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Human α-calcitonin gene-related peptide (CGRP) is a potent vasodilator in human mesenteric vasculature

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1 The effect of human α-calcitonin gene-related peptide (CGRP) and sodium nitroprusside have been measured on human isolated mesenteric vasculature and on rings of human superior mesenteric artery and saphenous vein.
2 When noradrenaline (10^{-5} M) was used as the vasoconstrictor in preparations perfused with Krebs solution at constant flow, human α-CGRP was 10 times more potent than sodium nitroprusside in evoking dose-dependent falls in perfusion pressure.
3 Human α-CGRP and sodium nitroprusside were about equipotent at relaxing rings of superior mesenteric artery contracted by noradrenaline (10^{-6} M). When the tone of saphenous vein rings was raised by noradrenaline (10^{-6} M), human α-CGRP did not relax the vascular smooth muscle.
4 The results show that human α-CGRP is a potent vasodilator in human arterial preparations and may act preferentially on arterioles rather than large arteries.

Keywords calcitonin gene-related peptide (CGRP) sodium nitroprusside vasodilators mesenteric vasculature

Introduction

Studies on the structure and expression of the rat calcitonin gene resulted in the identification of a novel neuropeptide, the α-calcitonin gene-related peptide (CGRP; Rosenfeld *et al.*, 1983). A second β-CGRP gene is now known to be present also (Rosenfeld *et al.*, 1984). In man, like the rat, two CGRP peptides have been predicted using gene cloning (Edbrooke *et al.*, 1985; Jonas *et al.*, 1985; Steenbergh *et al.*, 1984, 1985) and one, human α-CGRP, has been isolated from human medullary thyroid carcinoma tissue (Morris *et al.*, 1984). Immunocytochemical analysis has localised CGRP in the rat in discrete regions of the central nervous system (Skofitsch & Jacobowitz, 1985a, b) and at nerve endings within the vasculature and heart (Mulderry *et al.*, 1985; Sigrist *et al.*, 1986). In man CGRP has been localised with calcitonin producing cells in the thyroid (Schifter *et al.*, 1986), circulates at low levels in normal plasma (Girgis *et al.*, 1985), and at elevated levels in plasma of patients with lung and medullary thyroid carcinoma (Girgis *et al.*, 1985; Edbrooke *et al.*, 1985).

Rat and human have been shown in animal models to be potent inhibitors of gastric acid secretion (Lenz *et al.*, 1985), potent vasodilators (Brain *et al.*, 1985; Holman *et al.*, 1986; Marshall *et al.*, 1986b, c), and can modulate neurotransmission (Al-Kazwini *et al.*, 1986). So far studies on man have demonstrated a long lasting vasodilation of cutaneous vessels (Brain *et al.*, 1985), inhibition of gastric acid secretion (Kraenzlin *et al.*, 1984), and recently hypotension with an associated tachycardia after the intravenous administration of CGRP in normal volunteers (Struthers *et al.*, 1986; Gennari & Fischer, 1985). The aim of the present work was to study the effects of human α-CGRP in human isolated mesenteric vasculature and to compare it with a standard vasodilator, sodium nitroprusside.

Methods

Macroscopically normal human mesentery was obtained from males and females (aged 56–67 years) undergoing left hemicolectomy or anterior resection. Saphenous veins were from female patients (aged 45–66 years) undergoing coronary artery bypass surgery. Tissues were kept in Krebs solution on ice during removal to the laboratory from the operating theatre.

A section of mesentery was dissected free from the gastro-intestinal tract and perfused through a branch of the superior mesenteric artery with Krebs solution at 37°C and of the following composition (mM); NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4.7H_2O$ 1.2 and glucose 11. The Krebs solution was bubbled with 95/5% O_2/CO_2 and pumped at a constant flow of 5.8 ml min^{-1} (Watson-Marlow 502S flow inducer). The perfusion pressure was recorded with a Statham P23Id pressure transducer connected to a Grass polygraph. In most experiments the perfusion pressure was raised by adding the vasoconstrictor noradrenaline (10^{-5} M) to the Krebs solution. This procedure allows the effects of vasodilators to be more easily measured. Doses of vasodilator were injected into the perfusing Krebs solution in volumes of less than 100 μl. Human α-CGRP was added in a cumulative manner with doses spaced at 4 min intervals. Sodium nitroprusside was given in single doses due to its shorter duration of action. Changes in the perfusion pressure due to human α-CGRP or sodium nitroprusside were measured in mm Hg from the resting stable pressure preceding drug addition.

In other experiments, rings (about 3 mm length) were cut from a branch of the superior mesenteric artery and suspended between 200 μm diameter wires, one fixed and the other connected to a Grass FT.03 transducer to record isometric tension. The arterial rings were kept in Krebs solution (composition as above) at 37°C and bubbled with 95/5% O_2/CO_2. Tissues were equilibrated for at least 1 h under initial tension of 2.0 g. The rings were contracted using noradrenaline (10^{-6} M). After the contraction had plateaued either sodium nitroprusside or human α-CGRP was added cumulatively with doses spaced at 1 min intervals. The relaxation produced by each dose was calculated as the percentage fall in tension from that induced by noradrenaline immediately prior to the cumulative concentration-effect curve.

Rings of saphenous vein were set up and used as described above for rings of mesenteric artery except that the resting tension was set at 1.0g.

Human α-CGRP was obtained purified from Cambridge Research Biochemicals and purity and structure determined by amino acid analysis, h.p.l.c. and mass spectrometry. The peptide was dissolved in distilled water (10^{-3} M) and stored in aliquots at –20°C. Sodium nitroprusside (Sigma) was dissolved in distilled water and protected from light at all times and noradrenaline bitartrate (Sigma) was dissolved in Krebs solution.

All results are given as mean ± s.e. mean. Differences between treatments have been compared using Student's *t*-test. When $P<0.05$, values were considered to be statistically significant.

Results

Perfused mesenteric vasculature

The perfusion pressure of human isolated mesenteric vasculature was raised to 150 ± 35 mm Hg (mean ± s.e. mean) by the addition of a sub-maximal concentration of the vasoconstrictor noradrenaline, 10^{-5} M. This rise was usually well maintained and a stable pressure was recorded for at least 20 min prior to the addition of a vasodilator. Human α-CGRP caused dose-related falls in the perfusion pressure which were slow in onset (Figure 1).

Human α-CGRP was about 10 times more potent than sodium nitroprusside in lowering the perfusion pressure raised by noradrenaline, 10^{-5} M (Figure 2). Sodium nitroprusside evoked a quicker vasodilatation and more rapid recovery than human α-CGRP. For example the $t_{1/2}$ (time for 50% recovery of perfusion pressure) after sodium nitroprusside, 10^{-7} M, was 2.1 ± 0.4 min while after human α-CGRP the $t_{1/2}$ was greater than 60 min. During the prolonged vasodilation induced by human α-CGRP, in one tissue a dose of noradrenaline, 10^{-4} M, was given. This caused a short lived rise in the perfusion pressure, indicating that the vessels were still functional.

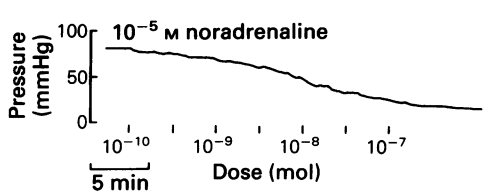

Figure 1 The effect of human α-CGRP on the perfusion pressure of human isolated mesenteric vasculature in the presence of vasoconstriction evoked by noradrenaline (10^{-5} M).

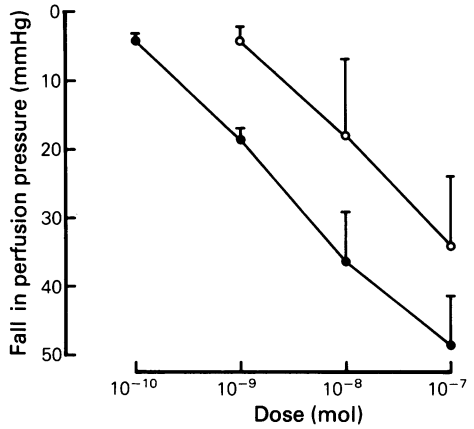

Figure 2 The fall in perfusion pressure evoked by human α-CGRP (•) and sodium nitroprusside (○) in human isolated mesenteric vasculature perfused with Krebs solution containing noradrenaline (10^{-5} M). Points represent means from four tissues and vertical bars are s.e. mean.

Mesenteric artery rings

The tone of rings cut from branches of the superior mesenteric artery of four patients was raised by noradrenaline, 10^{-6} M, to 1.8 ± 0.7 g. The cumulative addition of sodium nitroprusside relaxed this tension (Figure 3) with an IC_{50} (drug concentration to relax the noradrenaline-induced tone by 50%) of 6.1 ± 3.8 × 10^{-7} M. The onset of relaxation evoked by human α-CGRP was not as rapid as that to sodium nitroprusside. The human α-CGRP concentration-dependent relaxation (Figure 3) gave an IC_{50} of 2.3 ± 1.9 × 10^{-6} M. Therefore the peptide was of similar potency to sodium nitroprusside in rings of superior mesenteric artery.

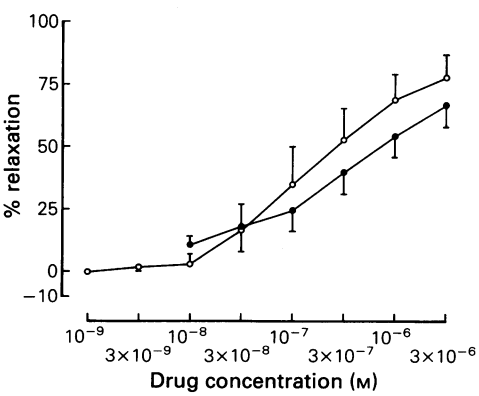

Figure 3 Cumulative concentration-response curves showing the relaxation of noradrenaline-induced tone (10^{-6} M) evoked by human α-CGRP (•) and sodium nitroprusside (○) in human mesenteric arterial rings. The results are plotted as the percentage reduction of the response to noradrenaline (1.8 ± 0.7 g) and are shown as mean ± s.e. mean from four preparations.

Saphenous vein rings

The mean tone induced by noradrenaline 10^{-6} M in rings of saphenous vein from two patients was 235 mg. Sodium nitroprusside (10^{-9}–10^{-6} M) caused the complete relaxation of this tone. In contrast, human α-CGRP (3 × 10^{-8}–3 × 10^{-6} M) evoked only a mean of 16% relaxation with the highest drug concentration.

Discussion

The distribution of CGRP-staining nerve endings associated with the guinea-pig and rat cardiovascular systems, has been described (Mulderry *et al.*, 1985; Wharton *et al.*, 1986), but as yet similar studies have not been reported for man. In the guinea-pig and rat arterial system the highest level of CGRP appears to be associated with the superior mesenteric artery as determined by a combination of immunocytochemical and quantitive radioimmunoassay techniques (Mulderry *et al.*, 1985; Wharton *et al.*, 1986). In general more perivascular CGRP-immunoreactive fibres were found around arteries than around veins (Uddman *et al.*, 1986). A dense meshwork of CGRP-containing fibres was associated with the rat superior mesenteric artery and with the arterioles (Sasaki *et al.*, 1986).

Human α-CGRP has recently been shown to be a potent vasodilator in rat and rabbit perfused mesenteric vasculature (Marshall *et al.*, 1986b) and in a number of other vascular beds from several species (for review see Marshall & Craig, 1988). Previous studies in man demonstrate that human α-CGRP when given intradermally elicited erythema lasting up to 12 h (Brain *et al.*, 1985), whilst intra-coronary administration produced dose-dependent increases in the diameter of human epicardial arteries (McEwan *et al.*, 1986). Studies on human volunteers have proved less definitive. In these CGRP when given intravenously caused a small drop in blood pressure and tachycardia (Gennari & Fischer, 1985; Struthers *et al.*, 1986).

The present results demonstrate that human α-CGRP is a potent vasodilator in the perfused human mesenteric vascular bed (when compared with sodium nitroprusside) and is relatively less effective on rings of branches of the superior mesenteric artery. This finding is in agreement with studies using rat perfused mesenteric

vasculature and rings of superior mesenteric artery where human α-CGRP also appears more potent, relative to nitroprusside, on the resistance arterioles than on the large conduit artery (Marshall *et al.*, 1986a, b). In both types of preparation nitroprusside had a short duration of action, whereas human α-CGRP differed in being long acting in the human perfused mesenteric vasculature. This duration of action was longer than that observed in previous studies on the rat and rabbit mesentery (Marshall *et al.*, 1986b), possibly in part a reflection of the age of the patients and the effect of the anaesthetic on the preparations. The limited study on rings of human saphenous vein showed, in agreement with previous studies on rings of rat femoral and hepatic portal veins (Al-Kazwini *et al.*, 1987) that human α-CGRP caused no relaxation of noradrenaline-induced tone. These results are possibly surprising in view of the presence of CGRP-containing neurones adjacent to some rat veins (Mulderry *et al.*, 1985; Wharton *et al.*, 1986). Thus it appears on the basis of the available data that the cardiovascular action of CGRP in rat and man is exerted primarily on the arterial side of circulation.

The site and mechanism of action of CGRP remains unclear. In rings of rat aorta and mesenteric artery, human α-CGRP acts in an endothelial dependent manner like acetylcholine (Brain *et al.*, 1985; Kubota *et al.*, 1985; Al-Kazwini *et al.*, 1987). In human radial, coronary and gastric arteries the relaxation evoked by human α-CGRP was also endothelium dependent (Thom *et al.*, 1987). In contrast to these observations, in rings of cat middle cerebral artery the response was independent of the endothelium (Hanko *et al.*, 1985). Whether this reflects the difference in species and/or vascular bed remains to be established.

The present experiments demonstrate the effectiveness of human α-CGRP as a vasodilator in the human mesenteric vascular bed, and add weight to the accumulating evidence which supports a role for human α-CGRP in the modulation of cardiovascular function in man.

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