Systemic and Regional Hemodynamic Effects of Calcitonin Gene–Related Peptide

DONALD J. DiPette, KATHRYN SCHWARZENBERGER, NANCY KERR, and O. BRYAN HOLLAND

SUMMARY Calcitonin gene–related peptide, a 37-amino-acid neuropeptide, has been shown to be widely distributed in periadventitial nerves throughout the cardiovascular system, particularly in association with coronary arteries. In vivo and in vitro studies have demonstrated that calcitonin gene–related peptide possesses potent vasodilator properties. Circulating calcitonin gene–related peptide is derived primarily from periadventitial nerves, though its systemic and regional hemodynamic effects are unknown. In this study, systemic and regional hemodynamics were determined by the radioactive microsphere technique prior to and following the intravenous administration of 65-pmol and 2.2-nmol doses of calcitonin gene-related peptide and vehicle to three groups of conscious, unrestrained rats. Vehicle administration did not change any systemic or regional organ hemodynamic parameter determined. In contrast, 65 pmol and 2.2 nmol of calcitonin gene–related peptide significantly decreased mean blood pressure and total peripheral resistance and increased heart rate in a dose-dependent manner, while only slightly increasing cardiac output. Both 65-pmol and 2.2-nmol doses of calcitonin gene-related peptide significantly increased blood flow (percentage of cardiac output) to the heart. There was no difference in blood flow to the heart between the two doses. In addition, the 2.2-nmol dose of calcitonin gene–related peptide significantly increased blood flow to the stomach, liver, and skin and decreased it to the brain, kidneys, and spleen. In conclusion, calcitonin gene–related peptide infusion decreases blood pressure in a dose-dependent manner primarily by peripheral vasodilation. In addition, calcitonin gene–related peptide selectively changes regional organ blood flow, particularly to cause coronary vasodilation. Therefore, calcitonin gene–related peptide may have an important role in blood pressure and regional organ blood flow regulation.

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KEY WORDS • calcitonin gene–related peptide • blood pressure • cardiovascular function • calcium • vasodilator

CALCITONIN gene–related peptide (CGRP), a 37-amino-acid neuropeptide thought to result from alternative processing of the primary transcript of the calcitonin gene, has been identified in multiple species including rats and humans.1,2 CGRP has been shown to possess marked cardiovascular activity. In vivo and in vitro studies have demonstrated that CGRP is a potent vasodilator.3,4 It dilates isolated rat aortic rings in a dose-dependent manner, and its local administration produces vasodilation in skin.3,5 The systemic administration of CGRP results in a lowering of blood pressure.4,6 In addition to its vasodilator properties, CGRP has been shown to have potent chronotropic and inotropic effects, both in normal subjects4,6 and in isolated tissues.7

Immunohistochemical and radioimmunoassay techniques have identified CGRP-containing nerve fibers throughout the cardiovascular system. CGRP immunoreactivity is found in periadventitial nerves in association with blood vessels, notably the coronary arteries, as well as in all regions of the heart, particularly around the sinoatrial and atrioventricular nodes.8,9,10 Circulating CGRP appears to be derived primarily from these periadventitial nerves.11 In none of these studies has the in vivo effect of circulating CGRP on systemic and regional hemodynamics been elucidated. Therefore, this study was undertaken to determine the hemodynamic effects of CGRP in the conscious, unrestrained rat.
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Methods

Systemic and regional hemodynamics were determined by the radioactive microsphere technique as previously described, but with the following modifications. The microspheres (15 ± 3 μm in diameter, from 3M, St. Paul, MN, USA) were obtained as a stock solution in a suspending vehicle of normal saline with 0.05% polysorbate 80 (Tween 80) added. Prior to use, the vials containing the spheres were sonicated for 10 to 15 minutes. Each rat received a 10-second intraventricular injection of 0.25 ml (approximately 110,000 spheres) of microspheres labeled with either cerium-141 or strontium-85 followed by a 0.5-ml saline flush. Fifteen seconds prior to microsphere injection, blood withdrawal was begun from the caudal artery at a rate of 0.44 ml/min and continued for a total of 1.5 minutes. Where indicated, a second microsphere injection with the alternate isotope was performed. Tissue samples were then dissected, weighed, and counted in a gamma counter. The left ventricle was examined for evidence of catheter-induced trauma. If such trauma was noted, the rat was discarded. Systemic and regional hemodynamics were then calculated according to previously described standard equations.

All animals studied were male Sprague-Dawley rats (Texas Animal Specialties, Humble, TX, USA) weighing between 325 and 425 g. All rats were housed in a climate-controlled and light-cycled room and were fed standard rat chow with water to drink ad libitum. Nineteen rats were divided into three groups and studied in the following manner. The control group (n = 7) received normal saline (0.4 ml), administered as an intravenous bolus injection. The two remaining groups received intravenous bolus injections of either 65 pmol (n = 6) or 2.2 nmol (n = 6) of CGRP (Peninsula Laboratories, Belmont, CA, USA) dissolved in 0.4 ml of normal saline. On the day of study, all rats were anesthetized with ether, and catheters (PE50, Clay-Adams, Parsippany, NJ, USA) were inserted in the left ventricle (from the right carotid artery), right jugular vein, and ventral caudal artery. All three catheters were buried subcutaneously and externalized through the back between the scapulae and secured. The rats were then allowed to awaken and placed in individual cages to allow full mobility. Three to four hours after the surgical procedure, the acute experiment was begun in rats that had attained stable blood pressure and heart rate.

Blood pressure and heart rate were continuously monitored and recorded in 15-minute intervals, and left ventricular pressures were determined immediately prior to each microsphere injection (Model P23Gb transducers, Model 13-4615-50 transducer preamplifier, and Model 2400S chart recorder, Gould Statham, Oxnard, CA, USA). All intravenous injections were administered in the jugular vein. Systemic and regional hemodynamics were determined immediately prior to and 3 minutes after injection of either the vehicle or the 65-pmol dose of CGRP, and 10 minutes after the 2.2-nmol dose of CGRP. These time points were determined based on pilot studies that demonstrated a maximal and stable decrease in blood pressure attained after each dose. The times differed because the blood pressure returned almost to baseline by 10 minutes with the 65-pmol dose, but a maximal blood pressure fall was not achieved at 3 minutes with the 2.2-nmol dose.

All data are expressed as the mean ± standard error of the mean. One-way analysis of variance followed by the Scheffe test and Student’s t test for paired data (before and after CGRP) were utilized for statistical analysis. A p value of less than 0.05 was considered significant. All animal studies were performed in accordance with institutional animal care guidelines.

Results

There were no significant differences in baseline weight (vehicle group, 363 ± 23 g; 65-pmol group, 370 ± 19 g; 2.2-nmol group, 376 ± 14 g) or in any baseline systemic or regional hemodynamic parameter among the three groups studied. The systemic and regional hemodynamic responses (expressed as the percent distribution of cardiac output) prior to and following administration of either vehicle or 65-pmol or 2.2-nmol doses of CGRP are shown in Table 1.

Vehicle administration did not change any systemic or regional hemodynamic parameter. In contrast, both 65-pmol and 2.2-nmol doses of CGRP significantly decreased mean blood pressure while increasing heart rate. Cardiac output increased slightly, but the increase only reached significance with the 2.2-nmol dose of CGRP. Both doses of CGRP significantly decreased total peripheral resistance. Both the 65-pmol and 2.2-nmol doses of CGRP decreased stroke volume, with the 2.2-nmol dose reaching significance (0.37 ± 0.03-0.34 ± 0.02 and 0.32 ± 0.02-0.27 ± 0.2 ml/beat [p < 0.05], respectively). Administration of 65 pmol of CGRP resulted in a significant increase in blood flow (percent of cardiac output) to the heart. This included increases to the left ventricle (2.93 ± 0.4-4.2 ± 0.7%), right ventricle (1.75 ± 0.3-2.49 ± 0.4%) and atria (0.71 ± 0.2-1.19 ± 0.2%). Similarly, the 2.2-nmol dose of CGRP increased blood flow to the left ventricle (3.42 ± 0.3-4.11 ± 0.4%), right ventricle (1.70 ± 0.2-3.5 ± 0.4%), and atria (0.78 ± 0.2-1.64 ± 0.2%).

The changes from baseline in systemic hemodynamic parameters for the three groups studied are shown in Figure 1. CGRP appeared to decrease mean blood pressure and total peripheral resistance and increase heart rate and cardiac output in a dose-dependent manner, though the changes with the 65-pmol dose were usually not significantly greater with evaluation by analysis of variance. The changes from baseline for organ blood flow (percent of cardiac output) and vascular resistance are shown in Figures 2 and 3. The coronary circulation was unusually sensitive to CGRP in that coronary flow markedly increased and coronary resistance decreased following both doses of CGRP. In contrast, a statistically significant change by analysis of variance was usually only seen with the 2.2-nmol dose in the other organs, though a modest, though
TABLE 1. Effect of Calcitonin Gene-Related Peptide or Vehicle Administration on Systemic and Regional Hemodynamics

<table>
<thead>
<tr>
<th>Hemodynamic parameter</th>
<th>Vehicle Pre</th>
<th>Vehicle Post</th>
<th>CGRP (65 pmol) Pre</th>
<th>CGRP (65 pmol) Post</th>
<th>CGRP (2.2 nmol) Pre</th>
<th>CGRP (2.2 nmol) Post</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systemic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>109 ± 3</td>
<td>111 ± 2</td>
<td>111 ± 2</td>
<td>105 ± 2*</td>
<td>113 ± 5</td>
<td>72 ± 5*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>326 ± 19</td>
<td>324 ± 16</td>
<td>348 ± 20</td>
<td>393 ± 15*</td>
<td>383 ± 25</td>
<td>488 ± 14†</td>
</tr>
<tr>
<td>CO (ml/min)</td>
<td>111 ± 5</td>
<td>113 ± 7</td>
<td>126 ± 6</td>
<td>133 ± 4</td>
<td>120 ± 7</td>
<td>131 ± 9†</td>
</tr>
<tr>
<td>TPR (mm Hg/ml/min)</td>
<td>1.00 ± 0.06</td>
<td>1.01 ± 0.05</td>
<td>0.89 ± 0.04</td>
<td>0.79 ± 0.02†</td>
<td>0.96 ± 0.07</td>
<td>0.36 ± 0.05*</td>
</tr>
<tr>
<td><strong>Regional (% CO)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>2.56 ± 0.25</td>
<td>2.39 ± 0.26</td>
<td>2.69 ± 0.41</td>
<td>3.63 ± 0.59*</td>
<td>2.83 ± 0.25</td>
<td>3.74 ± 0.35*</td>
</tr>
<tr>
<td>Brain</td>
<td>1.12 ± 0.17</td>
<td>1.16 ± 0.21</td>
<td>1.05 ± 0.07</td>
<td>0.91 ± 0.05*</td>
<td>1.06 ± 0.07</td>
<td>0.78 ± 0.06*</td>
</tr>
<tr>
<td>Kidney</td>
<td>6.64 ± 0.7</td>
<td>6.85 ± 0.7</td>
<td>6.39 ± 0.26</td>
<td>5.70 ± 0.14</td>
<td>8.05 ± 0.78</td>
<td>4.88 ± 0.50*</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.23 ± 0.05</td>
<td>1.19 ± 0.06</td>
<td>1.09 ± 0.09</td>
<td>1.24 ± 0.12</td>
<td>1.12 ± 0.08</td>
<td>1.60 ± 0.22†</td>
</tr>
<tr>
<td>Liver</td>
<td>0.07 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>0.09 ± 0.02</td>
<td>0.14 ± 0.03†</td>
<td>0.13 ± 0.04</td>
<td>0.25 ± 0.02†</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.47 ± 0.29</td>
<td>2.53 ± 0.22</td>
<td>2.27 ± 0.11</td>
<td>1.89 ± 0.08*</td>
<td>3.23 ± 0.43</td>
<td>1.27 ± 0.32†</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.08 ± 0.01</td>
<td>0.09 ± 0.02</td>
<td>0.08 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Skin</td>
<td>0.15 ± 0.02</td>
<td>0.15 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td>0.23 ± 0.02*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. CGRP = calcitonin gene-related peptide; MBP = mean blood pressure; HR = heart rate; CO = cardiac output; TPR = total peripheral resistance.

* p < 0.01, † p < 0.001, ‡ p < 0.05, compared to preinjection values.

Discussion

The potent vasodilator properties of CGRP have been previously reported. 1, 5, 6 Infradermal administration of CGRP causes vasodilation that is not blocked by indomethacin, suggesting a lack of prostaglandin mediation. 5 Moreover, CGRP is a thousandfold more potent as a vasodilator than other known vasodilators, such as acetylcholine or substance P, and 10 to 100 times more potent than isoprenaline. 5

CGRP had an unusually prominent effect on the heart. CGRP immunoreactivity is distributed throughout the heart, though it is concentrated in association with the sinoaortic and atrioventricular nodes. 5 Positive chronotropic and inotropic effects are seen when CGRP is administered to the isolated rat auricle as well as to humans. 6, 7 Most studies demonstrate that the chronotropic effect of CGRP cannot be blocked by either β- or combined α- and β-adrenergic receptor blockade. 5, 7 Our study confirms the dose-response chronotropic effect of CGRP. In addition to the possibility of its having a direct effect on the sinoatrial node, peripheral vasodilation should also have contributed to the chronotropic effect. Interestingly, only a slight increase in cardiac output was noted, despite the increase in heart rate and the reduction in afterload. The lack of a greater increase in cardiac output following CGRP injection was primarily due to the reduction in stroke volume. It is unlikely that the decrease in stroke volume could be secondary to a negative inotropic effect, since positive inotropic activity has been uniformly reported with CGRP administration. 6, 7 Therefore, other mechanisms should be responsible for the observed decrease in stroke volume. One possible explanation is that the increase in heart rate could in turn
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Figure 2. The change in regional organ blood flow and organ resistance in response to vehicle (shaded bar) and calcitonin gene-related peptide, 65 pmol (hatched bar) and 2.2 nmol (stippled bar). CO = cardiac output. See legend to Figure 1 for definition of other symbols.

Figure 3. The change in regional organ blood flow and organ resistance in response to vehicle (shaded bar) and calcitonin gene-related peptide, 65 pmol (hatched bar) and 2.2 nmol (stippled bar). CO = cardiac output. See legend to Figure 1 for definition of other symbols.

decrease diastolic filling time and thus lead to a reduction in stroke volume. This seems unlikely, particularly at the 65-pmol dose, since the increase in heart rate was minimal. An alternative explanation could be that CGRP administration led to a redistribution of intravascular volume. In this regard, although not as potent as substance P, CGRP has been shown to lead to extravasation of plasma. Such extravasation would decrease venous return and ultimately stroke volume. An earlier study noted extravasation of Evans blue dye from plasma within 5 minutes with intravenous CGRP doses of 2.6 and 7.4 nmol/kg. Thus, the 2.2-nmol dose that we used would be expected to produce similar effects, though it would probably be unlikely with the 65-pmol dose. Alternatively, CGRP may cause direct venodilation, since CGRP-containing nerve fibers have been demonstrated by immunochemical techniques to be present in the adventitia of veins. However, since hematocrit and central venous pressure were not measured in this study, these possibilities remain only speculative. Therefore, the blood pressure-lowering effect of CGRP seen in this study appears to result primarily from peripheral vasodilation. Lesser effects involving a relative blunting of the anticipated increase in cardiac output cannot be ruled out. Additionally, although the animals were studied approximately 4 hours after operative recovery, some of the small changes found could be related to an interaction of CGRP with the lingering effects of anesthesia. The apparent dose-dependent blood pressure-lowering effect of CGRP in this study confirms previous reports. In our study, CGRP exhibited prominent differences in regional organ blood flow. Interestingly, others have reported that CGRP immunoreactivity is not uniformly distributed in arterial vascular structures and that, although total CGRP concentrations are low in the heart, CGRP-containing nerve fibers are particularly prominent around the coronary arteries. Our data demonstrate that the coronary vasculature is particularly sensitive to vasodilation induced by CGRP. A marked increase in flow and decrease in resistance was seen in both the right and left ventricles and atria following both the 65-pmol and 2.2-nmol doses of CGRP. Furthermore, the increase in flow in the two doses was similar. This response differs markedly from the pattern found in the other vascular beds studied, where the 65-pmol dose caused smaller changes in flow or resistance or both. Though we did not demonstrate a dose-response relationship between CGRP and coronary flow, we suspect that administration of lower dosages may allow this to be demonstrated. One explanation for the observed increase in blood flow in the heart could be that the increase in flow is secondary to the increase in heart rate and a resultant increase in cardiac demand. However, although there was a sig-
significant increase in heart rate, cardiac output increased only minimally, particularly at the lower (65-pmol) dose of CGRP, and this increase was not statistically significant. Furthermore, despite the fact that the 2.2-nmol dose increased both heart rate and cardiac output to a greater extent than did the 65-pmol dose, the 65-pmol dose, if anything, increased heart blood flow more than did the larger dose. These findings strongly suggest that the coronary vasodilation following CGRP injection is secondary to a direct effect.

In addition to its effect on the heart, CGRP caused vasodilation in the stomach, liver, and skin. High concentrations of CGRP have previously been noted in the superior mesenteric artery. Interestingly, despite other reports demonstrating prominent cutaneous vasodilation, skin flow increased only at the higher dose of CGRP. In contrast, CGRP administration resulted in a decrease in flow in the brain, kidneys, and spleen. Since minimal changes in vascular resistance were noted in the brain and kidneys, the decrease in flow appears to be secondary to the reduction in systemic blood pressure following CGRP administration. Thus, these beds, in addition to muscle, appear to be particularly insensitive to CGRP. The spleen was the only organ in which a definite increase in resistance was associated with a reduction in organ blood flow. One possible explanation for decreased flow in all of these organs is that reactive increases in other pressor systems, such as the sympathetic nervous system and the renin-angiotensin system, may have occurred in response to the marked decrease in blood pressure induced by CGRP. Additional studies are necessary to explore this possibility.

CGRP has been shown in humans to circulate at approximately 25 ± 1.2 pmol/L, with some subjects having values of up to 70 pmol/L. Interestingly, these levels are approximately five times greater than circulating levels of calcitonin. Since CGRP is a neuropeptide, levels achieved locally (in coronary arteries, etc.) may be much higher than circulatory levels. The cardiovascular effects of circulating CGRP remain to be determined. Similarly, the effect of CGRP in the physiological control of cardiovascular function in humans is unknown. The possibility that circulating CGRP levels may change with changes in calcium balance is of obvious interest.

In conclusion, the results of our study confirm the potent vasodilator properties of CGRP. The vasodilation seen with CGRP is not equally distributed throughout the vascular system. Instead, CGRP causes selective vasodilation and thus a redistribution of cardiac output. The coronary vasculature appears to be particularly sensitive to the vasodilator effects of CGRP. Thus, CGRP may have an important role in blood pressure and regional organ blood flow regulation, particularly in the heart.

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