenylate cyclase but that any vasodilator effect may have been negated by the stimulation of constrictor receptors.

Isoprenaline, PACAP, PGE_1 and PGE_2 all caused a dose-dependent elevation in intracellular cyclic AMP in rabbit cultured vascular smooth muscle cells. This supports the hypothesis, although does not prove it, that they act via this mechanism. It is possible to measure cyclic AMP in snap-frozen vascular bioassay rings which allows the elevation in cyclic AMP to be correlated with biological effect, but the maximal elevation in cyclic AMP observed with this method is too small to generate dose-response curves (Vuorinen *et al.*, 1992). For this reason, we chose to measure cyclic AMP generation in cultured cells in the presence of a phosphodiesterase inhibitor as the magnitude of the response allows agonist dose-response curves to be compared. Rabbit aorta was chosen as the source of vascular smooth muscle cells as they grow readily from explants and their identity can be confirmed by their morphology and staining characteristics. Repeating these studies with cultured microvascular smooth muscle cells would be of interest but it is not technically possible to grow these cells in sufficient numbers for such an experiment.

It is important not to over interpret the cyclic AMP radioimmunoassay data. The correlation between an elevation of intracellular cyclic AMP concentration and biological activity for different agonists can vary because of the phenomenon of compartmentalization (Buxton & Bruton, 1979). For example, some agonists cause a highly localized but effective elevation in cyclic AMP concentration with little change in total intracellular cyclic AMP content. Others, such as forskolin, affect many compartments causing an increase in total intracellular cyclic AMP which appears disproportionate to the biological effect. One group has used cell fixation by microwave irradiation to study cyclic nucleotide accumulation and shown that intracellular accumulation patterns are agonist-specific and dose-dependent (Barsony & Marx, 1990). For example, the effect of forskolin was maximal in the nucleus whereas isoprenaline and PGE_2 caused accumulation of cyclic AMP along the plasma membrane (Barsony & Marx, 1990).

Nitroprusside was the most potent relaxant tested in rabbit aorta but was the weakest when tested in the microcirculation. Similarly, the nitric oxide donor, isosorbide dinitrate, in a dose which causes a fall in systemic blood pressure through its action on large arteries, has little effect on flow in most microvascular beds (Wanless *et al.*, 1987). However, the microcirculation of rabbit skeletal muscle does dilate in response to endogenous nitric oxide released in response to acetylcholine (Persson *et al.*, 1990). The microvessels of rat and rabbit skin are capable of synthesizing and responding to nitric oxide, for instance after challenge with endotoxin or ultraviolet light (Pons *et al.*, 1992; Warren *et al.*, 1992b; 1993). The response to these inflammatory stimuli is mediated by the inducible form of nitric oxide synthase. This enzyme generates greater quantities of nitric oxide than the constitutive nitric oxide synthase, which may explain the effectiveness of inflammatory stimuli in causing vasodilatation. All of the present experiments were carried out in rabbit tissue but it seems likely that similar findings occur in man. PACAP38 is a powerful vasodilator of the human microcirculation as well as forearm resistance vessels (Warren *et al.*, 1992a). Prostaglandins and β_2-adrenoceptor agonists have similar vasodilator potency in man and rabbit. Nitroprusside causes arterial relaxation at low concentrations in man but is a weak vasodilator in the skin microcirculation (Warren *et al.*, unpublished observations).

In conclusion, aorta, coeliac artery and skin microvessels of the rabbit were all responsive to stimulators of adenylate cyclase. Tissue receptor differences appear to account for the variations seen with the agonists used, but in general the importance of adenylate cyclase-mediated vasodilatation increased with decreasing vessel size. The role of adenylate cyclase within the microcirculation has implications for our understanding of the mechanism of action of anti-inflammatory drugs, such as cyclo-oxygenase inhibitors, which suppress increased blood flow in inflamed tissue yet have little effect on systemic blood pressure.

Supported by the British Heart Foundation.

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(Received April 6, 1993
Revised May 27, 1993
Accepted June 2, 1993)