Human calcitonin gene related peptide: a potent endogenous vasodilator in man


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Summary

1. In addition to calcitonin and katacalcin, it is now known that the human calcitonin gene encodes a novel peptide called calcitonin gene related peptide (CGRP). In experimental animals, CGRP produces vasodilatation and complex changes in plasma calcium.

2. We have now assessed its biological activity in man by infusing human CGRP (hCGRP) into six normal volunteers. hCGRP (545 pmol/min) caused the diastolic pressure to fall from 64±5 to 55±7 mmHg (P<0.05), the heart rate to increase from 61±7 to 87±5 beats/min (P<0.05) and the skin temperature to increase from 33.7±0.9 to 34.9±0.5°C. Plasma noradrenaline increased from 481±126 to 835±65 pg/ml (P<0.05) and plasma adrenaline from 57±17 to 82±12 pg/ml (P<0.05). There were no significant changes in the albumin-corrected plasma calcium.

3. hCGRP is thus a potent endogenous vasodilator in man and is in fact more potent than any other known vasodilator. Together with the observations that CGRP circulates in normal subjects at relatively high concentration (approximately 25 pmol/l) and that CGRP is present in perivascular nerves, this study suggests a possible role for CGRP in controlling peripheral vascular tone in man.

Key words: blood pressure, calcitonin, calcium, catecholamines, vasodilatation.

Abbreviations: CGRP, calcitonin gene related peptide; FAB, fast atom bombardment; hCGRP, human calcitonin gene related peptide.

Introduction

The techniques of molecular biology are being increasingly applied in clinical medicine and are producing a dramatic increase in our understanding of the regulatory processes within cells. The single calcitonin gene transcript has now been shown to be able to produce different messenger RNA species [1, 2]. Thus the primary RNA transcript from the calcitonin gene can be processed to produce either calcitonin itself or a novel 37 amino acid peptide called calcitonin gene related peptide (CGRP) [3]. In the thyroid, the calcitonin gene produces both CGRP and calcitonin, while in nervous tissue the main product is CGRP.

CGRP had been found to be widely distributed throughout the central nervous system, especially in the posterior horn of the spinal cord where it appears to be co-stored with substance P [4, 5]. A possible role for CGRP as a neurotransmitter has been suggested by the observations that stimulated rat trigeminal ganglion cells release immunoreactive CGRP and that intracerebroventricular CGRP stimulates sympathetic outflow in rats [6, 7].

Peripherally, CGRP is also widespread throughout the body, especially in the thyroid and perivascular nerves. In animal work, CGRP has been found to be a potent vasodilator when injected intradermally in both rabbits and man, while its effects on plasma calcium are complex and vary from one species to the next [8, 9]. In man, rat
CGRP has previously been shown to inhibit gastric secretion and release of gastrointestinal hormones [10]. We have recently reported on the first isolation of CGRP from human tissue and have determined its amino acid sequence by a novel mass spectrometric approach called fast atom bombardment (FAB) mapping [11]. This has shown that human CGRP (hCGRP) and rat CGRP differ in four out of 37 amino acids. Now that human CGRP has been sequenced and synthesized, we have been able to assess its biological activity in man by infusing synthetic hCGRP into human volunteers and measuring its cardiovascular, sympathetic-adrenal and endocrine effects.

Methods

These studies were approved by the Research and Ethical Committee of the Hammersmith Hospital and each volunteer gave written informed consent. Six normal male volunteers (aged 30–39 years) were studied on one occasion each. They remained supine throughout the study, which was performed in a quiet clinical laboratory with a constant thermostatically controlled temperature of 20°C. Intravenous cannulae were inserted into a vein in each forearm, one for infusion and the other for blood sampling. After a 10 min rest period, baseline blood samples were taken and measurements were made of blood pressure, heart rate, and skin temperature. They were then infused for 10 min with a control vehicle solution (containing 0.9% NaCl and 5% human serum albumin) and subsequently with synthetic hCGRP. The synthetic hCGRP give identical results on FAB mass spectroscopy as the original hCGRP isolated from medullary carcinoma tissue. The hCGRP infusion rate began at 5.5 pmol/min and the rate was increased at 10 min intervals to produce incremental infusion rates of 5.5, 13, 27, 68, 136, 272 and 545 pmol/min. Each infusion solution was made up separately to contain hCGRP, 1 ml of human albumin and 20 ml of 0.9% NaCl so that the same volume was infused at each infusion rate by a Braun infusion pump. Blood pressure, heart rate and skin temperature were recorded before and during each infusion. Skin temperature was measured throughout by a temperature probe (Hewlett Packard) which was taped to each subject’s left cheek. At the end of the 545 pmol/min infusion, each volunteer underwent a longer infusion of 30 min duration at a rate of 272 pmol/min. Blood samples were subsequently taken at 2, 5, 10, 15 and 20 min after the end of this long infusion to help define the half-life of CGRP.

Blood samples were taken into chilled heparinized tubes and immediately spun in a refrigerated centrifuge. A small aliquot of blood was placed in glass bottles at room temperature and the serum was separated for albumin analysis. The plasma was separated and kept at either −70°C, −20°C or 4°C before assay as appropriate for each biochemical assay. Plasma calcium was measured by direct-current plasma emission on a Beckman Spectrspan emission spectrometer. Serum albumin was measured on a Technicon RA-1000 using the standard bromocresol green method (Technicon). The albumin-corrected calcium level was calculated by the standard formula Ca_corr = Ca_measured − 0.02 (40 − [Alb]) with albumin measured in g/l and Ca in mmol/l. Plasma noradrenaline and plasma adrenaline were measured by the double isotope enzymatic method of Brown & Jenner [12].

Levels of immunoreactive CGRP were measured in the plasma samples using a specific and sensitive radioimmunoassay. Antibodies were raised in rabbit to the synthetic human peptide coupled by carbodi-imide to bovine serum albumin. The antibody designated CCG2/1 was used in a non-equilibrium assay at a final dilution of 1 in 240 000 with the synthetic human peptide as standard. [125I]-iodohistidyl]CGRP obtained from Amersham International was used as label. The sensitivity of the assay was 1.0 fmol/tube, and 12 fmol displaced initial binding by label by 50%. No cross-reactivity was found with any of 24 control peptides tested.

The data for each parameter were analysed by one-way analysis of variance to detect any overall effect of CGRP. If analysis of variance showed an overall significant result, paired t-tests were then used to detect which time points were significantly different from the baseline values.

Results

Fig. 1 shows the haemodynamic effects of hCGRP in normal volunteers. hCGRP caused no change in systolic blood pressure, a small fall in diastolic blood pressure and a marked dose-related increase in heart rate. hCGRP also caused marked flushing over the whole body accompanied by a significant increase in skin temperature from 33.7 ± 0.9 to 34.9 ± 0.5°C (P < 0.05). It is worth noting also that the tachycardia only reversed slowly once the hCGRP infusion was stopped and had not returned to baseline 20 min after the end of the infusion. In addition, after the infusion was terminated, there was a significant rise in diastolic pressure. Plasma noradrenaline and adrenaline both increased significantly during the hCGRP
Calcitonin gene related peptide in man

Fig. 1. Effect of hCGRP infusion on blood pressure (BP) and heart rate in six normal volunteers. There were significant changes in both diastolic pressure and heart rate (one-way analysis of variance). *Represents $P < 0.05$ (paired $t$-test) from baseline (10 min reading).

Fig. 2. Effect of hCGRP infusion on plasma catecholamines in six normal volunteers. Plasma noradrenaline and adrenaline both increased significantly (one-way analysis of variance). *Represents $P < 0.05$ (paired $t$-test) from baseline (10 min reading).

infusion (Fig. 2). The albumin-corrected plasma calcium was $2.26 \pm 0.11$ mmol/l before CGRP, $2.24 \pm 0.09$ mmol/l at the highest dose, $2.19 \pm 0.09$ mmol/l at the end of the long infusion and $2.20 \pm 0.08$ mmol/l 20 min after the CGRP infusion; none of these small changes was statistically significant.

Fig. 3 shows the levels of immunoreactive CGRP achieved during these studies. The initial fall was due mainly to the data from one individual; if genuine, this could be due to changing posture as the subjects became supine at the beginning of the study or to haemodynamic effects increasing the clearance of CGRP. During the long infusion, steady state plasma levels of $165 \pm 59$ pmol/l were achieved. The half-life of the first phase of elimination of hCGRP was $9.7 \pm 2.9$ min.

Discussion

The haemodynamic effects of hCGRP along with the marked flushing which it produces suggest that hCGRP acts principally as a peripheral vasodilator. This is further supported by the reflex increase in plasma catecholamines. In fact, the absolute fall in blood pressure in this study was small, but this is usually the case in normal volunteers who are able to maintain their blood pressure in the presence of vasodilators by activation of their sympathetic nervous system. Indeed we have previously found as here that plasma noradrenaline is a more sensitive indicator of vasodilating activity than changes in blood pressure themselves [13, 14]. In accordance with this, there was a dose-related increase in both heart rate and in plasma catecholamines, although this was less obvious for diastolic pressure. Indeed after the infusion was terminated, there was a surprising rise in diastolic pressure. The mechanism of this is unknown but it may be due to the persistence of some counter-regulatory response or to down-regulation of receptors.
On a molar basis CGRP is a more potent vasodilator in man than any other endogenous or synthetic compound including prostacyclin, adrenaline, histamine, vasoactive intestinal polypeptide and sodium nitroprusside (Table 1). Furthermore, CGRP immunofluorescence is present in the adventitial nerves surrounding most peripheral blood vessels including coronary and cerebral vessels [3, 20]. Intradermal injections have confirmed that CGRP is a potent vasodilator, while studies in isolated aortic strips suggest that endothelial cells may be required to produce this effect [8]. This suggests that CGRP may stimulate the release of an unknown secondary mediator from vascular endothelial cells. Since indomethacin only partially suppresses this intradermal vasodilatation, it is likely that prostaglandin generation is responsible for only a small part of this response to CGRP. Furthermore, hCGRP circulates in normal man at relatively high concentration (approximately 25 pmol/l) and at approximately five times the circulating level of calcitonin [21]. This potent vasodilator activity, along with its endogenous localization in peripheral vessels and the fact that CGRP is a circulating hormone, suggests that CGRP could be an important regulator of peripheral vascular tone in man. Generalized or localized alterations in CGRP may therefore contribute to blood vessel tone in such pathophysiological states as hypertension, arterial spasm, endotoxic shock or Raynaud's phenomenon.

In medullary carcinoma of the thyroid excess secretion of calcitonin is associated with flushing and diarrhoea. CGRP has also been isolated from medullary carcinoma tissue and plasma levels of CGRP have been found to be elevated in these patients [1]. Although the circulating levels of CGRP are usually lower than those of calcitonin, CGRP is a much more potent vasodilator than calcitonin and it is therefore quite possible that some of the flushing and vasodilatation found in this condition is due to CGRP rather than calcitonin. Certainly the average plasma levels of CGRP in most patients with medullary carcinoma of thyroid (3.4 nmol/l) are about 20 times those found at the highest dose used in this study. We do not, however, yet know whether those patients who do complain of flushing are those with the highest levels of CGRP.

Another feature of interest in this study is that after the end of the CGRP infusion the offset of the tachycardia was much slower than the offset of the plasma CGRP concentration. This dissociation of concentration and effect is not seen with most other vasodilators, where the blood pressure and heart rate return to baseline 2-3 min after terminating the infusion [16, 18, 19]. The likely cause of this dissociation in our study is that CGRP is tightly bound to a receptor on peripheral vascular tissue so continuing to exert its effect while not detectable in the plasma. Although specific receptors for CGRP have been identified in the central nervous system and the pituitary gland, no such receptors have yet been described in vascular tissue [22, 23]. From our study, a high affinity binding site might be predicted from both the potency of CGRP and its slow offset of effect.

In animal work, the effects of CGRP on calcium homeostasis are complex. CGRP lowers plasma calcium in the rat but increases it in the chick, while in the rabbit low dose CGRP has an immediate hypocalcaemic effect followed at higher dose by a prolonged hypercalcaemia [9]. These complex effects are unique with no other known hormone producing such diverse effects on calcium homeostasis. This suggests that CGRP may produce two separate effects on bone cells with each effect having different species specificity. Therefore the absence of any effect of CGRP on plasma calcium in man does not necessarily imply the absence of any effect on bone cells since CGRP could be producing two separate but opposing effects on plasma calcium, as in the rabbit. In fact, CGRP may cause a small fall in plasma calcium in patients with severe Paget's disease, although much less than is observed following an

### Table 1. Comparison of the rates of infusion and the achieved plasma levels of several vasodilators required to produce a reflex tachycardia of approximately 15 beats/min

<table>
<thead>
<tr>
<th>Vasodilator</th>
<th>Infusion rate (pmol min⁻¹ kg⁻¹)</th>
<th>Plasma concn. (pmol/l)</th>
<th>Heart rate (beats/min)</th>
<th>Diastolic blood pressure (mmHg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGRP</td>
<td>1.8</td>
<td>56</td>
<td>+14</td>
<td>-8</td>
<td>Present work</td>
</tr>
<tr>
<td>Vasoactive intestinal polypeptide</td>
<td>6.7</td>
<td>130</td>
<td>+13</td>
<td>-5</td>
<td>Krejs &amp; Fordtran [15]</td>
</tr>
<tr>
<td>Prostacyclin</td>
<td>11</td>
<td>508</td>
<td>+15</td>
<td>-5</td>
<td>FitzGerald et al. [16]</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>550</td>
<td>4610</td>
<td>+15</td>
<td>-15</td>
<td>FitzGerald et al. [17]</td>
</tr>
<tr>
<td>Histamine</td>
<td>1153</td>
<td>17 700</td>
<td>+16</td>
<td>-9</td>
<td>Ind et al. [18]</td>
</tr>
<tr>
<td>Nitroprusside</td>
<td>5030</td>
<td>—</td>
<td>+15</td>
<td>-9</td>
<td>Dean et al. [19]</td>
</tr>
</tbody>
</table>

**Comparison of the rates of infusion and the achieved plasma levels of several vasodilators required to produce a reflex tachycardia of approximately 15 beats/min**
equimolar dose of calcitonin (A. D. Struthers, unpublished work).

In conclusion, human CGRP has been found to be the most potent vasodilator described in man. Since CGRP is an endogenous compound found in abundance in nerve fibres associated with peripheral blood vessels, this raises the possibility that CGRP is an important neuromodulator of vascular tone in man.

Acknowledgments

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References