Central Human Cocaine- and Amphetamine-Regulated Transcript Peptide 55–102 Increases Arterial Pressure in Conscious Rabbits

Kiyoshi Matsumura, Takuya Tsuchihashi, Isao Abe

Abstract—We determined cardiovascular and neurohormonal responses to intracerebroventricular administration of human cocaine- and amphetamine-regulated transcript (CART) peptide 55–102 in conscious rabbits. Intracerebroventricular injection of CART 55–102 elicited dose-related increases in mean arterial pressure and renal sympathetic nerve activity. Peak values of mean arterial pressure and renal sympathetic nerve activity induced by intracerebroventricular injection of 1 nmol of CART 55–102 (+5.0±2.6 mm Hg and +72.5±20.8%) were obtained 40 and 60 minutes after injection, respectively. Plasma epinephrine and glucose concentrations significantly increased 30 and 60 minutes after intracerebroventricular injection of CART 55–102 (control versus 60 minutes for epinephrine, 77.0±62.4 versus 1067.5±329.3 pg/mL, P<0.01; for glucose, 6.25±0.33 versus 11.57±0.93 mmol/L, P<0.01). Plasma norepinephrine concentrations also significantly increased at 30 minutes. Plasma insulin, vasopressin, and cortisol concentrations increased at 60 minutes but did not attain significant values. However, pretreatment with intravenous injection of pentolinium (5 mg/kg), a ganglion-blocking agent, eliminated these cardiovascular and neurohormonal responses. In contrast, intracerebroventricular injection of the same dosage of CART 55–102 (1 nmol) as that used in the intracerebroventricular experiment failed to cause any cardiovascular and renal sympathetic nerve responses. These results suggest that intracerebroventricular human CART 55–102 acts in the central nervous system and activates sympathoadrenal outflow, which results in increases in arterial pressure and plasma glucose levels in conscious rabbits. (Hypertension. 2001;38:1096–1100.)

Key Words: peptides • catecholamines • central nervous system • glucose • renal sympathetic nerve activity

Appetite and feeding behavior are regulated by many neuropeptides, such as neuropeptide Y, corticotrophin-releasing factor, α-melanocyte stimulating hormone, and melanin-concentrating hormone.1–2 Cocaine- and amphetamine-regulated transcript (CART) was originally described as mRNA induced in rat striatum after acute administration of cocaine or amphetamine.3 At least 6 CART proteins and peptides have been identified in rodents and humans, and some have been purified and sequenced.4,5 CART peptides have been found in brain areas known to be involved in physiological control of feeding behavior.4,5 Moreover, intracerebroventricular (ICV) administration of CART peptide fragment 55–86, other fragments (54–102, 55–102, 61–102, and 62–102), and recombinant CART peptide 55–102 cause dose-dependent inhibition of food intake in mice or rats,6,7 which suggests that these peptides participate in regulation of food intake and feeding behaviors. Amino acid sequences of CART peptides in rat and human are similar, and 95% identity exists between them.4,5 In the C-terminal region of peptide (55–102), a difference of only 1 amino acid exists: a Val→Ile substitution at position 55.4,5 CART-immunoreactive cells have been shown to be present not only in hypothalamic nuclei, but also in the medulla, particularly in the nucleus of the solitary tract and area postrema8; these regions of the medulla constitute one of the most important cardiovascular centers of the brain. In addition, ICV administration of CART has been shown to cause increases in plasma corticosterone, oxytocin, and glucose concentrations.9 We anticipated that CART peptides would be found to participate in cardiovascular and neuroendocrine regulations in addition to feeding behavior in the brain, and we hypothesized that central administration of CART peptides would modulate the hypothalamic–pituitary adrenal axis and change arterial pressure. Accordingly, the present study was designed to investigate the central effects of CART peptides on blood pressure, sympathetic nervous system, and blood variables, including plasma catecholamines, vasopressin, insulin, cortisol, and glucose concentrations. Experiments were conducted in conscious rabbits and included a direct recording of renal sympathetic nerve activity (RSNA), because sympathetic nerve activity or the baroreceptor reflex is strongly influenced by anesthesia.10,11

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1096
Methods

Preparation of Animals
Experiments were conducted in 21 male Japanese White rabbits weighing 2.5 to 2.7 kg. All experiments were performed according to the institutional guidelines for animal experimentation at Kyushu University.

Three days before experimentation, in each rabbit, bipolar electrodes were implanted on the left renal sympathetic nerve and a stainless steel cannula was placed in the right lateral cerebral ventricle. The following experiments were performed on conscious rabbits. On the day of the experiment, catheters were inserted into the car artery and vein of each animal, which had been given 1% lidocaine local anesthesia. RSNA was recorded and analyzed as described previously.12–14 Experiments were started at 9:00 AM and finished before 2:00 PM. All drugs for ICV injection were dissolved in artificial cerebrospinal fluid (aCSF).13,14

Relationship Between Dosage Level of Intracerebroventricular CART and Cardiovacular Responses
To determine the dosage level of human CART (55-102; Peptide Institute) needed to increase arterial pressure, aCSF, 0.1 and 1 nmol CART were given by ICV injection (n = 5 for each). These doses of CART were dissolved in 80 µL of aCSF. Administration of each dose of CART was separated by 90 minutes.

Effect of ICV CART on Cardiovacular and Neurohormonal Responses
A blood sample (3.0 mL) was drawn from the arterial catheter to measure plasma catecholamines, vasopressin, glucose, insulin, cortisol, osmolality, and hematocrit. CART (1 nmol per 80 µL) was then injected (n = 6). Additional blood samples were drawn 30 and 60 minutes after ICV injection of CART. Blood samples were replaced by the same volume of 0.9% saline. Our previous study had demonstrated that ICV administration of aCSF fails to change arterial pressure, RSNA, and blood variables.14 Therefore, in the present study, responses of arterial pressure, RSNA, and blood variables induced by ICV CART were compared with those of the control period.

Effect of Pentolinium on Cardiovascular Responses Induced by ICV Injection of CART
Rabbits were injected with pentolinium (Sigma Chemical Co; 5 mg/kg in 0.3 mL IV), a ganglion-blocking agent. Five minutes later, a blood sample (3.0 mL) was drawn to measure blood variables. Ten minutes after intravenous pentolinium, CART (1 nmol ICV) was injected (n = 5). Additional blood samples were drawn 30 and 60 minutes after ICV injection of CART. Additional blood samples were drawn 30 and 60 minutes after ICV injection of CART (2.5 mg/kg IV) was injected after the third blood-sample drawing.

Effect of Intravenous Injection of CART on Cardiovacular and Sympathetic Responses
To evaluate leakage of ICV-injected CART into the systemic circulation, the same dosage level of CART (1 nmol) used in the ICV injection experiment was injected intravenously (n = 5).

Blood Analysis
Plasma catecholamine concentrations were measured by high-performance liquid chromatography,15 and plasma vasopressin, insulin, and cortisol concentrations were measured by radioimmunoassay.12,16,17 Plasma glucose levels were measured with a Glucose Analyzer 2 (Beckman Instruments).12,13 Plasma osmolality was measured with a freezing-point osmometer.12,13

Statistics
All values are expressed as mean±SE. To determine effects of ICV and intravenous injections of CART on cardiovascular and RSNA responses or blood variables, 1-way ANOVA with repeated measurements was performed. A value of P<0.05 was considered significant.

Results

Relationship Between Dosage of ICV CART and Cardiovacular Responses
Baseline values for mean arterial pressure (MAP) and heart rate (HR) before ICV injection of CART were 83.6±1.3 mm Hg and 222.0±9.7 bpm, respectively. ICV injection of CART elicited dose-related increases in MAP and RSNA (Figure 1). Results shown in Figure 1 illustrate peak responses for MAP, HR, and RSNA obtained during a 90-minute recording period. Because 1 nmol of CART caused significant increases in MAP and RSNA, we used this dosage level of CART in the following experiments.

Effect of ICV CART on Cardiovacular and Neurohormonal Responses
Figure 2 shows typical responses of MAP, HR, and RSNA elicited by ICV injection of CART (1 nmol). ICV injection of 1 nmol of CART provoked increases in MAP and RSNA, and peak values of these variables were obtained after 40 and 60 minutes, respectively (Figure 3). After peak values were obtained, MAP and RSNA decreased and returned to their baseline levels within 90 to 120 minutes. However, HR did not show any significant changes. Table 1 shows the effects of ICV injection of CART on blood variables. Plasma epinephrine and glucose concentrations significantly increased at 30 and 60 minutes. Plasma norepinephrine concentrations also increased significantly at 30 minutes. Plasma vasopressin, insulin, and cortisol concentrations increased at 60 minutes but did not attain significant values.

Figure 1. Bar graphs showing central effects of 2 doses (0.1 and 1 nmol) of CART peptide and aCSF (80 µL) on changes in MAP (top), HR (middle), and integrated RSNA (bottom) in 5 rabbits. Values are mean±SE. **P<0.01 vs respective responses to aCSF by Duncan’s multiple-range test.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.
osmolality and hematocrit showed no changes. Rabbits sat quietly in the box, and behavioral effects of ICV CART were not observed throughout the experimental period.

Effect of Pentolinium on Cardiovascular Responses Induced by ICV Injection of CART

After pentolinium administration, MAP decreased from $80.0 \pm 2.2$ to $54.4 \pm 1.7$ mm Hg and HR increased from $203.0 \pm 11.6$ to $250.0 \pm 12.8$ bpm. However, ICV injection of CART failed to cause any further responses in MAP or HR, and RSNA was almost completely suppressed until 120 minutes after injection of CART. Table 2 shows effects of pentolinium on blood variables induced by ICV injection of CART. Plasma epinephrine, norepinephrine, insulin, and glucose levels showed no significant changes. Intravenous injection of pentolinium increased plasma vasopressin concentrations; however, ICV injection of CART failed to cause further changes in plasma vasopressin concentrations.

Effect of Intravenous Injection of CART on Cardiovascular and Sympathetic Responses

The same dosage level of CART (1 nmol) used in the ICV experiment was injected intravenously. After intravenous injection of CART, arterial pressure, HR, and RSNA remained within 5% of control values.

Discussion

The present study demonstrated that ICV injection of CART causes significant increases in arterial pressure, RSNA, and plasma epinephrine and norepinephrine concentrations. Intravenous injection of pentolinium, a ganglion-blocking agent, abolished responses of arterial pressure and plasma epinephrine and norepinephrine concentrations. These results suggest that the pressor response induced by the ICV injection of CART can be attributed primarily to enhanced sympathetic outflow. Furthermore, intravenous injection of the same dose of CART used in the ICV injection experiment failed to cause any cardiovascular and sympathetic responses, which suggests that the responses induced by ICV injection of CART were not caused by a leakage of CART into the systemic circulation. To the best of our knowledge, this is the first study to demonstrate cardiovascular and sympathetic responses induced by central administration of CART in conscious animals.

Central CART inhibits food intake\(^6,7\); however, effects of CART on blood glucose and insulin levels remain to be investigated. In the present study, ICV injection of CART caused significant increases in plasma epinephrine and glucose concentrations. In contrast, plasma insulin and cortisol concentrations increased but did not attain significant values. The response of plasma glucose levels elicited by ICV CART in the present study was similar to that reported by Vrang et al.\(^9\) although this group did not determine the other endocrine factors regulating blood glucose levels.\(^9\) In the present study,
we did not perform the experiment involving ICV administration of aCSF. However, our previous study demonstrated that ICV administration of aCSF fails to cause any significant changes in arterial pressure, RSNA, and plasma catecholamine and insulin concentrations. In addition, ICV administration of aCSF failed to cause any significant changes in arterial pressure, HR, and RSNA in the dose-response experiment of the present study. Therefore, we believe that the responses of arterial pressure, RSNA, and plasma catecholamine and glucose concentrations in the present study can be attributed to the effects of ICV CART itself. The present study has an advantage in that the experiments were conducted on conscious animals and that serial changes in plasma catecholamine concentrations were determined. The parallel changes in plasma glucose levels and plasma epinephrine concentrations suggest a close relationship between these 2 variables. Hyperglycemia has been shown to be evoked by an increase in plasma epinephrine concentration; thus, this response of plasma glucose level in the present study was probably due to the increased plasma epinephrine concentration. Subsequently, this increase in plasma glucose level may have induced the slight increase in plasma insulin level in the present study.

ICV administration of CART is reported to increase c-Fos positive corticotropin-releasing hormone–immunoreactive neurons in the paraventricular nucleus and also plasma corticosterone concentration in rats. Therefore, we hypothesized that central CART would increase plasma adrenocorticotropic hormone concentrations and, subsequently, increase plasma cortisol concentrations. However, in the present study, ICV CART failed to change plasma cortisol concentrations. Interpretation of the dissociation of the present and previous findings is difficult. Central CART may participate in a differential regulation of corticosterone and cortisol secretions. Further studies are necessary to determine simultaneously the central effects of CART on changes in plasma adrenocorticotropic hormone, corticosterone, and cortisol concentrations. ICV CART also did not cause any significant changes in plasma vasopressin concentrations in the present study, and these results are consistent with the findings reported by Vrang et al. CART-immunoreactive neurons have been shown to be abundant in the paraventricular nucleus and supraoptic nucleus; however, these neurons may not be involved in vasopressin secretion.

A limitation of the present study was that specific antagonists for CART peptide were not available. Therefore, it is difficult to conclude that ICV-injected CART activates specific CART receptors to increase arterial pressure and sympathetic nerve activity. In addition, the present study did not clarify the exact site at which CART acts in the central nervous system nor the mechanisms of pressor response and activation of the sympathetic nervous system induced by ICV CART. High-density CART mRNA has been shown not only

### TABLE 1. Effects of Injection of CART Peptide (1 nmol ICV) on Blood Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>0</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine, pg/mL</td>
<td>77.0 ± 62.4</td>
<td>736.5 ± 330.0†</td>
<td>1067.5 ± 329.3†</td>
</tr>
<tr>
<td>Norepinephrine, pg/mL</td>
<td>278.0 ± 55.0</td>
<td>984.8 ± 241.8*</td>
<td>524.8 ± 175.6</td>
</tr>
<tr>
<td>Vasopressin, pg/mL</td>
<td>4.7 ± 1.2</td>
<td>4.3 ± 0.9</td>
<td>6.7 ± 1.3</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.357 ± 0.004</td>
<td>0.358 ± 0.005</td>
<td>0.355 ± 0.005</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>6.25 ± 0.33</td>
<td>8.20 ± 0.47*</td>
<td>11.57 ± 0.93†</td>
</tr>
<tr>
<td>Insulin, μU/mL</td>
<td>13.5 ± 4.6</td>
<td>12.3 ± 3.8</td>
<td>16.3 ± 3.5</td>
</tr>
<tr>
<td>Cortisol, ng/mL</td>
<td>15.7 ± 1.5</td>
<td>19.2 ± 1.1</td>
<td>20.2 ± 3.3</td>
</tr>
<tr>
<td>Osmolality, mOsm/L</td>
<td>287.2 ± 0.9</td>
<td>288.8 ± 0.8</td>
<td>293.3 ± 2.8</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

\*P < 0.05, †P < 0.01 vs control (0 min) by Duncan’s multiple-range test.

### TABLE 2. Effects of Pentolinium on Responses of Blood Variables Induced by ICV Injection of CART Peptide

<table>
<thead>
<tr>
<th>Variables</th>
<th>0</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine, pg/mL</td>
<td>10.4 ± 0.4</td>
<td>10.4 ± 0.4</td>
<td>14.6 ± 2.6</td>
</tr>
<tr>
<td>Norepinephrine, pg/mL</td>
<td>240.2 ± 168.3</td>
<td>169.0 ± 60.9</td>
<td>298.0 ± 183.4</td>
</tr>
<tr>
<td>Vasopressin, pg/mL</td>
<td>39.4 ± 8.0</td>
<td>14.9 ± 3.6</td>
<td>21.7 ± 6.9</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.327 ± 0.006</td>
<td>0.326 ± 0.006</td>
<td>0.325 ± 0.007</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.98 ± 0.26</td>
<td>6.02 ± 0.22</td>
<td>6.27 ± 0.25</td>
</tr>
<tr>
<td>Insulin, μU/mL</td>
<td>6.6 ± 1.7</td>
<td>7.4 ± 1.5</td>
<td>8.6 ± 2.7</td>
</tr>
<tr>
<td>Cortisol, ng/mL</td>
<td>15.2 ± 1.6</td>
<td>18.0 ± 1.1</td>
<td>19.8 ± 1.1</td>
</tr>
<tr>
<td>Osmolality, mOsm/L</td>
<td>285.6 ± 2.0</td>
<td>286.8 ± 1.7</td>
<td>285.6 ± 1.9</td>
</tr>
</tbody>
</table>

Values are mean ± SE.
in the hypothalamus but also in the medulla oblongata, eg, in the nucleus of the solitary tract and area postrema, which suggests that these brain regions might be involved in the pressor responses induced by ICV CART. The primary site in the brain in which CART acts to inhibit food intake is considered to be the hypothalamus; therefore, ICV CART might primarily act at the hypothalamic nuclei, subsequently stimulating the cardiovascular center of the medulla to increase arterial pressure. A study that focuses on microinjection of CART into the hypothalamus and medulla oblongata will be necessary to clarify the role of CART in the brain with regard to cardiovascular and sympathetic regulations.

Neuropeptides such as neuropeptide Y, agouti-related protein, orexins, α-melanocyte stimulating hormone, and CART have been shown to be involved in feeding in the central nervous system and to interact with leptin. These neuropeptides seem to function together for cardiovascular and sympathetic regulation as well as for the regulation of food intake and energy expenditure. ICV administration of leptin activates sympathoadrenal outflow and increases arterial pressure. These effects were accompanied by increases in plasma glucose and insulin. Further studies are necessary to determine the exact site of the brain at which CART acts to augment the sympathoadrenal outflow and increases arterial pressure. A study that focuses on microinjection of CART into the hypothalamus and medulla oblongata will be necessary to clarify the role of CART in the brain with regard to cardiovascular and sympathetic regulations.

In conclusion, CART was found to exert a central pressor action mediated primarily by enhanced sympathoadrenal outflow, and these effects were accompanied by increases in plasma glucose levels. CART may participate in central cardiovascular and sympathetic regulation as well as in the regulation of appetite and food intake, although physiological implications have not yet been determined. Further studies are necessary to determine the exact site of the brain at which CART acts to augment the sympathoadrenal outflow and the interactions of leptin and CART in central cardiovascular and sympathetic regulations.

Acknowledgments
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