Chronic Administration of Ghrelin Improves Left Ventricular Dysfunction and Attenuates Development of Cardiac Cachexia in Rats With Heart Failure

Noritoshi Nagaya, MD; Masaaki Uematsu, MD; Masayasu Kojima, MD; Yoshihiko Ikeda, MD; Fumiki Yoshihara, MD; Wataru Shimizu, MD; Hiroshi Hosoda, MD; Yuki Hirota, PhD; Hideyuki Ishida, MD; Hidezo Mori, MD; Kenji Kangawa, PhD

Background—Ghrelin is a novel growth hormone (GH)–releasing peptide that may also induce vasodilation and stimulate feeding through GH-independent mechanisms. We investigated whether ghrelin improves left ventricular (LV) dysfunction and attenuates cachexia in rats with chronic heart failure (CHF).

Methods and Results—Ligation of the left coronary artery or sham operation was performed; 4 weeks after surgery, rat ghrelin (100 μg/kg SC BID) or saline was administered for 3 weeks. Echocardiography and cardiac catheterization were performed. Serum GH and insulin-like growth factor-1 were significantly higher in both CHF and sham rats treated with ghrelin than in those given placebo (P<0.05 for both). CHF rats given placebo showed an impaired increase in body weight compared with sham rats given placebo (P<0.05). CHF rats treated with ghrelin, however, showed a significantly greater increase in body weight than those given placebo (+10% versus +3%, P<0.05). They showed significantly higher cardiac output (315±49 versus 266±31 mL · min⁻¹ · kg⁻¹, P<0.05) and LV dP/dt max (5738±908 versus 4363±973 mm Hg/s, P<0.05) than CHF rats given placebo. Ghrelin increased diastolic thickness of the noninfarcted posterior wall, inhibited LV enlargement, and increased LV fractional shortening in CHF rats (from 15±3% to 19±3%, P<0.05).

Conclusions—Chronic subcutaneous administration of ghrelin improved LV dysfunction and attenuated the development of LV remodeling and cardiac cachexia in rats with CHF. (Circulation. 2001;104:1430-1435.)

Key Words: heart failure ■ hormones ■ growth substances ■ nutrition
whether ghrelin attenuates the development of cardiac cachexia.

Methods

Model of CHF
Myocardial infarction was produced in male Wistar rats weighing 200 to 240 g by left coronary ligation as described previously. The control rats underwent a sham operation consisting of thoracotomy and cardiac exposure but without coronary artery ligation. The surviving rats were maintained on standard rat chow. There was a 33% mortality rate within 48 hours after coronary ligation, and the subsequent mortality was 15% for 4 weeks. Four weeks after surgery, 31 infarct rats were randomly divided into 2 groups and given either ghrelin (n=16) or placebo (n=15) for 3 weeks. Similarly, 26 sham-operated rats were randomized to receive ghrelin (n=13) or placebo (n=13).

Administration of Ghrelin
Rat ghrelin was obtained from the Peptide Institute Inc. Four weeks after surgery, rat ghrelin (100 μg/kg BID for 3 weeks) or saline was injected subcutaneously in both CHF rats and sham-operated rats.

Echocardiographic Studies
Echocardiographic studies were performed before and after 3-week supplementation with ghrelin. 2D targeted M-mode tracings were obtained at the level of the papillary muscles. Anterior and posterior end-diastolic and end-systolic wall thickness, LV end-diastolic and end-systolic dimensions, and LV fractional shortening were measured by the American Society for Echocardiology leading-edge method from 3 consecutive cardiac cycles. LV meridional wall stress was estimated as 0.334×LV pressure×[LV dimension/(1+PWT/LV dimension)], where PWT is posterior wall thickness. LV pressure was recorded within 12 hours of the final echocardiogram.

Hemodynamic Studies
Hemodynamic studies were performed after 3 weeks of treatment with ghrelin or placebo. After anesthesia with pentobarbital, polyethylene catheters were inserted into the right femoral artery and the right ventricle (RV). Hemodynamic variables were measured with a thermomicroprobe for measurement of cardiac output. A 1.5F micromanometer-tipped catheter was advanced into the LV and then replaced with a thermocouple for measurement of cardiac output.

After completion of these measurements, blood was drawn from the femoral artery for measurements of GH and IGF-1. Serum GH was measured with a radioimmunoassay kit (Biotrak RIA, Amersham). Serum IGF-1 was determined with an enzyme immunoassay kit (Active Rat IGF-1 EIA, DSL Inc).

Acute Hemodynamic Effects of Ghrelin
Acute hemodynamic responses to intravenous injection of ghrelin (10 μg/kg) or placebo were examined in sham rats, CHF rats, and spontaneous diabetic rats, a GH-deficient rat model that carries a disrupted GH gene (n=5 each).

Isolated Myocyte Contractile Function
To investigate whether ghrelin has direct inotropic effects on cardiac myocytes, adult cardiac myocytes (n=6) were isolated from the LV of normal rats by collagenase digestion. Cardiac myocytes were also isolated from the LV noninfarcted region of CHF rats that received 3-week treatment with ghrelin (n=40) or placebo (n=38) to examine the chronic effects of ghrelin treatment on myocyte contractile function. Myocyte contraction was monitored every 4 ms by a high-speed CCD camera (Fastcam-net, Photron) and measured with NIH Image software (version 1.62, NIH). Myocytes were stimulated at 0.5 Hz with 7-ms pulses.

Results

Effects of Ghrelin on Somatotropic Function and Body Weight
Moderate to large infarcts were observed in both CHF groups, and the mean infarct size was similar in the two (Table 1). Serum GH and IGF-1 were significantly higher in both CHF and sham rats treated with ghrelin than in those given placebo (Figure 1). CHF rats given placebo showed an impaired increase in body weight compared with sham rats given placebo (Figure 2). CHF rats treated with ghrelin, however, showed a significantly greater increase in body weight than those given placebo. Tibial length was significantly increased in rats treated with ghrelin compared with those given placebo (Table 1). LV weight/tibial length was significantly higher in sham rats treated with ghrelin than in those given placebo. RV weight/tibial length was significantly lower in CHF rats treated with ghrelin than in those given placebo. The ratios of gastrocnemius muscle weight to tibial length and muscle protein content to tibial length were significantly lower in CHF rats given placebo than in sham rats given placebo, suggesting the presence of cachexia in this model of CHF. Both parameters, however, were significantly higher in CHF rats treated with ghrelin than in those given placebo.

Effects of Ghrelin on Hemodynamics
Heart rate, mean arterial pressure, RV systolic pressure, and mean right atrial pressure tended to be lower in CHF rats treated with ghrelin than those given placebo, although these changes did not reach statistical significance (Table 2). Cardiac output, stroke volume, and LV dP/dt max were signif-
significantly higher in CHF rats treated with ghrelin than in those given placebo (Figure 3), whereas LV end-diastolic pressure and LV dP/dt max were significantly lower in CHF rats given ghrelin (Table 2). Systemic vascular resistance was significantly lower in CHF rats treated with ghrelin than in those given placebo (Figure 3).

Effects of Ghrelin on LV Geometry and Function
Ghrelin significantly increased diastolic thickness of the noninfarcted posterior wall in both CHF and sham rats (Figure 4). LV diastolic dimension tended to decrease in CHF rats treated with ghrelin, although it tended to increase in those given placebo. Thus, LV diastolic dimension was significantly smaller in CHF rats treated with ghrelin than in those given placebo. As a result, LV wall stresses in systole and diastole were significantly lower in CHF rats treated with ghrelin than those given placebo (Table 3). LV fractional shortening increased significantly in CHF rats treated with ghrelin, although it tended to decrease in CHF rats given placebo (Figure 4).

Histological Analysis
Muscle fiber diameter of the noninfarcted myocardium was significantly larger in CHF rats treated with ghrelin than those given placebo (Figure 5). There was no significant difference in collagen volume fraction between CHF rats treated with ghrelin and those given placebo.

Acute Hemodynamic Response to Ghrelin
A single injection of ghrelin significantly decreased mean arterial pressure in both sham-operated rats (−8 mm Hg, P<0.05) and CHF rats (−7 mm Hg, P<0.05). The hypotensive effect was also observed in GH-deficient rats (−6 mm Hg, P<0.05). Ghrelin significantly decreased systemic vascular resistance in sham, CHF, and GH-deficient rats (−12%, −13%, and −10%, P<0.05, respectively). There were no significant changes in heart rate, LV end-diastolic pressure, or LV dP/dt max in each group (data not shown). Placebo injection had no effect on these hemodynamic parameters (data not shown).

Isolated Myocyte Contractile Function
Fractional cell shortening relative to baseline value was not significantly altered by each dose of ghrelin (99±6% for 1 pmol/mL, 97±17% for 10 pmol/mL, and 91±15% for 100 pmol/mL), suggesting that ghrelin has no direct inotropic

**TABLE 1. Characterization of the Animals**

<table>
<thead>
<tr>
<th></th>
<th>Sham-Placebo</th>
<th>Sham-Ghrelin</th>
<th>CHF-Placebo</th>
<th>CHF-Ghrelin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>13</td>
<td>13</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Infarct size, %</td>
<td></td>
<td></td>
<td>37±6</td>
<td>36±7</td>
</tr>
<tr>
<td>Body weight, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>285±12</td>
<td>291±17</td>
<td>241±24*</td>
<td>245±23*</td>
</tr>
<tr>
<td>After treatment</td>
<td>315±13‡</td>
<td>338±16‡</td>
<td>249±33*</td>
<td>270±32‡‡</td>
</tr>
<tr>
<td>Tibial length, mm</td>
<td>40.9±1.3</td>
<td>42.6±1.4‡</td>
<td>40.3±1.9</td>
<td>42.3±2.0‡</td>
</tr>
<tr>
<td>LV wt/body wt, g/kg</td>
<td>1.80±0.10</td>
<td>1.94±0.09</td>
<td>2.23±0.15</td>
<td>2.28±0.25</td>
</tr>
<tr>
<td>RV wt/body wt, g/kg</td>
<td>13.9±1.0</td>
<td>15.4±1.2‡</td>
<td>13.7±1.5</td>
<td>14.5±1.6</td>
</tr>
<tr>
<td>RV wt/tibial length, mg/mm</td>
<td>0.54±0.05</td>
<td>0.56±0.06</td>
<td>1.47±0.32*</td>
<td>0.96±0.31†</td>
</tr>
<tr>
<td>Gastrocnemius muscle wt/tibial length, mg/mm</td>
<td>4.2±0.4</td>
<td>4.5±0.6</td>
<td>8.9±1.5*</td>
<td>6.0±1.5†</td>
</tr>
<tr>
<td>Gastrocnemius muscle protein/tibial length, mg/mm</td>
<td>6.8±0.2</td>
<td>7.3±0.3</td>
<td>4.5±0.8*</td>
<td>5.7±0.4†</td>
</tr>
</tbody>
</table>

Sham-Placebo indicates sham rats given placebo; Sham-Ghrelin, sham rats treated with ghrelin; CHF-Placebo, CHF rats given placebo; and CHF-Ghrelin, CHF rats treated with ghrelin. Gastrocnemius muscle wt/tibial length and gastrocnemius muscle protein/tibial length were calculated from the data of each group (n=5). Data are mean±SD.

*P<0.05 vs respective sham group; †P<0.05 vs respective placebo group; ‡P<0.05 vs baseline.

---

**Figure 1.** Effects of ghrelin on circulating GH and IGF-1 in CHF and sham rats. Data are mean±SD. *P<0.05 vs respective placebo group.

**Figure 2.** Effects of ghrelin on relative changes in body weight in CHF and sham rats. Data are mean±SD. *P<0.05 vs sham group; †P<0.05 vs placebo group.
effects. In contrast, fractional cell shortening in myocytes of CHF rats treated with ghrelin for 3 weeks was significantly greater than that in CHF rats given placebo (19±1% versus 17±1%, P<0.05). Shortening velocity in ghrelin-treated CHF rats was significantly increased compared with that in CHF rats given placebo (278±14 versus 213±10 μm/s, P<0.001).

Discussion

Ghrelin is a novel GH-releasing peptide, isolated from the stomach, that acts through a mechanism independent of that of hypothalamic GH-releasing hormone. The GH-releasing effects of ghrelin are thought to be mediated by specific receptors, GHS-R, present mainly in the pituitary. Taken together with our results that administration of ghrelin increased serum GH and IGF-1 in both CHF and sham rats, this molecule may reach and act on the anterior pituitary via the blood circulation.

In the present study, chronic treatment with ghrelin tended to decrease mean arterial pressure and significantly decreased systemic vascular resistance in CHF rats. IGF-1 has been shown to cause vasodilation through a stimulatory effect on nitric oxide synthesis, suggesting that the vasodilator effects of ghrelin may be mediated at least in part via IGF-1. Conversely, we recently found that GHS-R exists in blood vessels and that a single injection of ghrelin induces a hypotensive effect without an increase in IGF-1.

This study also demonstrated that long-term administration of ghrelin improved cardiac performance in CHF rats, as indicated by increases in cardiac output, stroke volume, LV dP/dt max, and LV fractional shortening and by increases in fractional cell shortening and shortening velocity of isolated myocytes. Although the GHS-R gene has been shown to be abundantly expressed in the myocardium, the present study could not demonstrate direct inotropic effects of ghrelin on isolated cardiac myocytes. Thus, a decrease in systemic vascular resistance by ghrelin may be responsible for the increased cardiac output. Alternatively, because GH upregulates sarcoplasmic Ca2+ -ATP and thereby enhances myocardial contractility, part of the cardiac effects of ghrelin may be mediated by GH. In the present study, treatment with ghrelin increased posterior wall thickness, inhibited the progressive LV enlargement, and thereby reduced the LV wall stress in rats with CHF. Histological analysis also demonstrated that ghrelin induced a cardiac hypertrophic response without development of significant fibrosis. GH and IGF-1 have been shown to enhance physiological compensatory hypertrophy in rats after myocardial infarction, resulting in a decrease in LV wall stress leading to improvement in cardiac function. Thus, ghrelin may also improve cardiac function through GH/IGF-1–dependent mechanisms. Taken together, an improvement in cardiac function by ghrelin may be related not only to its effects on the GH/IGF-1 axis but also to a result of its vasodilatory effects.
Cardiac cachexia, which is a catabolic state characterized by weight loss and muscle wasting, occurs frequently in patients with end-stage CHF and is a strong independent risk factor for mortality in patients with CHF. In the present study, CHF rats given placebo showed an impaired increase in body weight and a significant decrease in the muscle/bone ratio, suggesting the presence of cardiac cachexia. In contrast, CHF rats treated with ghrelin revealed an appropriate increase in body weight and a preserved muscle-to-bone ratio similar to those observed in sham-operated rats. The ratios of gastrocnemius muscle weight to body weight and of protein content to body weight were significantly higher in CHF rats treated with ghrelin than in those given placebo (0.53 ± 0.04% versus 0.46 ± 0.07% and 0.09 ± 0.01% versus 0.07 ± 0.01%, both P<0.05), and mean right atrial pressure did not increase significantly in those given ghrelin. These data indicate that the increase in body weight by ghrelin was not due to peripheral fluid retention but rather to muscle weight gain.

Tschop et al showed that administration of ghrelin induces a positive energy balance and weight gain by decreasing fat utilization and increasing carbohydrate utilization through a GH-independent mechanism. Furthermore, ghrelin enhanced production of GH and IGF-1, both of which cause anabolic effects such as maintenance of skeletal muscle mass and myocardial growth. These findings suggest that ghrelin may play an important role in the regulation of metabolic balance and in preventing cardiac cachexia through GH-dependent or -independent effects.

Although many animal studies have documented beneficial effects of GH, controlled studies in humans have been predominantly negative. Nevertheless, ghrelin may have additional therapeutic potential because it has GH-independent effects, such as vasodilatory actions and anticahesive effects.

Conclusions

Chronic subcutaneous administration of ghrelin improved LV dysfunction and attenuated the development of LV remodeling and cardiac cachexia in rats with CHF. Administration of ghrelin may be a new therapeutic approach to the treatment of CHF.

Acknowledgments

This work was supported in part by a Research Grant for Cardiovascular Disease (12C-2) from the Ministry of Health, Labor, and Welfare; the Uehara Memorial Foundation; JSPS-RFIF 97I 00 201, Science Frontier program of MECSST, NEDO; and the Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research (OPSR) of Japan.

References

Chronic Administration of Ghrelin Improves Left Ventricular Dysfunction and Attenuates Development of Cardiac Cachexia in Rats With Heart Failure
Noritoshi Nagaya, Masaaki Uematsu, Masayasu Kojima, Yoshihiko Ikeda, Fumiki Yoshihara, Wataru Shimizu, Hiroshi Hosoda, Yuki Hirota, Hideyuki Ishida, Hidezo Mori and Kenji Kangawa

_Circulation_. 2001;104:1430-1435
doi: 10.1161/hc3601.095575

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/104/12/1430

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/