Central Ghrelin Modulates Sympathetic Activity in Conscious Rabbits

Kiyoshi Matsumura, Takuya Tsuchihashi, Koji Fujii, Isao Abe, Mitsuo Iida

Abstract—Ghrelin is an orexigenic peptide originally isolated from the stomach. Intravenous administration of ghrelin has been shown to elicit a decrease in arterial pressure without a significant change in heart rate (HR), suggesting that ghrelin may act on the central nervous system to modulate sympathetic activity. The aim of the present study was to determine the central effects of ghrelin on cardiovascular and sympathetic responses in conscious rabbits. Intravenous injection of ghrelin elicited dose-related decreases in arterial pressure and HR, without a significant change in renal sympathetic nerve activity. On the other hand, intracerebroventricular injection of 1 nmol of ghrelin decreased arterial pressure, HR, and renal sympathetic nerve activity. Peak depressor or sympathoinhibitory responses of mean arterial pressure and renal sympathetic nerve activity (−19.0±1.5 mm Hg and −43.3±5.4%) were observed at 50 and 40 minutes, respectively, after intracerebroventricular injection of 1 nmol of ghrelin. Furthermore, a subdepressor dose of intracerebroventricular infusion of ghrelin (0.3 nmol/150 μL per hour) significantly augmented the baroreflex sensitivities assessed by renal sympathetic nerve activity and HR compared with those of vehicle infusion (Gmax; −17.8±3.1 versus −9.4±1.6% mm Hg, P<0.05; −12.5±1.8 versus −6.6±1.2 bpm/mm Hg, P<0.05; respectively). These results suggest that intravenous injection of ghrelin acts, at least in part, on the central nervous system to decrease arterial pressure and renal sympathetic nerve activity, and that central ghrelin participates in the regulations of the sympathetic nerve activity to the kidney and the baroreceptor reflex in conscious rabbits. (Hypertension. 2002;40:694-699.)

Key Words: baroreceptors ■ blood pressure ■ central nervous system ■ ghrelin ■ nervous system, sympathetic renal

G

hrelin, an acylated 28-residue peptide originally isolated from the rat stomach, is an endogenous ligand for the growth hormone (GH) secretagogue receptor.1,2 Although ghrelin is likely to regulate pituitary GH secretion along with GH-releasing hormone and somatostatin,2,3 GH secretagogue receptors have also been identified in hypothalamic neurons and in the brainstem.4,5 Intracerebroventricular (ICV) administration of ghrelin has been shown to generate a dose-dependent increase in food intake and body weight,6,7 suggesting that ghrelin participates in the regulation of food intake and GH secretion. Furthermore, ICV administration of ghrelin has been shown to increase plasma vasopressin level without a significant change in arterial pressure in conscious rats.8

It has also been reported that intravenous injection of human ghrelin elicits a decrease in blood pressure without an increase in heart rate (HR) in healthy men.9 In addition, plasma ghrelin concentration is increased in patients with cachexia associated with chronic heart failure.10 These previous findings suggest that ghrelin may participate not only in feeding behavior but also in cardiovascular and sympathetic regulation. Although ghrelin has been reported to have a vasodilatory effect in humans,11 the underlying mechanisms of depressor response induced by intravenous injection of ghrelin have not yet been determined. Because the depressor response was not accompanied by tachycardia, it is likely that mechanisms other than direct vasodilating effects, at least in part, are involved in this depressor response. To clarify the mechanisms for this depressor response, the effects of ghrelin on sympathetic activity and on the baroreceptor reflex should be determined. We hypothesized that intravenous administration of ghrelin acts at the central nervous system to modulate the sympathetic nervous system, resulting in a decrease in arterial pressure without tachycardia. Accordingly, in the present study, we focused on the central effect of ghrelin on sympathetic activity and baroreceptor reflex. To evaluate the sympathetic nervous system precisely, the present study was conducted on conscious rabbits with direct recording of renal sympathetic nerve activity (RSNA), because the sympathetic nervous system and baroreceptor reflex are greatly affected by anesthesia.12,13

Methods

Preparation of Animals

The experiments were conducted with 24 male Japanese White rabbits (Biotek, Saga, Japan) weighing 2.5 to 2.7 kg. All experiments were performed according to the institutional guidelines for animal
experimentation at Kyushu University. Rabbits were anesthetized with pentobarbital sodium (30 mg/kg IV). Three days before experimentation, bipolar electrodes were implanted on the left renal sympathetic nerve, and a stainless steel cannula was placed in the right lateral cerebral ventricle.14,15 RSNA was recorded as described previously.14,15 At least 3 days after the surgical procedures, the following experiments were performed on conscious rabbits placed in a box. On the day of the experiment, polyethylene catheters (PE-50) were inserted into the central ear artery and the marginal ear vein under 1% lidocaine local anesthesia. Each experiment was started in the morning, and a 30-minute control period was obtained to get baseline levels of mean arterial pressure (MAP), HR, and RSNA. All drugs for ICV injection were dissolved in artificial cerebrospinal fluid (aCSF).15–17

Effects of Intravenous Ghrelin on Cardiovascular and Sympathetic Responses
To determine the systemic effect of ghrelin on cardiovascular and sympathetic responses, vehicle (0.9% saline; 0.2 mL) and human ghrelin (Peptide Institute, Osaka, Japan), 1 or 5 nmol, were injected intravenously in ascending concentration order (n=6 for each). These doses of ghrelin were dissolved in 0.9% saline (0.2 mL). The administration of each dose of ghrelin was separated by a period of 60 minutes. MAP, HR, and RSNA were confirmed to return to their baseline values before each injection.

Effects of ICV Ghrelin on Cardiovascular and Sympathetic Responses
To determine the central effect of ghrelin on cardiovascular and sympathetic responses, aCSF (80 μL) or 1 nmol of human ghrelin was ICV injected (n=6 for each). This dose of ghrelin was dissolved in 80 μL aCSF.

Effects of ICV Infusion of Ghrelin on Baroreceptor Reflex
Three days after the surgical procedure, the effects of ghrelin on the baroreflex control of RSNA and HR were determined (n=6). Either aCSF or ghrelin was infused with a compact syringe pump at a flow rate of 150 μL/hour. Fifteen minutes after the beginning of the ICV infusion of either aCSF or ghrelin (0.3 nmol/hour), the sensitivities of the baroreflex control of RSNA and HR were determined as described previously.15

A progressive infusion of sodium nitroprusside (5 to 80 μg/kg per minute diluted in 0.9% NaCl) was performed to induce a 25 to 30 mm Hg decrease in MAP. Phenytoine (2 to 32 μg/kg per minute diluted in 0.9% NaCl) was infused for 3 minutes to induce a 30 mm Hg increase in MAP. Half of the rabbits were infused first with sodium nitroprusside and then phenylephrine; the remaining rabbits received an infusion of phenylephrine before sodium nitroprusside. At least 30 minutes elapsed between the infusion of each vasoactive agent to allow MAP, HR, and RSNA to return to the baseline values.

The values of the mean RSNA before each infusion were defined as 100%. Data for the MAP-RSNA or MAP-HR relations during increases and decreases in MAP were fitted to a sigmoid logistic function curve. The equation used for the data analysis was based on the following mathematical model:

\[ \text{RSNA or HR} = \frac{P_4}{1 + \exp\left(\frac{P_3 - \text{MAP}}{P_2}\right)} + P_1 \]

In this equation, \( P_1 \) is the range between the upper and lower plateau; \( P_2 \), a range-independent measure of slope or normalized gain; \( P_3 \), the blood pressure at the midpoint of the logistic function curve; and \( P_4 \), the lower plateau. Data were fit to the logistic function curve using a nonlinear regression program in the Statistical Analysis System (NLIN procedure, SAS Institute). In the present study, the maximum slope (\( G_{\text{max}} = -P_3 / P_2 P_4 \)) calculated from the parameters of the logistic function curve was considered to be the sensitivity of the baroreceptor reflex.

Statistics
All values are expressed as mean±SE. To determine the intravenous and central effects of ghrelin on cardiovascular and sympathetic responses, 1-way ANOVA with repeated measurements was performed, followed by Duncan’s multiple range test. In addition, to compare the responses induced by ICV aCSF and ghrelin, 2-way ANOVA with repeated measurements was applied. A paired t test was used to determine the effects of ICV ghrelin on baroreflex control. A value of \( P<0.05 \) was considered significant.

Results
Effects of Intravenous Ghrelin on Cardiovascular and Sympathetic Responses
Baseline values for MAP and HR before the intravenous injection of ghrelin were 87.0±1.9 mm Hg and 232.5±11.7 bpm, respectively. Intravenous injection of ghrelin elicited dose-related decreases in MAP without a significant change in RSNA. Intravenous injection of 5 nmol ghrelin decreased HR (Figure 1). Peak depressor and bradycardiac responses induced by intravenous injection of 5 nmol ghrelin were obtained at 20 and 40 minutes after injection, respectively, and MAP and HR returned to the baseline levels at 60 minutes.

Effects of ICV Ghrelin on Cardiovascular and Sympathetic Responses
Figure 2 shows the typical responses of MAP, HR, and RSNA elicited by ICV injection of ghrelin (1 nmol). ICV injection of 1 nmol of ghrelin provoked decreases in MAP, HR, and RSNA, and peak responses (−19.0±1.5 mm Hg, −80.0±11.6 bpm, and −43.3±5.4%) were obtained at 40 to 50 minutes (Figure 3). After peak responses were observed, MAP, HR, and RSNA gradually returned to baseline levels, although these variables were still significantly decreased at
90 minutes after administration. These depressor, bradycardic, and sympathoinhibitory responses to ICV ghrelin were also significantly different from those to ICV aCSF by 2-way ANOVA with repeated measurements (P<0.01 for each variables).

Effects of ICV Infusion of Ghrelin on Baroreceptor Reflex

ICV infusion of ghrelin (0.3 nmol/hour) did not cause any significant changes in MAP, HR, or RSNA, but it significantly augmented the baroreflex control of RSNA \((G_{max} = 17.8 \pm 3.1 \text{ versus } -9.4 \pm 1.6 \% / \text{mm Hg}; P<0.05)\) and HR \((G_{max} = 12.5 \pm 1.8 \text{ versus } -6.6 \pm 1.2 \text{ bpm/mm Hg}; P<0.05)\) (Table and Figure 4). \(P_2\) value in the logistic function curve of RSNA significantly increased during ICV infusion of ghrelin. In addition, \(P_1\) and \(P_2\) values in the logistic function curve of HR also significantly increased during ICV infusion of ghrelin (Table).

Discussion

The present study demonstrated that intravenous injection of ghrelin elicited dose-related decreases in MAP without a significant change in RSNA. It has been shown that ghrelin has a vasodilating effect; however, it seems difficult to attribute this depressor response solely to the direct vasodilating effect of ghrelin, because in the present study the depressor response was not accompanied by activations of RSNA and HR mediated by baroreceptor reflex. In contrast, ICV injection of 1 nmol ghrelin caused significant decreases in arterial pressure, HR, and RSNA. These findings suggest that systemically administered ghrelin acted, at least in part, at the central nervous system to suppress sympathetic activity, resulting in decreases in arterial pressure and HR. Slightly dissociated responses of RSNA elicited by intravenous or ICV injected ghrelin might be attributed to the different depressor mechanisms. The direct vasodilating effect and the suppression of sympathetic nervous system in the brain might participate in depressor response of intravenous injection of ghrelin. Moreover, ICV infusion of ghrelin augmented the baroreflex controls of RSNA and HR in conscious rabbits. To the best of our knowledge, this is the first study to demon-
strate the central cardiovascular and sympathetic modulation by ghrelin in conscious animals.

ICV administration of ghrelin not only suppressed sympathetic outflow to the kidney but also augmented the baroreflex control of RSNA and HR in conscious rabbits. In the present study, to eliminate the direct effects of ghrelin on the heart or blood vessels and to evaluate the contribution of exogenous brain ghrelin to the baroreceptor reflex, a subpressor dose of ghrelin was ICV infused. Accordingly, P1 values in baroreflex curves for MAP-RSNA and MAP-HR relationships did not change during ICV infusion of ghrelin. These findings support the idea that central ghrelin participates in central cardiovascular and sympathetic modulation in 2 different manners: by suppressing the sympathetic nervous system and by augmenting the baroreceptor reflex. On the other hand, intravenous administration of ghrelin has been reported to increase the cardiac index in healthy men.9 These hemodynamic and sympathetic modulations by ghrelin may be beneficial in the treatment of the patients with congestive heart failure, because activation of the sympathetic nervous system and decreased cardiac function need to be treated in these patients. In fact, intravenous infusion of human ghrelin significantly increased cardiac index and stroke volume in patients with congestive heart failure.19

In the present study, ICV infusion of ghrelin significantly increased P1 value of HR, although it failed to change P1 value of RSNA. The different response of P1 values of baroreflex curve might be explained by the different regulatory mechanisms between RSNA and HR. RSNA is regulated by sympathetic nervous system, whereas HR is dually regulated by the sympathetic and parasympathetic nervous system. The effect of ghrelin on the parasympathetic nervous system might be attributable to the different response of P1 values of RSNA and HR.

ICV and intravenous injections of ghrelin caused decreases in HR. It seems difficult to evaluate separately the roles of sympathetic and parasympathetic nervous system in the present study. However, bradycardiac effects during ICV administration of ghrelin might be primarily attributable to the suppression of sympathetic nervous system, because time courses of MAP, HR, and RSNA were similar. In contrast, bradycardiac effect during intravenous administration of ghrelin might be dually modulated by sympathetic and parasympathetic nervous system, because a little dissociated response between HR and RSNA was observed. Ghrelin may have some different effects between sympathetic and parasympathetic nervous system.

Ghrelin acts at the GH secretagogue receptor, the G protein–coupled receptor, to release GH to the systemic circulation.2,3 In fact, intravenous or ICV administration of

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**Table 1: Parameters and Maximum Gain of Baroreflex Control of RSNA and HR During ICV Infusion of Ghrelin or aCSF**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>aCSF</th>
<th>Ghrelin</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>P1, %</td>
<td>262.2±15.9</td>
<td>272.5±25.8</td>
</tr>
<tr>
<td>P2</td>
<td>0.13±0.023</td>
<td>0.25±0.028*</td>
</tr>
<tr>
<td>P3, mm Hg</td>
<td>78.6±2.4</td>
<td>80.9±2.5</td>
</tr>
<tr>
<td>P4, %</td>
<td>-2.5±5.7</td>
<td>1.4±1.4</td>
</tr>
<tr>
<td>Gmax, %/mm Hg</td>
<td>-9.4±1.6</td>
<td>-17.8±3.1*</td>
</tr>
<tr>
<td>HR</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>P1, beats/min</td>
<td>172.6±13.5</td>
<td>210.5±15.9*</td>
</tr>
<tr>
<td>P2</td>
<td>0.16±0.035</td>
<td>0.23±0.031*</td>
</tr>
<tr>
<td>P3, mm Hg</td>
<td>77.5±2.5</td>
<td>80.3±2.5</td>
</tr>
<tr>
<td>P4, beats/min</td>
<td>168.0±10.6</td>
<td>153.4±5.4</td>
</tr>
<tr>
<td>Gmax, beats/min/mm Hg</td>
<td>-6.6±1.2</td>
<td>-12.5±1.8*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SE. aCSF indicates artificial cerebrospinal fluid; P1, range of renal sympathetic nerve activity (RSNA) or heart rate (HR); P2, slope coefficient; P3, mean arterial pressure at midrange; P4, minimum RSNA or HR; Gmax, maximum gain of RSNA or HR.

*P<0.05; **P<0.01 vs aCSF by paired t test.
Ghrelin has been shown to increase plasma GH concentration.\textsuperscript{9,20,21} Therefore, although plasma GH concentrations were not determined in the present study, plasma GH concentration could increase after the ICV administration of ghrelin. On the other hand, GH activity has been proposed to be associated with myocardial growth and cardiac function.\textsuperscript{22,23} It may be important to discriminate the relative role of released GH and ghrelin itself on the cardiovascular and sympathetic effects of ICV ghrelin. In regard to feeding behavior, GH release is not considered to be involved in the ghrelin-induced orexigenic effect, because ICV injection of ghrelin can increase food intake in GH-deficient spontaneous dwarf rats.\textsuperscript{24} Furthermore, Bisi et al\textsuperscript{25} reported that intravenous injection of GH failed to change arterial pressure, HR, and left ventricular ejection fraction in healthy men. Based on these previous findings, the cardiovascular and sympathetic responses induced by ghrelin in the present study could be attributable to the effect of ghrelin by itself.

The present study was limited by which brain regions are involved in the regulation of blood pressure and baroreceptor reflex by ghrelin. Ghrelin is predominantly produced by the stomach, whereas substantially lower amounts are derived from bowel, pituitary, kidney, placenta, and hypothalamus.\textsuperscript{3} In contrast, GH secretagogue receptors have also been identified on hypothalamic neurons and in the brainstem.\textsuperscript{4,5} ICV administration of ghrelin has been shown to induce c-fos expression in the neurons of the nucleus of the solitary tract and dorsomotor nucleus of the vagus,\textsuperscript{26} suggesting that centrally administered ghrelin activated the neurons of these regions. The nucleus of the solitary tract, where baroreceptor and chemoreceptor afferents terminate, is one of the most important brain regions to regulate blood pressure and the sympathetic nervous system. In addition, activation of neurons in the nucleus of the solitary tract by l-glutamate, an excitatory neurotransmitter in the brain, decreases arterial pressure and inhibits RSNA.\textsuperscript{27} These anatomical and physiological findings support the idea that central ghrelin might act at the medulla oblongata, such as at the nucleus of the solitary tract and dorsomotor nucleus of the vagus, and at the arcuate hypothalamic nucleus, to modulate the sympathetic nervous system and the baroreceptor reflex. A study that focuses on the microinjection of ghrelin into the hypothalamus and medulla oblongata will be necessary to clarify the role of ghrelin in the brain with regard to cardiovascular and sympathetic regulations.

Appetite and feeding behavior are regulated by many factors such as leptin and neuropeptide Y.\textsuperscript{28,29} ICV administration of ghrelin has been shown to generate a dose-dependent increase in food intake and body weight,\textsuperscript{6,7} suggesting that ghrelin participates in the regulation of food intake and in the regulation of GH secretion. Leptin and ghrelin are released from white adipose tissue and the stomach, respectively, both of which enter into the systemic circulation and reach to the brain.\textsuperscript{3} During food deprivation, plasma leptin levels rapidly decline,\textsuperscript{30} neuropeptide Y/agouti-related protein production is elevated, and circulating ghrelin levels increase, suggesting that leptin and ghrelin coregulate the hypothalamic peptidergic system of feeding behavior in opposite ways.\textsuperscript{30,31} ICV ghrelin has been shown to antagonize the leptin-induced inhibition of food intake through the activation of the hypothalamic neuropeptide Y\textsubscript{1} receptor pathway.\textsuperscript{24} On the other hand, these neuropeptides involved in feeding behavior have been suggested to participate in central cardiovascular and sympathetic regulations.\textsuperscript{16,17,32–34} ICV administration of leptin activates sympathetic nervous system, resulting in an increase in arterial pressure.\textsuperscript{33,34} In contrast, in the present study, ICV administration of ghrelin suppressed the sympathetic nervous system, resulting in a decrease in arterial pressure. Based on these findings, leptin and ghrelin may interact with each other and play roles in central cardiovascular and sympathetic regulations and in food intake and energy expenditure.

Chronic central administration of ghrelin has been shown to increase both neuropeptide Y and agouti-related protein (AGRP) mRNA levels in the arcuate nucleus.\textsuperscript{35} ICV administration of neuropeptide Y decreases arterial pressure and RSNA in conscious rabbits.\textsuperscript{36} On the other hand, AGRP is an endogenous melanocortin-3 and -4 receptor (MC3-R and MC4-R) antagonist, and central cardiovascular and sympathetic responses to ICV leptin are reportedly to be mediated through activation of hypothalamic MC3-R and MC4-R.\textsuperscript{37–39} Based on these previous findings, central cardiovascular effects of ghrelin could be explained by the mechanisms of the activation of hypothalamic neuropeptide Y and the antagonism of the MC3-R and MC4-R by activated AGRP.

Plasma ghrelin concentration has been shown to be decreased in obese patients\textsuperscript{40} and to be increased in patients with cachexia associated with congestive heart failure,\textsuperscript{10} suggesting that plasma ghrelin concentration could be influenced by catabolic-anabolic balance. It has been reported that circulating levels of ghrelin are in the low nanogram range,\textsuperscript{10,40} and data for ghrelin levels in CSF have not been available yet. In the present study, ghrelin levels in plasma and CSF were not determined. Therefore, it seems difficult to conclude that physiological levels of central ghrelin directly decrease arterial pressure and sympathetic activity. Neuropeptides participating in feeding behavior—such as leptin, neuropeptide Y, orexins, AGRP, and ghrelin—may function together to regulate sympathetic outflow and arterial pressure.

In conclusion, intravenous injection of a high dose of ghrelin (5 nmol) decreased arterial pressure and HR. ICV injection of a smaller dose of ghrelin (1 nmol) decreased arterial pressure and suppressed RSNA. Furthermore, ICV infusion of ghrelin augmented the baroreceptor control of RSNA and HR in conscious rabbits. These findings indicate that systemically administered ghrelin reaches the brain and modulates the sympathetic activity. The present study is an acute experiment; therefore, chronic studies are required to better elucidate the importance of ghrelin on the cardiovascular system. Further studies will be needed to determine the full physiological implications of the role of ghrelin on the regulations of appetite and sympathetic activity in the brain, especially in interaction with leptin.

**Perspectives**

Ghrelin is primarily found as an endogenous ligand for the GH secretagogue receptor and stimulates food intake.\textsuperscript{1} Recent studies also demonstrated that systemically administered
ghrelin decreases arterial pressure and increases cardiac index and stroke volume, suggesting that it participates in cardiovascular and sympathetic regulations. The present study demonstrates that central ghrelin suppressed sympathetic activity and augmented the baroreceptor reflex, although dose of ICV-injected ghrelin might not be in physiological range. However, the results of the present study support the idea that ghrelin may be beneficial effects for the treatment of the patients with hypertension or congestive heart failure. The effects of chronic administration of small dose of ghrelin on cardiovascular and sympathetic regulation should be determined in the near future.

References

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