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The actions of calcitonin gene related peptide and vasoactive intestinal peptide as vasodilators in man *in vivo* and *in vitro*

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1 The two peptides calcitonin gene related peptide (CGRP) and vasoactive intestinal peptide (VIP) produced marked dilatation of the forearm vascular bed when infused via the brachial artery.

2 CGRP relaxed preconstricted segments of human radial, coronary, gastric and cerebral arteries in an endothelium dependent manner.

3 VIP relaxed human gastric and transverse cervical arteries in an endothelium dependent manner, but relaxation of the human pulmonary artery was not dependent on endothelium.

4 The characteristics of the endothelium dependent relaxation of these medium-sized muscular arteries indicated involvement of the endothelium derived relaxing factor *in vitro*.

5 Caution is expressed in drawing comparisons between the mechanisms involved in the *in vivo* and *in vitro* vascular responses.

Keywords calcitonin gene related peptide vasoactive intestinal peptide vasodilatation endothelium derived relaxing factor

Introduction

The two peptides calcitonin gene related peptide (CGRP) and vasoactive intestinal peptide (VIP) are widely distributed in animal species including man and a number of diverse actions of the peptides have been described (Goodman & Iversen, 1986; Said, 1983). They share vasoactive properties (Brain *et al.*, 1985; Said & Mutt, 1970a,b) and may have important functions as neurotransmitters or neuromodulators in a non-adrenergic non-cholinergic nervous system (Burnstock, 1985).

VIP was first discovered in 1970 in hog small intestine (Said & Mutt, 1970b) and has subsequently been located in perivascular nerves supplying several tissues (Uddman *et al.*, 1981), and within the heart and its conducting system (Weihe & Reinecke, 1981). In the parasympathetic system, VIP is costored with acetylcholine (ACh) (Burnstock, 1983). Studies with cat cerebral arterioles suggested that its vasodilator properties might be prostaglandin mediated (Wei *et al.*, 1980), but in man the cardiac inotropic, peripheral vasodilator and renin elevating response to intravenously administered VIP is not blocked by either indomethacin or propranolol (Calam *et al.*, 1984).

Molecular genetics led to the discovery of CGRP before any understanding of its function. It is a product of alternative mRNA transcription of the calcitonin gene which in thyroidal C cells produces calcitonin and in non-thyroidal tissue, CGRP (Amara *et al.*, 1982). It has also been widely identified in the nervous system (Tschopp *et al.*, 1985) and the cardiovascular system; in the heart (Rosenfeld *et al.*, 1983) and perivascular nerves (Mulderry *et al.*, 1985), where it is co-stored with substance P (Lundberg *et al.*, 1985). Given

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intravenously to normal volunteers, CGRP causes peripheral vasodilatation and hypotension (Struthers *et al.*, 1986), whereas central administration in experimental animals appears to activate sympathetic outflow with a pressor response and an increase in plasma noradrenaline levels (Fisher *et al.*, 1983). Both peptides have been found in human plasma—CGRP in the normal circulation (Girgis *et al.*, 1985) with higher levels occurring in normal pregnancy (Stevenson *et al.*, 1986), and VIP after exercise (Galbo *et al.*, 1979). In the case of CGRP there is evidence that the circulating peptide originates from perivascular nerves (Zaidi *et al.*, 1985).

The purpose of this study was to assess the direct activity of these vasodilator peptides in a human vascular resistance bed, the forearm, and to investigate their mechanism of action by studying the responses of human isolated blood vessels.

Methods

In vitro

Segments of pulmonary, gastric, coronary, radial and transverse cervical arteries were freshly obtained from surgical resection specimens and cerebral arteries were obtained from autopsy tissue within 4 h of death. Material was obtained from 16 patients (age range 19–72 years, 10 male). The vessels were collected into cold Krebs buffer of composition (mM) NaCl 118, KCl 4.7, $MgSO_4.7H_2O$ 1.2, $NaH_2PO_4.2H_2O$ 1.0, glucose 11.1, $NaHCO_3$ 25, $CaCl_2.6H_2O$ 2.5, Na_2 EDTA 0.03, cleaned of excess connective tissue and mounted as 2–4 mm rings (internal diameter 1–4 mm) under resting tension 1–4 g in 6 ml organ baths containing Krebs buffer at 37° C aerated with 95% O_2, 5% CO_2 (Towart, 1982). Vessels were then allowed to equilibrate for 1 h prior to starting experiments. Arteries were preconstricted using approximate EC_{50} of noradrenaline (1–3 μmol l^{-1}) or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (1–10 μmol l^{-1}). After stable contraction CGRP or VIP were added to the tissue bath in a cumulative fashion. Experiments were performed in the presence of indomethacin (10 μmol l^{-1}) to block cyclooxygenase activity. All arterial segments used for these studies relaxed in response to acetylcholine (0.1–3 μmol l^{-1}) or the calcium ionophore A23187 (0.1–0.3 μmol l^{-1}) and this was regarded as indicative of functional endothelial integrity. The endothelium was subsequently deliberately removed from some rings by rubbing the lumen with watchmakers forceps, and the effectiveness of this procedure was demonstrated by abolition of relaxation in response to ACh or A23187. In other preparations, haemoglobin (5 μmol l^{-1}) or methylene blue (10 μmol l^{-1}) were added to the tissue bath after the arterial rings were effectively relaxed by CGRP or VIP.

In vivo

Ten healthy male volunteers (aged 20–40 years) participated in studies which were approved by the St Mary's Hospital Ethics Committee. Each subject attended the Pickering Clinical Investigation Unit once, and the observations were made with the subject recumbent in a room at a steady temperature (23° C). Forearm blood flow (FBF) was measured by venous occlusion plethysmography using temperature compensated mercury in rubber strain gauges (Whitney, 1953). The hand circulation was excluded during measurements by inflation of a wrist cuff to a pressure 30 mmHg above systolic. The brachial artery of one arm was cannulated under lignocaine local anaesthesia with a 23 gauge needle to allow intra-arterial infusion of drugs and blood pressure monitoring. The other arm served as a control throughout the study. CGRP (10, 30, 100 ng min^{-1}, i.a.) or VIP (10, 30, 100 ng min^{-1}, i.a.) were infused for 5 min periods at each dose level into the study arm. FBF was measured in both arms every 15 s during the last 3 min of each infusion sequence. Results were expressed as percentage change in FBF in the study arm, minus any change in the control arm.

Drugs

Synthetic VIP (Peninsula U.K.) and synthetic human αCGRP (Peninsula U.K.) were prepared as aliquots in 0.1 M acetic acid, stored at –20° C, thawed and neutralized with sodium hydroxide immediately prior to use *in vitro*. For the forearm studies the peptides were diluted in a 50:50 solution of normal saline and Haemaccel plasma substitute (Behring, Hoechst, U.K.). For the tissue bath studies, stock solutions of indomethacin (10 mmol l^{-1}) (Sigma Chemical Co.) were prepared in 50% ethanol and of A23187 (10 mmol l^{-1}) (Sigma Chemical Co.) in 100% dimethylsulphoxide. These solutions were appropriately diluted in distilled water before use. Noradrenaline was dissolved in Kreb's buffer. Methylene blue (Sigma Chemical Co.), $PGF_{2\alpha}$ (Upjohn), ACh (Sigma Chemical Co.) and human haemoglobin (Sigma Chemical Co.) were prepared in distilled water.

Statistics

Where appropriate, statistical evaluation of the blood flow responses was made using analysis of variance.

Results

In vitro

CGRP (1 nmol l^{-1}–1 μmol l^{-1}) relaxed preconstricted segments of human radial (n = 2), coronary (n = 4), gastric (n = 5) and cerebral (n = 3) arteries in an endothelium dependent manner (Figure 1). VIP (1 nmol l^{-1}–1 μmol l^{-1}) also relaxed human gastric (n = 2), splenic (n = 2), transverse cervical (n = 3) (Figure 2) and pulmonary (n = 5) arteries (Figure 3). VIP relaxation of the gastric, splenic and transverse cervical arteries was dependent on the presence of endothelium; however, VIP induced relaxation of pulmonary artery was not dependent on functional endothelium. The endothelium dependent relaxations could be abolished either by luminal rubbing, addition of haemoglobin or addition of methylene blue.

In vivo

Infusion of CGRP into the brachial artery of six subjects (10–100 ng min^{-1}) produced a marked dose dependent increase in FBF (Figure 4). The FBF responses produced by CGRP at 30 and 100 ng min^{-1} were significantly different from control FBF ($P < 0.01$). At 100 ng min^{-1} the mean net increase in FBF was 174 ± 24% (mean ± s.e. mean); at this dose marked flushing of the skin was noted in all six subjects. Infusion of VIP into the brachial artery of four subjects (10–100 ng min^{-1}) also produced a marked dose dependent increase in FBF (Figure 5). At 100 ng min^{-1} the net increase in FBF was 223 ± 34% (mean ± s.e. mean). The effects of the peptides were confined to the infused arm at these dose ranges; there was no significant change in the control arm blood flow.

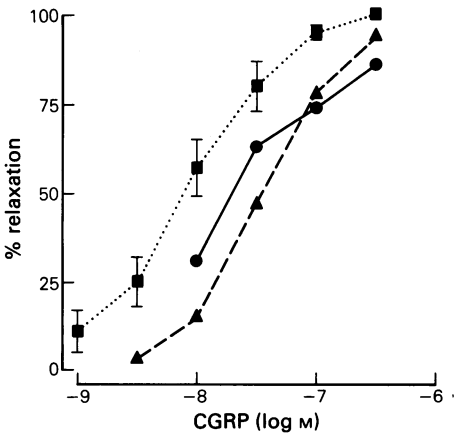

Figure 1 Endothelium dependent relaxation by CGRP in coronary (●——●) (n = 4) (means ± s.e. mean), gastric (■----■) (n = 5) and radial (▲----▲) (n = 2) arteries.

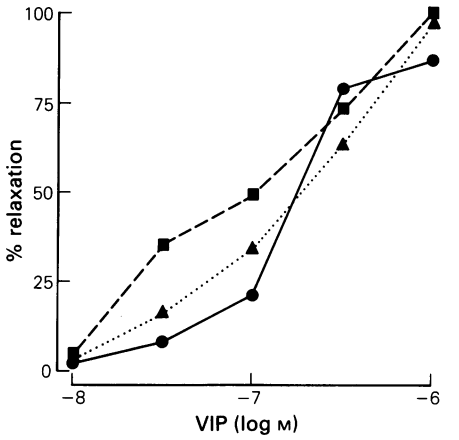

Figure 2 Endothelium dependent relaxation by VIP in splenic (■----■) (n = 2), gastric (▲----▲) (n = 2) and transverse cervical (●——●) (n = 2) arteries.

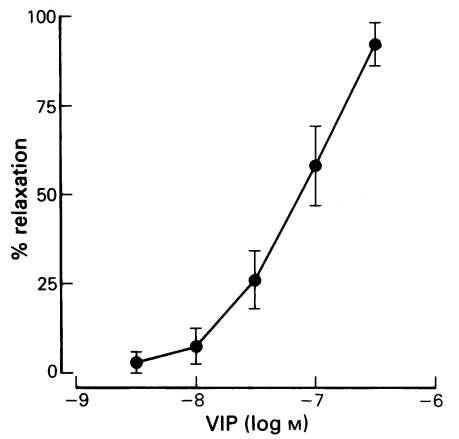

Figure 3 Endothelium independent relaxation of pulmonary arteries by VIP (n = 6) (means ± s.e. mean).

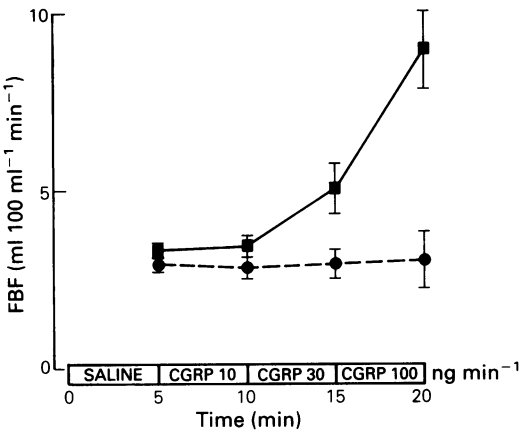

Figure 4 The effect of CGRP (10–100 ng min^{-1}) infused via the brachial artery on FBF. Study arm ■——■; Control arm ●----● (means ± s.e. mean, $n = 6$).

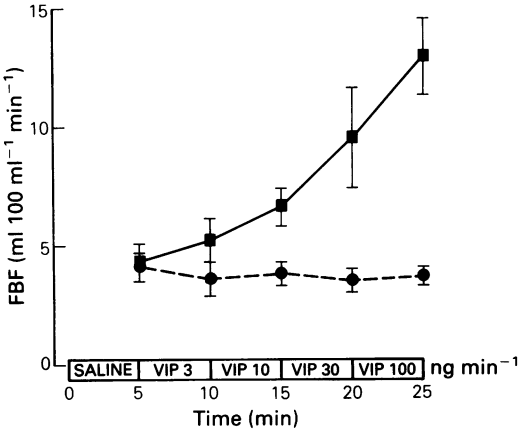

Figure 5 The effect of VIP (3–100 ng min^{-1}) infused via the brachial artery on FBF. Study arm ■——■; Control arm ●----● (means ± s.e. mean, $n = 4$).

Discussion

These studies demonstrate that CGRP and VIP are potent vasodilators in the human forearm vascular bed. The *in vitro* observations suggest that the effects of both peptides may in some sites be partly or wholly mediated by the release of endothelium derived relaxing factor (EDRF). The reversal of the response by haemoglobin and methylene blue and its abolition by luminal rubbing support this conclusion (Martin *et al.*, 1985). Within medium-sized muscular arteries there are clearly differences dependent on site as illustrated here by the endothelium independent pulmonary artery relaxation by VIP. Smaller human pial arteries of internal diameter 250–600 μM have been demonstrated to relax in response to CGRP and VIP even after endothelial removal (Hardebo *et al.*, 1985). Inter-species differences may also be manifest; whilst VIP and CGRP are endothelium dependent in relaxing rat aorta (Davies & Williams, 1983; Said, 1983), they relax human, cat and rabbit pial arteries independently of endothelium (Hardebo *et al.*, 1985) and VIP relaxation of the dog carotid is endothelium independent (D'Orleans-Juste *et al.*, 1985). These variations may depend as much on site and vessel size as species, and comparative studies must take these factors into account.

Perhaps of greater interest is the apparently variable contribution of the endothelium in different vascular beds and different calibre vessels within the human circulation. However, considerable caution is necessary in extrapolating from *in vitro* observations of large vessels to the *in vivo* response of a resistance vascular bed. Bubbled Krebs buffer bears little resemblance to circulating blood and if EDRF proves to be an oxygen-derived free radical (Furchgott *et al.*, 1981), the two situations might, in this context, be unrelated. The EDRF mechanism has been shown to be cyclic GMP linked (Rapoport *et al.*, 1983) and if the response to these peptides involves EDRF, a rise in intracellular cyclic GMP might be expected, but most of the studied functions of VIP and CGRP seem to be linked to a rise in cyclic AMP (Güild & Drummond, 1984; Wohlwend *et al.*, 1985). Both peptides stimulate cyclic AMP formation in cultured rat aortic smooth muscle cells—the site in which the whole tissue relaxant responses are paradoxically endothelium dependent (Kubota *et al.*, 1985). However, rises in cellular levels of cyclic AMP are not invariably associated with a functional response, and do not preclude the involvement of an additional second messenger (Vegesna & Diamond, 1984). Methylene blue and haemoglobin are held to be inhibitors of soluble guanylate cyclase and their use in these studies suggests the involvement of cyclic GMP in the endothelium dependent relaxation responses.

A further conceptual difficulty in accepting the role of EDRF in the response to VIP and CGRP arises with consideration of their storage in perivascular nerves, some considerable diffusion distance away from the endothelium. Whilst there is evidence that acetylcholine, the archetypal releaser of EDRF, may be generated and stored in the endothelial layer (Parnavelas *et al.*, 1985), there is none such for VIP or CGRP. Clearly the diffusion distance diminishes with progression along the vascular tree towards the

resistance vessels, and the peptides have been demonstrated in the circulation, but this would seem rather an extravagant and circuitous route for a neuro-transmitter.

The prolonged duration of the red wheal response to intradermal CGRP has been documented (Brain *et al.*, 1985), and the skin flushing that appeared in these subjects in response to intra-arterial infusion was a little more intense with CGRP than with VIP, but in both cases it had disappeared within 30 min. The forearm vasodilatation also diminished over this same time course, although it is noteworthy that the vasodilator response to intra-arterial acetylcholine is much more evanescent. This may imply a difference in mechanism and we have recently found that CGRP relaxes human resistance vessels taken from subcutaneous fat in a manner independent of endothelium (unpublished data). The use of a specific EDRF antagonist *in vivo* would clarify the nature of these responses. However, until such an agent becomes available, the role of EDRF in the responses found *in vivo* in this study must remain speculative.

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