Cardiovascular Effects of Calcitonin Gene-Related Peptides I and II in Man

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Calcitonin gene-related peptide (CGRP) is present in sensory nerve fibers in the heart and around peripheral arteries. On interaction with specific CGRP binding sites and activation of adenylate cyclase, CGRP causes vasodilation and has positive inotropic and chronotropic effects on the heart. In the present study, human CGRP I and II exerted positive inotropic effects on isolated human right auricles and relaxed small arteries from human skeletal muscle precontracted with norepinephrine (EC50 for CGRP I 0.59 nM and for CGRP II 0.37 nM). CGRP I and II (3.2 nmol) administered i.v. to 6 normal subjects exerted positive inotropic actions on the human heart concomitant with positive chronotropic effects, hypotension, and vasodilation. CGRP may, therefore, be of importance for cardiovascular control in man. (Circulation Research 1987;60:393-397)

Materials and Methods

In Vitro Studies

Pieces of human right auricles (approximately 0.2 g) were obtained from 8 patients undergoing coronary artery bypass grafting for ischemic heart disease. Small skeletal muscle arteries were collected from 4 patients undergoing hip joint surgery. Collection of human tissue was approved by the Ethics Committee of the Karolinska Hospital. The patients were premedicated with morphine-scopolamine and anesthetized with thiopental and nitrous oxide or isoflurane. The tissues were transported in aerated Tyrode’s solution for in vitro experiments. Auricles and dissected arterial segments (length 2-3 mm, diameter 0.3-0.5 mm) from skeletal muscles were preloaded with a tension of 5 mN (mN = g/m·sec-2) in a 2-ml organ bath also containing aerated Tyrode’s solution at 37°C for 15-20 minutes after resection. The longitudinal and circular contractile forces were continuously monitored in the auricles and arteries, respectively, using a Statham transducer (Grass Instrument Co., Quincy, Mass., model FT 03) in combination with a Grass polygraph (model 7C).

Electrical stimulation was applied to the auricles, using values (1 Hz, 1 millisecond, 50 V) found to yield reproducible contractile responses. After stabilization for 30 minutes, CGRP I and II and norepinephrine (NE) were cumulatively added to the preparations in alternate order.

After stable tension of the arteries was obtained, 5 μM NE (Sigma Chemical Co., St. Louis, Mo.) was added for 15 minutes to elicit control contractions. The arteries were rinsed and NE readded. Relaxant effects of CGRP I and II and of acetylcholine (ACh; Sigma) were then studied on cumulative addition of increasing concentrations. Data are expressed as percent relaxation in relation to the slight spontaneous fall in the NE contraction of the controls. The effects of preincubation with propranolol (ICI, Macclesfield, United Kingdom) and atropine (Sigma) on the CGRP-induced relaxation were also tested.
In Vivo Studies

Six normal human subjects (3 males and 3 females, mean age 50 ± 2 years, weight 67 ± 5 kg, height 168 ± 4 cm) received 3.2 nmol human CGRP I and II in 50 ml 0.9% NaCl by 10-minute i.v. infusions separated by a 3-day period according to a cross-over design alternating the use of CGRP I and II. Informed consent about the experimental procedure was obtained from all subjects.

Heart rate, arterial blood pressure, and facial flushing were monitored, and blood (15 ml) was collected from 0 (no effect) to 4 (maximal facial reddening). The intensity of readily observable facial flushing was evaluated in arbitrary units according to a severity score ranging from 0 (no effect) to 4 (maximal facial reddening).

Plasma was assayed for NE and epinephrine by a radioenzymatic method (The Upjohn Co., Kalamazoo, Mich.), and for cAMP and cGMP after acetylation by specific radioimmunoassays (Amersham International, Amersham, United Kingdom). Total serum calcium was measured by atomic absorption spectrophotometry.

Results are expressed as mean values ± SEM. Statistical analysis was done by paired t test and analysis of variance.6

Materials

Over 95% of synthetic immunoreactive CGRP I and II (Peninsula Laboratories) eluted as single peaks on reversed phase high pressure liquid chromatography.6

Results

CGRP I and II caused concentration dependent positive inotropic effects on pieces of the electrically driven human right auricles in vitro (Figure 1). The two related peptides were equally potent. Equimolar amounts of NE yielded a significantly smaller response (p < 0.05). Furthermore, CGRP I and II caused relaxation of skeletal muscle arterial segments precontracted with 5 μM NE, with EC50 values of 0.59 nM and 0.37 nM, respectively. Pretreatment with propranolol (10⁻⁶ M) and atropine (10⁻⁶ M) did not alter the effects of the CGRPs. The EC50 value of acetylcholine (21.4 nM) added for comparison was significantly higher than that of CGRP (p < 0.05).

In response to i.v. infusions of 3.2 nmol CGRP I and II, the heart rate was increased and the systolic arterial pressure was lowered (p < 0.01) (Table 1). The decrease of the diastolic arterial pressure was statistically significant with CGRP I (p < 0.01) but not CGRP II (p < 0.1). Marked facial flushing obtained with both peptides was associated with these effects (p < 0.001). A reduction of the duration of the pre-ejection period, the electromechanical systole, and the left ventricular ejection period was also recognized in response to the i.v. administration of CGRP I and II (p < 0.1 to p < 0.001). Plasma levels of cAMP and cGMP were increased, and serum calcium was slightly lowered (p < 0.05 to p < 0.001) by both CGRP I and II. Plasma levels of NE and epinephrine were raised with CGRP I (p < 0.01 to p < 0.001) but not with CGRP II.

Discussion

In this study, we have demonstrated cardiovascular responses in normal human subjects after i.v. administration of CGRP I and II, which include an increase of the heart rate, hypotension, and skin vasodilation (facial flushing). Furthermore, the observed shortening of the duration of the pre-ejection period, the electromechanical systole, and the left ventricular ejection are indicative of positive inotropic effects on the heart as recorded by noninvasive techniques. Positive inotropic effects of CGRP I and II in isolated auricles in vitro and relaxation of skeletal muscle arteries have been demonstrated for the first time using human tissues obtained surgically.

Immunoactive CGRP is present in both the central and peripheral nervous systems.6-8 In the heart, CGRP is localized in sensory nerve fibers within the myocardium of the atria and around coronary arteries of the ventricles from which the peptide is released by the administration of capsaicin in rats and guinea

Figure 1. Stimulation by CGRP I (○) and II (●) of right auricular tension of the human heart (top panel) and relaxation of norepinephrine (NE) precontracted human skeletal muscle arteries (bottom panel). For comparison, effects of NE (▲) on the heart and acetylcholine (△) on the arteries were tested. Tension was measured in pieces (n = 6 or 8 weighing approximately 0.2 g) of human auricles obtained at surgery from 8 patients with ischemic heart disease. Arterial segments (n = 4 or 6; length 2-3 mm, diameter 0.3-0.5 mm) were obtained at surgery from 4 patients and precontracted with 5 μM NE. Relaxation is expressed as a percent of the control contractions induced by NE. Values are presented as means ± SEM.
Table 1. Cardiovascular Effects of 3.2 nmol CGRP I and II in Normal Human Subjects (n = 6)

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Time (minutes)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>120</th>
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<tr>
<td>Heart rate (min⁻¹)</td>
<td></td>
<td>71 ± 4*</td>
<td>70 ± 3</td>
<td>85 ± 4†</td>
<td>77 ± 6</td>
<td>72 ± 4</td>
</tr>
<tr>
<td>CGRP I</td>
<td></td>
<td>72 ± 2</td>
<td>74 ± 3</td>
<td>85 ± 4†</td>
<td>75 ± 3</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>CGRP II</td>
<td></td>
<td>129 ± 4</td>
<td>130 ± 5</td>
<td>106 ± 3†</td>
<td>117 ± 3†</td>
<td>118 ± 5</td>
</tr>
<tr>
<td>Arterial pressure, systolic (mm Hg)</td>
<td></td>
<td>129 ± 6</td>
<td>122 ± 4</td>
<td>109 ± 4†</td>
<td>111 ± 6</td>
<td>116 ± 3</td>
</tr>
<tr>
<td>CGRP I</td>
<td></td>
<td>80 ± 3</td>
<td>84 ± 3</td>
<td>63 ± 3‡</td>
<td>78 ± 3</td>
<td>81 ± 3</td>
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<tr>
<td>CGRP II</td>
<td></td>
<td>84 ± 7</td>
<td>79 ± 3</td>
<td>73 ± 4</td>
<td>71 ± 4</td>
<td>74 ± 4</td>
</tr>
<tr>
<td>Arterial pressure, diastolic (mm Hg)</td>
<td></td>
<td>130 ± 5</td>
<td>122 ± 4</td>
<td>106 ± 3†</td>
<td>117 ± 3†</td>
<td>118 ± 5</td>
</tr>
<tr>
<td>Flushing (arbitrary units)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>3.0 ± 0.4†</td>
<td>2.8 ± 0.2†</td>
<td>1.5 ± 0.3‡</td>
</tr>
<tr>
<td>CGRP I</td>
<td></td>
<td>0</td>
<td>0</td>
<td>3.5 ± 0.2†</td>
<td>2.8 ± 0.3†</td>
<td>1.5 ± 0.4‡</td>
</tr>
<tr>
<td>CGRP II</td>
<td></td>
<td>117 ± 7</td>
<td>120 ± 7</td>
<td>99 ± 5‡</td>
<td>103 ± 4‡</td>
<td>119 ± 6</td>
</tr>
<tr>
<td>Prejection period (msec)</td>
<td></td>
<td>112 ± 4</td>
<td>112 ± 5</td>
<td>99 ± 2</td>
<td>109 ± 3</td>
<td>111 ± 5</td>
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<tr>
<td>CGRP I</td>
<td></td>
<td>405 ± 8</td>
<td>409 ± 7</td>
<td>377 ± 7†</td>
<td>401 ± 9</td>
<td>405 ± 8</td>
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<tr>
<td>CGRP II</td>
<td></td>
<td>397 ± 8</td>
<td>409 ± 9</td>
<td>354 ± 6†</td>
<td>387 ± 10†</td>
<td>385 ± 7†</td>
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<tr>
<td>Electromechanical systole (msec)</td>
<td></td>
<td>289 ± 5</td>
<td>289 ± 3</td>
<td>278 ± 7</td>
<td>298 ± 7</td>
<td>286 ± 5</td>
</tr>
<tr>
<td>CGRP I</td>
<td></td>
<td>286 ± 7</td>
<td>288 ± 7</td>
<td>258 ± 5‡</td>
<td>278 ± 8</td>
<td>272 ± 8</td>
</tr>
<tr>
<td>CGRP II</td>
<td></td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>3.3 ± 0.2†</td>
<td>2.8 ± 0.2‡</td>
<td>2.6 ± 0.2‡</td>
</tr>
<tr>
<td>Plasma cyclic AMP (ng/ml)</td>
<td></td>
<td>3.0 ± 0.4</td>
<td>3.1 ± 0.4</td>
<td>4.6 ± 0.5</td>
<td>4.9 ± 0.2‡</td>
<td>4.4 ± 0.2</td>
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<tr>
<td>CGRP I</td>
<td></td>
<td>3.6 ± 0.7</td>
<td>3.6 ± 0.6</td>
<td>5.1 ± 0.8</td>
<td>4.8 ± 0.7‡</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>CGRP II</td>
<td></td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>3.3 ± 0.2†</td>
<td>2.8 ± 0.2‡</td>
<td>2.6 ± 0.2‡</td>
</tr>
<tr>
<td>Plasma cyclic GMP (ng/ml)</td>
<td></td>
<td>1.9 ± 0.3</td>
<td>2.1 ± 0.3</td>
<td>3.0 ± 0.4§</td>
<td>2.3 ± 0.3</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>CGRP I</td>
<td></td>
<td>8.9 ± 0.1</td>
<td>8.8 ± 0.1</td>
<td>8.7 ± 0.1§</td>
<td>8.5 ± 0.1†</td>
<td>8.5 ± 0.1†</td>
</tr>
<tr>
<td>CGRP II</td>
<td></td>
<td>8.9 ± 0.2</td>
<td>8.9 ± 0.2</td>
<td>8.7 ± 0.2</td>
<td>8.6 ± 0.1</td>
<td>8.6 ± 0.2‡</td>
</tr>
<tr>
<td>Serum calcium (mg/ml)</td>
<td></td>
<td>1.9 ± 0.3</td>
<td>2.1 ± 0.3</td>
<td>3.0 ± 0.4§</td>
<td>2.3 ± 0.3</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>CGRP I</td>
<td></td>
<td>178 ± 7</td>
<td>161 ± 10</td>
<td>223 ± 16†</td>
<td>212 ± 13‡</td>
<td>170 ± 9</td>
</tr>
<tr>
<td>CGRP II</td>
<td></td>
<td>192 ± 18</td>
<td>180 ± 18</td>
<td>195 ± 23</td>
<td>199 ± 10</td>
<td>181 ± 17</td>
</tr>
<tr>
<td>Plasma norepinephrine (pg/ml)</td>
<td></td>
<td>17 ± 4</td>
<td>25 ± 3</td>
<td>35 ± 5‡</td>
<td>24 ± 2</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>CGRP I</td>
<td></td>
<td>17 ± 2</td>
<td>17 ± 1</td>
<td>17 ± 1</td>
<td>18 ± 2</td>
<td>19 ± 2</td>
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<td>CGRP II</td>
<td></td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

*Values are means ± SEM; †p < 0.001, ‡p < 0.01, §p < 0.05 as compared to basal values.

CGRP is also present in nerve fibers innervating peripheral arteries. In the heart, specific binding sites for CGRP linked to stimulation of adenylate cyclase activity have been identified in highest concentrations in the atrium. CGRP has, moreover, been shown to stimulate the production of cAMP but not of cGMP in aortic smooth muscle cells and cerebral arteries, which may be related to the arterial relaxation. The increase of plasma levels of cAMP could, therefore, be the result of the stimulation of adenylate cyclase activity by CGRP in peripheral tissues such as the heart and blood vessels. The rise of plasma cGMP is consistent with a stimulation of the secretion of atriopeptin.

Evidence indicates that the positive inotropic and chronotropic effects in the atrium are brought about through direct actions of CGRP. Thus, the increase of rate and tension of the isolated rat atrium in response to CGRP are still obtained in the presence of adrenergic and histaminergic blocking agents and of indomethacin blocking prostaglandin synthesis. Relaxation of skeletal muscle arteries, as shown here, seems to be independent of adrenergic and cholinergic mechanisms. Unlike relaxation in the aorta of the rat, which
required the presence of the endothelium, relaxation persisted in the absence of the endothelium in cerebral arteries of the cat.\textsuperscript{10,11}

Small, but statistically significant, rises of circulating levels of norepinephrine and epinephrine indicating reflex activation of sympathetic tone were only seen after the administration of CGRP I and not of CGRP II. This difference may be related to the slightly less pronounced hypotensive action of CGRP II than I. Adrenergic blocking agents do not affect positive chronotropic and inotropic effects of CGRP on isolated atria of rat and guinea pig in vitro.\textsuperscript{12,13} Moreover, the positive chronotropic and hypotensive effects and skin vasodilation (facial flushing) of CGRP I were left unchanged during the administration of the combined \(\alpha\)- and \(\beta\)-adrenergic blocking agent labetalol in vivo in man, but the ventricular inotropic effect of CGRP I was prevented with labetalol.\textsuperscript{14} The ventricular inotropic response of CGRP, which is negligible in the rat,\textsuperscript{12} therefore, probably depends on sympathetic activation and catecholamine release due to the concomitant fall in blood pressure.\textsuperscript{15,16,20}

Small decreases of serum calcium levels may be the result of inhibition of bone resorption mediated through calcitonin receptors on osteoclasts. In this respect, calcitonin is much more potent than CGRP in suppressing the release of \(45\)Ca from bone explants and in lowering blood calcium levels.\textsuperscript{24,25} Furthermore, specific binding of CGRP to rat bone explants is lower than that of calcitonin.\textsuperscript{26,27} The decrease in serum calcium levels observed in the present experiments and the cross-tachyphylaxis between the two peptides\textsuperscript{28} are probably the result of limited structural homology between calcitonin and CGRP.\textsuperscript{29}

The combination of positive inotropic action of CGRP on the heart and relaxation of arteries may be beneficial in the treatment of congestive heart failure and vascular obstruction.\textsuperscript{29} To this end, CGRP is more potent than NE in raising the tension of the musculature of human heart auricles and acetylcholine in causing arterial relaxation. CGRP, as well as NE and ACh, are probably released in high concentrations from nerve fibers to interact with the cardiac and arterial musculature through local modes of action. Since CGRP is likely to be present in C-fiber afferents in the heart, local release of this peptide may induce changes in cardiac contractility and blood flow during episodes of cardiac pain.

References


**Key Words** • calcitonin gene-related peptides • chronotropic action • inotropic action • vasodilation
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