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VASOACTIVE INTESTINAL POLYPEPTIDE IS MORE POTENT THAN PROSTAGLANDIN E_2 AS A VASODILATOR AND OEDEMA POTENTIATOR IN RABBIT SKIN

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1 The 28 amino acid polypeptide, vasoactive intestinal polypeptide (VIP) induced increased local blood flow when injected intradermally in the rabbit.

2 VIP was found to be even more potent than prostaglandin E_2 (PGE_2) in increasing blood flow; VIP induced a significant effect at a 1 pmol dose.

3 VIP was shown to be poor in increasing microvascular permeability but very potent in enchancing local oedema induced by two substances which increase permeability, bradykinin and C5a des Arg. VIP was more potent than PGE_2 as an oedema potentiator.

4 Indomethacin had no effect on oedema potentiation induced by VIP, suggesting that a release of endogenous prostaglandins was not involved.

5 These results support our hypothesis for the regulation of oedema formation by arteriolar vasodilators, although the observations do not exclude the possibility of additional regulation by agonist interaction in the region of the venule.

6 VIP may be involved in the physiological control of normal blood flow and in hyperaemia in some types of inflammatory reactions.

Introduction

Vasoactive intestinal polypeptide (VIP) is a 28 amino acid substance which was originally isolated from hog small intestine (Said & Mutt, 1970). Intra-arterial and intravenous injections of VIP were shown to cause a marked decrease in peripheral resistance in the dog. In addition, it was demonstrated that VIP induces relaxation of several different isolated gut smooth muscle preparations and relaxation of isolated tracheal muscle (Piper, Said & Vane, 1970). It became increasingly apparent that the occurrence of VIP was not confined to the gut and that perhaps its most important location was in central and peripheral nerves (Bryant, Bloom, Polak, Albuquerque, Modlin & Pearse, 1976). For this reason attention has become diverted away from VIP as a potential circulating hormone (as are structurally related peptides such as secretin) and redirected towards a role as a local transmitter.

In our investigations of mechanisms involved in inflammation, we have found that prostaglandin E_1 (PGE_1), PGE_2 and, more recently, PGI_2, have the ability to enhance greatly protein leakage from skin vessels induced by substances that increase permeability such as histamine and bradykinin (Williams & Morley, 1973; Peck & Williams, 1978). This enhancing effect was found to correlate with the vasodilator activity of prostaglandins (Williams & Peck, 1977; Kopaniak, Hay & Movat, 1978; Williams, 1979). It was suggested that enhancement resulted from arteriolar dilatation producing increased intravenular hydrostatic pressure and passive venular distension.

In order to evaluate this hypothesis, we have tested a number of potential vasodilator substances in rabbit skin in order to investigate whether substances other than prostaglandins are able to enhance oedema formation. Substances were injected intradermally and their vasodilator activity assessed by their ability to increase local blood flow measured by a multiple-site ^{133}xenon clearance technique (Williams, 1979).

Most substances tested showed weak vasodilator activity in skin (e.g. acetylcholine, theophylline, adenosine, adenosine diphosphate, isoprenaline and substance P). However, one substance, VIP, showed striking vasodilator potency which exceeded that of PGE_2, the standard substance used in the test.

In this paper a comparison of the blood flow-increasing activity of VIP and PGE_2 is made. Further, using the accumulation of intravenously-injected ^{125}I-albumin to measure local oedema formation in

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skin, it is shown that VIP is able to potentiate oedema induced by bradykinin, although VIP itself does not induce increased microvascular permeability.

Synergism between putative mediators to induce local oedema is most clearly demonstrable using a substance which increases permeability without itself having vasodilator activity (unlike histamine and bradykinin which have both activities). We have discovered that the complement-derived polypeptide, C5a des Arg, is such a substance (Williams & Jose, 1981; Jose, Forrest & Williams, 1981). It is shown here that the two peptides C5a des Arg and VIP can act synergistically to induce oedema in skin and that VIP is a more potent potentiator than PGE_2.

VIP was originally shown to induce a lowering of peripheral resistance when injected intravascularly. The present observations showing the potent effects of the substance applied extravascularly are relevant to the current view of VIP as a potential local hormone.

These observations suggest that VIP could be involved in the physiological control of normal blood flow. In addition, VIP may have a role in the control of blood flow and protein leakage in certain pathophysiological conditions.

Methods

Male New Zealand White rabbits, 3.5–4 kg body weight were used in all experiments. Blood flow changes were measured by a multiple site ^{133}xenon clearance technique, as previously described (Williams, 1979). VIP (Sigma London Chemical Company) and PGE_2 (Sigma London Chemical Company) were mixed with a solution of ^{133}xenon (The Radiochemical Centre, Amersham) to give an activity of 5–10 μCi/0.1 ml and injected intradermally in 0.1 ml volumes into previously-clipped dorsal skin. Injections were given rapidly in randomized order according to a fixed balanced site pattern, with six replicates for each dose. A short-acting anaesthetic, methohexitone sodium (approximately 10 mg/kg) was given prior to injections to facilitate animal handling. After 20 min, each rabbit was killed using an overdose of pentobarbitone sodium, the back skin was removed and injection sites punched out with a 16 mm diameter punch. Samples of skin and injection fluid were stored under paraffin oil in sealed tubes for counting in an automatic γ-counter. Clearance in test sites was calculated as a percentage of clearance in phosphate-buffered saline control sites, as previously described (Williams, 1979).

Local plasma exudation was measured using the 30 min accumulation of intravenously-injected ^{125}I-human serum albumin, approximately 15 μCi/kg body weight (The Radiochemical Centre, Amersham). VIP, PGE_2, bradykinin triacetate (Sigma London Chemical Company), rabbit C5a des Arg (i.e. C5a without carboxyl terminal arginine, prepared as in Williams & Jose, 1981) or combinations of these agents, were injected intradermally and the amount of protein leakage determined in each skin sample using an automatic γ-counter, as above. Results were expressed in terms of local plasma volumes by dividing each skin ^{125}I count by the count of 1 μl of plasma at death (Williams, 1979).

In some experiments arachidonic acid (sodium salt, Sigma London Chemical Company) and indomethacin (Merck Sharp & Dohme) were mixed with other agents and injected intradermally.

Results

Figure 1 shows a comparison of the blood flow increase induced by VIP and PGE_2 measured over a 20 min period in rabbit skin. Both substances showed potent vasodilator activity. There was no significant difference between their effects at a dose of 100 pmol, however, VIP was more active than PGE_2 at 1 and 10 pmol ($P<0.001$ and $P<0.05$ respectively by Student's t test). At the lowest dose, 1 pmol, the effect of PGE_2 was not significant but VIP induced a $68\pm8\%$ increase in flow compared with control ($P<0.01$).

We have found previously that the arachidonic acid cyclo-oxygenase products PGE_2 and PGI_2 have similar high vasodilator potency in rabbit skin,

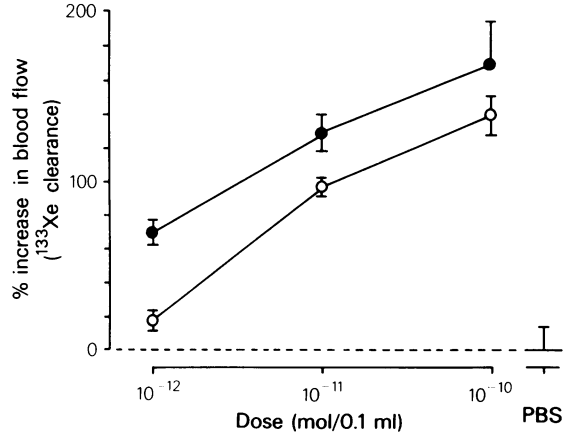

Figure 1 An experiment showing a comparison of vasoactive intestinal polypeptide (VIP, ●) and prostaglandin E_2 (PGE_2, ○) in increasing blood flow in rabbit skin over a period of 20 min following intradermal injection. The dashed line represents the control value obtained by injections of phosphate-buffered saline (PBS). The values are represented as means for $n=6$ sites; vertical lines show s.e.mean.

whereas PGD_2, $PGF_{2\alpha}$, histamine and bradykinin have relatively low activity (Williams & Peck, 1977; Williams, 1979). None of the arachidonic acid lipoxygenase products has been shown to have vasodilator activity; on the contrary some of the leukotrienes are vasoconstrictors (Drazen, Austen, Lewis, Clark, Goto, Marfat & Corey, 1980; Williams & Piper, 1980; Dahlen, Björk, Hedqvist, Arfors, Hammarström, Lindgren & Samuelsson, 1981; Peck, Piper & Williams, 1981). Thus, the vasodilator potency of VIP applied extravascularly in the skin compares favourably with any known arachidonic acid metabolite.

Vasodilator prostaglandins have the ability to enhance oedema induced by permeability-increasing substances (Williams & Morley, 1973). Vasodilators such as adenosine also potentiate oedema (Williams & Peck, 1977) but their potency is low in the skin so that endogenous release of these substances is unlikely to be of biological significance. On the other hand, because of its vasodilator potency, VIP could be of importance in the control of oedema formation. In our initial experiments we used combinations of VIP and bradykinin to test for synergism. VIP at a dose of 100 pmol induced little oedema alone, but caused a $107 \pm 15\%$ increase in responses to bradykinin at a dose of 250 pmol ($n = 6$ rabbits). Responses to bradykinin plus VIP were not significantly reduced by locally-injected indomethacin at 3 nmol (a reduction of $3.9 \pm 4.3\%$, $n = 9$ rabbits) indicating that VIP is not acting by releasing endogenous prostaglandins. In the same animals, matching responses to bradykinin (250 pmol) + AA(3 nmol) were reduced by $42 \pm 9\%$.

We have, as yet, no evidence to indicate that the nonapeptide bradykinin is released in inflammatory reactions in the rabbit. We have, however, evidence for the involvement of a larger polypeptide (approximately 70 amino acid residues), C5a des Arg. This peptide is interesting in that, unlike histamine and bradykinin, C5a des Arg has no detectable vasodilator activity (Williams & Jose, 1981). Hence, although it is potent in increasing venular permeability (the most potent endogenous substance that we know of), C5a des Arg induces little oedema when tested alone. As shown in Figure 2, striking synergism was produced using a mixture of the vasodilator peptide VIP and the permeability-increasing peptide C5a des Arg to produce oedema. VIP was again more potent than PGE_2; VIP inducing significantly more potentiation at 1 and 10 pmol ($P < 0.01$ and $P < 0.05$ respectively). Potentiation induced by VIP was not affected by indomethacin (results are shown in the legend to Figure 2). As shown, a mixture of PGE_2 and VIP induced insignificant oedema, demonstrating that neither substance produces significant increased venular permeability.

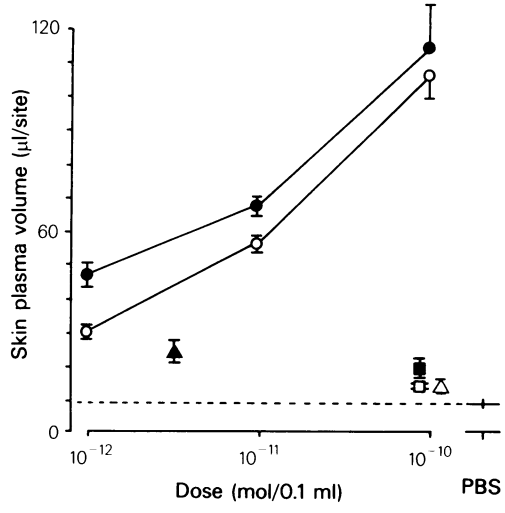

Figure 2 An experiment to demonstrate oedema formation in rabbit skin induced by synergism between C5a des Arg and vasoactive intestinal polypeptide (VIP, ●), and for comparison C5a des Arg and prostaglandin E_2 (PGE_2, ○). C5a des Arg was used at a dose of 2×10^{-12} mol throughout. The small response induced by C5a des Arg alone is shown (▲). Note that PGE_2 (□), VIP (■) or a mixture of the two substances (△) induced little oedema when compared with phosphate-buffered saline (PBS) controls. Locally-injected indomethacin (3×10^{-9} mol) had no significant effect ($1.4 \pm 7.4\%$ increase, $n = 8$ rabbits) on responses to C5a des Arg (2×10^{-12} mol) plus VIP (10^{-10} mol) but reduced matching responses to C5a des Arg (2×10^{-12} mol) + arachidonic acid (3×10^{-9} mol) by $50 \pm 9\%$ when tested in the same animals. The values are represented as means for $n = 6$ sites; vertical lines show s.e.mean.

All these results are consistent with the hypothesis that arteriolar tone is important in the control of plasma protein leakage from venules.

Discussion

VIP is shown here to be a potent vasodilator when applied extravascularly to the microvasculature of rabbit skin. The potency of VIP compares favourably with the vasodilator prostaglandins which have been widely regarded as the most potent arteriolar relaxants. Increased blood flow was highly significant at a VIP dose of 1 pmol, a dose at which the PGE_2 effect was not significant.

We have previously shown that E-type prostaglandins have the ability to enhance oedema induced by substances such as histamine and bradykinin. This potentiating effect has been correlated with the vasodilator activity of prostaglandins (Williams & Peck, 1977; Kopaniak *et al.*, 1978) which is supported by

the later observation that the vasodilator, prostacyclin, is also able to enhance oedema (Peck & Williams, 1978; Williams, 1979). It is possible that prostaglandins are acting at two sites to produce potentiation (Williams, 1979): prostaglandins could sensitize receptors to other agonists on venular endothelial cells as well as dilating arterioles. Our experimental techniques cannot differentiate these two effects if present together, however, other workers have provided evidence for synergism which is independent of hyperaemia (Amelang, Prasad, Raymand & Grega, 1981).

The present observations emphasize the importance of vasodilatation in oedema formation, although it is possible that VIP has an additional modulating effect directly on venular endothelial cells, as has been suggested for prostaglandins.

The phenomenon of oedema induced by synergism between two substances was originally described for histamine or bradykinin together with an E-type prostaglandin (Williams & Morley, 1973). We now know of several examples of the phenomenon. Two interesting examples are: synergism between two lipid metabolites of arachidonic acid, the permeability-increasing leukotriene B_4 together with the vasodilator PGE_2 (Bray, Cunningham, Ford-Hutchinson & Smith, 1981; Higgs, Salmon & Spayne, 1981; Wedmore & Williams, 1981); and synergism between two polypeptides, C5a des Arg and VIP as shown here.

The high potency of VIP in increasing skin blood flow implies a biological function. We have, as yet, no evidence for release of the peptide in inflammatory reactions in the skin, although release of substances like VIP would explain why vasodilatation and oedema in some reactions are not effectively suppressed by compounds which inhibit prostaglandin synthesis.

Mast cells (Cutz, Chan, Track, Goth & Said, 1978), PMN leukocytes (O'Dorisio, O'Dorisio, Cataland & Balcerzak, 1980) or cutaneous nerves (Polak & Bloom, 1980) are possible sources of VIP in inflammatory reactions.

In a previous study the potent vasodilator action of certain prostaglandins was demonstrated, but since indomethacin had no effect on normal blood flow (whilst able to inhibit arachidonic acid-induced increased flow) a role for prostaglandins in the control of normal flow appeared unlikely (Williams, 1979). The results presented here demonstrate that the potent effects induced by VIP in the skin are not inhibited by indomethacin. This observation and the known occurrence of high concentrations of VIP in cutaneous nerves suggest the possibility that this peptide could have a physiological role in the control of normal blood flow.

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