GH-releasing peptides improve cardiac dysfunction and cachexia and suppress stress-related hormones and cardiomyocyte apoptosis in rats with heart failure

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GH-releasing peptides improve cardiac dysfunction and cachexia and suppress stress-related hormones and cardiomyocyte apoptosis in rats with heart failure. The discovery of a GH secretagogue (GHS) receptor (GHSR) (17) and its natural ligand ghrelin (19), with wide distribution and various functions in the body, suggests that GHSR and ghrelin may have a regulatory role in many organs (8, 13, 44). Chronic administration of ghrelin improves LV dysfunction and attenuates development of cardiac cachexia in rats with CHF (28). However, a recent report indicates that ghrelin was far less effective than hexarelin (a modified GHRP-6) in terms of cardioprotection in rats with combined GH deficiency and cardiac ischemia-reperfusion (42). This finding raises the clinical potential of GHRP in CHF management.

The GHRP family includes hexarelin, GHRP-1, GHRP-2, and GHRP-6. GHRP-binding sites (GHSR) have been identified in the myocardium (37). Several studies have shown that GHRP also mediate a GH-independent cardioprotective effect in humans with idiopathic or ischemic cardiomyopathy (6) and animals with postischemic ventricular dysfunction (39) or myocardial stunning (46). The cardioprotective action of GHRP in CHF has not been examined.

Stress-related hormonal activation in CHF is characterized by increased activities of sympathoadrenal and renin-angiotensin-aldosterone axes and increased levels of plasma atrial natriuretic peptide (ANP) and endothelin-1 (ET-1), which determine symptom severity and ventricular dysfunction (4, 5, 12, 34). Strategies such as use of a β-blocker to alleviate hormonal activation have been demonstrated to benefit CHF (4, 5, 12, 34). The effect of GHRP on the hormonal responses in CHF is unknown.

Cardiomyocyte apoptosis increases in CHF and contributes to cardiomyocyte loss and cardiac dysfunction (29, 31, 40). We recently demonstrated that hexarelin suppresses ANG II-induced cardiomyocyte apoptosis in vitro (36). GHRP may have a similar action in CHF.

This study is designed to investigate whether chronic administration of GHRP 1) improves LV dysfunction and structural remodeling, 2) suppresses cardiomyocyte apoptosis, 3) attenuates stress-related hormonal activation, and 4) suppresses development of cardiac cachexia in rats with pressure-overload CHF.

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Materials and Methods

Model of pressure-overload CHF. Sprague-Dawley rats (252 ± 6 g body wt) underwent suprarenal abdominal aortic banding or sham surgery as described elsewhere (7). Briefly, under pentobarbital sodium (40 mg/kg ip) anesthesia, the abdominal aorta was banded with an open plastic tube above the renal artery to ~50% of its original diameter with a suture surrounding the tube. Sham-operated rats underwent the same procedure, except the banding tube was removed after 5 min. Mortality was 5% within 48 h after banding and 6% over 9 wk. The surviving rats were maintained on a standard rat feeding regimen. All animal experiments were approved by the Institutional Animal Experimental Ethics Committee of Peking Union Medical College.

Animal groups. At 9 wk after surgery, 48 surviving rats (40 CHF and 8 sham rats) were assigned to 6 groups (n = 8 for each group): CHF-GHRP-1, CHF-GHRP-2, CHF-GHRP-6, CHF-hexarelin, CHF-saline (placebo), and sham-saline. Body weight was measured before surgery, 9 wk after surgery (before GHRP or saline injection), and after the last GHRP or saline injection.

Administration of GHRP. At 9 wk after the banding surgery, GHRP-1, GHRP-2, GHRP-6, hexarelin (all 100 μg/kg, ~115 nmol/kg, a commonly used dose in previous studies), or saline was injected subcutaneously twice a day for 3 wk into animals of the respective groups.

Echocardiographic studies. Echocardiographic studies were performed before and 3 wk after administration of GHRP. Two-dimensional targeted M-mode traces were obtained at the level of the papillary muscles. The diastolic anterior wall thickness, diastolic posterior wall thickness, LV end-systolic dimension (LVESD), LV end-diastolic dimension (LVEDD), and ejection fraction were measured by the American Society for Echocardiology "leading-edge" method from three consecutive cardiac cycles. LV fractional shortening was calculated as follows: (LVEDD,LVESD)/LVEDD × 100%.

In vivo hemodynamic studies. Acute in vivo hemodynamic studies were performed after 3 wk of treatment with GHRP or placebo. After anesthesia, a catheter was inserted into the right carotid artery and connected and dissolved in 500 l of Tris-EDTA buffer and then extracted and dissolved in 10% formic acid. The DNA was collected by centrifugation, dried, dissolved in water and used to calculate the respective RIA kit (PLA General Hospital) with a protocol similar to that for the hGH RIA. The direct reaction between sample plasma and ANG I antiserum served as control. The radioactivity (counts/min) in each tube was converted to nanograms per milliliter with reference to the ANG I standard curve. PRA was expressed as follows: PRA (ng·ml⁻¹·h⁻¹) = (ANG I concentration in test tube − ANG I concentration in control tube)/incubation time (h). All assays were performed in duplicate.

Plasma catecholamine (CA) was measured fluorometrically by the trihydroxyindole (THI) method with minor modification. Briefly, blood was drawn from the femoral artery, anticoagulated with heparin (12,500 U/ml), and then centrifuged to obtain plasma. Perchloric acid (0.6 mol/l) was added to 1 ml of plasma to reach a final concentration of 5%, and the sample was centrifuged at 4,500 g for 15 min. The supernatant (0.1 ml) was diluted 10-fold with 3 M acetate buffer to obtain an appropriate norepinephrine (NE) concentration. The plasma CA concentration was measured by the THI method. The fluorescence intensity of the samples was determined by a luminescence spectrometer (model LS55, Perkin-Elmer) and normalized to nanograms per milliliter. Because THI is insensitive to dopamine, this method mainly measured plasma epinephrine and NE.

Agarose gel electrophoresis of DNA fragmentation. DNA laddering was examined by agarose gel electrophoresis, as described by Prigent et al. (38). Briefly, the freshly isolated or frozen myocardium (200–500 mg) was minced and homogenized in an equal volume of homogenization buffer at 0°C. A 100-μl aliquot of the homogenate was mixed with 1.25 ml of lysis buffer and centrifuged, and the supernatant was collected. The DNA in the supernatant was precipitated and dissolved in 500 μl of Tris-EDTA buffer and then extracted by phenol-chloroform saturated with Tris-EDTA buffer. The DNA solution was precipitated again in 60% ethanol and 0.5 mol/l NaCl at −20°C. The DNA was collected by centrifugation, dried, dissolved in Tris-EDTA buffer, treated with RNase (100 μg/ml) for 30 min at 37°C, and subjected immediately to electrophoresis on agarose gel to differentiate DNA fragments.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling. LV tissue was frozen with liquid nitrogen and stored at −80°C until required. Frozen tissue sections (4 μm thick) were cut with a cryostat. Cardiomyocytes with DNA nick end labeling, as described by Roche (Philadelphia, PA), according to the manufacturer’s instructions. Briefly, the tissue sections were fixed in 1% acetone for 10 min. After they were washed with PBS, the sections were incubated with blocking solution (3% H₂O₂ in methanol) for 10 min at room temperature and then in permeable solution (0.1% Triton X-100 and 0.1% sodium citrate) for 2 min. The sections were rinsed with PBS and incubated with the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) reaction mixture for 1 h at 37°C in a humidified chamber. As a positive control, fixed and permeated sections were treated with DNase I (1 mg/ml; Sigma) for 10 min to introduce nicks...
in the genomic DNA. After converter peroxidase (POD) was added, the sections were incubated for 30 min at 37°C in a humidified chamber. Then the 3,3-diaminobenzidine substrate was added for visualization of nuclei with DNA nick end labeling. The sections were counterstained with methyl green to show normal nuclei. The percentage of myocytes with DNA nick end labeling was analyzed by counting the cells exhibiting brown nuclei at \( \times \)1000 magnification in 10 randomly chosen fields (1 mm\(^2\)) in triplicate plates.

**RT-PCR for determining mRNA levels of GHSR, bax, and bcl-2.**

Total RNA was extracted with Trizol reagent. RNA (1 \( \mu \)g) was reverse transcribed using random primers and Superscript RT. The single-stranded cDNA was amplified by PCR with Taq DNA polymerase. The primer sequences were as follows: \( 5'\)-GAGATCGCTCAGATCAGCCAGTAC-3' (sense) and \( 5'\)-TAATCCCCATCTTCTTCAG-3' (antisense) for GHSR (27), \( 5'\)-GCAGAGGATGATTGCTGATG-3' (sense) and \( 5'\)-CTCACGCCCATTCTTCTTCAG-3' (antisense) for bax (41), and \( 5'\)-TCACGCCCATTCTTCTTCAG-3' (sense) and \( 5'\)-GAAGAGTTCCTCCACCAC-3' (antisense) for bcl-2 (33). GAPDH cDNA was amplified as internal control.

**Western blotting.**

Total protein extracts were prepared by homogenization of myocardial biopsies in lysis buffer. Protein (60 \( \mu \)g/sample) electrophoresis was performed with an SDS-polyacrylamide gel; then the sample was transferred to a polyvinylidine difluoride membrane. Primary antibodies (rabbit caspase-3 polyclonal IgG, and rabbit p38 MAPK polyclonal IgG, dilution 1:500; Santa Cruz Technology) were added to the membrane, which was allowed to react with the membrane. The blots were then washed and incubated with horseradish peroxidase-conjugated anti-rabbit antibodies. The bands were visualized using an enhanced chemiluminescence detection system.

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**Table 1. Improvement of hemodynamics by GHRP in CHF rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>LVESP, mmHg</th>
<th>LVEDP, mmHg</th>
<th>LV dP/dt(_{max}), mmHg/s</th>
<th>LV dP/dt(_{min}), mmHg/s</th>
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<tbody>
<tr>
<td>Sham-saline</td>
<td>393 ± 2</td>
<td>122 ± 4</td>
<td>130 ± 8</td>
<td>113 ± 2</td>
<td>6 ± 1</td>
<td>7,354 ± 66</td>
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<tr>
<td>CHF-saline</td>
<td>395 ± 5</td>
<td>179 ± 5*</td>
<td>109 ± 10*</td>
<td>113 ± 8*</td>
<td>11 ± 2*</td>
<td>5,289 ± 110*</td>
</tr>
<tr>
<td>CHF-GHRP-1</td>
<td>389 ± 7</td>
<td>164 ± 3*</td>
<td>113 ± 8*</td>
<td>11 ± 2*</td>
<td>6,067 ± 121*</td>
<td>-6,204 ± 161*</td>
</tr>
<tr>
<td>CHF-GHRP-2</td>
<td>401 ± 12</td>
<td>166 ± 5*</td>
<td>115 ± 11*</td>
<td>10 ± 3*</td>
<td>6,083 ± 99*†</td>
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</tr>
<tr>
<td>CHF-GHRP-6</td>
<td>394 ± 4</td>
<td>165 ± 6*</td>
<td>114 ± 9*</td>
<td>10 ± 2*</td>
<td>6,105 ± 130*†</td>
<td>-6,200 ± 176*†</td>
</tr>
<tr>
<td>CHF-hexarelin</td>
<td>388 ± 8</td>
<td>166 ± 6*</td>
<td>114 ± 8*</td>
<td>11 ± 1*</td>
<td>6,098 ± 145*†</td>
<td>-6,189 ± 128*†</td>
</tr>
</tbody>
</table>

Values are means ± SD. GHRP, growth hormone-releasing peptide(s); CHF, chronic heart failure; HR, heart rate; MAP, mean arterial pressure; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; dP/dt\(_{max}\), maximum rate of pressure development; dP/dt\(_{min}\), maximal dilatory velocity. *P < 0.05 vs. sham-placebo. †P < 0.05 vs. CHF-placebo.
overnight at 4°C and then incubated with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology; dilution 1:2,500) for 1 h. The immunoreactive bands were visualized using Western blot luminal reagents and scanned with Image Analysis software (Alpha Innotech). The blot was replicated on three to four rats in each group for each protein.

**Hypophysectomy, acute global myocardial ischemia, and creatine kinase measurement.** Hypophysectomy (Hys) was performed on an additional 14 normal adult Sprague-Dawley rats. Under pentobarbital anesthesia, a medial incision was made in the neck to expose the base of the skull through the lateral sternohyoid muscle. A small hole was made with a cranial drill at the hormion to reach the pituitary fossa. The pituitary was sucked out, and the incision was closed. Care (i.e., room temperature control) and feeding (glucose/salt) were closely monitored. The 12 rats that survived the surgery were divided into 2 groups: Hys-GHRP (n = 6) and Hys-placebo (n = 6). In the Hys-GHRP group, at 2 wk after Hys, hexarelin (100 μg/kg sc) was injected twice a day for 7 days; then the heart was removed and mounted on a Langendorff apparatus and perfused retrogradely with oxygenated Tyrode solution (constant perfusion at 10 ml/min). The heart was paced with a stimulus of 150 beats/min. Global “ischemia” was achieved by reducing the perfusion flow to 2 ml/min for 40 min. The myocardium was then homogenized. Ischemic injury of cardiomyocytes was evaluated by release of creatine kinase (CK) from the heart. CK release was indicated by CK activity in the outflow perfusate, which was measured spectrophotometrically using a CK measurement kit (Institute of Nanjing Jiancheng Bioengineering, Nanjing, China). CK phosphorylates creatine, yielding creatine phosphate, which dissociates into inosine and inorganic phosphate. Inorganic phosphate reacts with ammonium molybdate to produce phosphomolybdic acid, which is reduced to molybdenum blue. CK activity is indicated by the production of molybdenum blue [measured as optical density (OD)]

![Fig. 2. Effects of GHRP on circulatory levels of stress-related hormones in CHF rats. NE, noradrenaline; ET-1, endothelin-1; ANP, atrial natriuretic peptide. *P < 0.05 vs. sham-saline. †P < 0.05 vs. CHF-saline.](image)

### Table 2. Effect of chronic GHRP treatment on cardiac cachexia

<table>
<thead>
<tr>
<th>Group</th>
<th>BW, g</th>
<th>TL, mm</th>
<th>LVW/BW, mg/kg</th>
<th>LVW/TL, mg/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-saline</td>
<td>251 ± 6</td>
<td>353 ± 19</td>
<td>49.8 ± 1.4</td>
<td>0.30 ± 0.01</td>
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<tr>
<td>CHF-saline</td>
<td>251 ± 6</td>
<td>333 ± 9*</td>
<td>355 ± 11*</td>
<td>49.6 ± 1.8</td>
</tr>
<tr>
<td>CHF-GHRP-1</td>
<td>248 ± 5</td>
<td>333 ± 12*</td>
<td>365 ± 16†</td>
<td>51.4 ± 0.7†</td>
</tr>
<tr>
<td>CHF-GHRP-2</td>
<td>251 ± 6</td>
<td>334 ± 11*</td>
<td>367 ± 6†</td>
<td>51.9 ± 1.2†</td>
</tr>
<tr>
<td>CHF-GHRP-6</td>
<td>251 ± 5</td>
<td>333 ± 11*</td>
<td>367 ± 13†</td>
<td>51.5 ± 1.0†</td>
</tr>
<tr>
<td>CHF-hexarelin</td>
<td>250 ± 6</td>
<td>334 ± 9*</td>
<td>367 ± 8†</td>
<td>51.4 ± 0.8†</td>
</tr>
</tbody>
</table>

Values are means ± SD. BW, body weight; TL, tibial length; LVW, LV weight. *P < 0.05 vs. sham-saline. †P < 0.05 vs. CHF-saline.
and was calculated as follows: \( \text{CK (U/ml)} = \frac{([\text{sample OD} - \text{blank OD}] - [\text{standard OD} - \text{standard blank OD}])}{\text{standard CK activity}} \). In the Hys-placebo group, the procedure was the same as that described for the Hys-GHRP group, except saline, instead of hexarelin, was injected.

Statistical analysis. Values are means ± SD. Parameters among different groups were compared by one-way ANOVA followed by the Newman-Keuls test. Changes in parameters during treatment were analyzed with two-way ANOVA for repeated measures followed by the Newman-Keuls test. \( P < 0.05 \) was considered significant.

RESULTS

Improvement of LV geometry remodeling and dysfunction by GHRP in CHF. In the CHF-saline group, LVESD and LVEDD increased compared with the sham surgery group, suggesting heart enlargement and impairment of systolic and diastolic function. GHRP treatment decreased LVESD and LVEDD in CHF rats (Fig. 1). Diastolic posterior wall thickness significantly decreased in the CHF-saline group and tended to increase in the CHF-GHRP groups but did not reach statistical significance. No significant differences in diastolic anterior wall thickness were observed among the six groups (Fig. 1). Ejection fraction and fractional shortening significantly decreased in the CHF-saline group compared with the sham surgery group and significantly increased in the CHF-GHRP groups compared with the CHF-saline group (Fig. 1). There was no significant difference in heart rate among the six groups (Table 1).

Improvement of hemodynamics by GHRP in CHF. Mean arterial pressure increased significantly in the CHF rats but decreased in the CHF-GHRP groups (Table 1). LVESP, LV dP/dt max, and LV dP/dt min decreased and LVEDP increased in the CHF-saline group compared with the sham surgery group. GHRP treatment significantly reduced the declines of LVESP, LV dP/dt max, and LV dP/dt min and the increase of LVEDP (Table 1).

![Fig. 3. Suppression of GHRP in cardiomyocyte apoptosis in rats with CHF. A: DNA fragmentation (laddering) shown by agarose gel electrophoresis. B: Western blot showing decrease in 17-kDa subunit of caspase-3 in GHRP-treated CHF rats. C and D: RT-PCR showing increase in bcl-2 and decrease in bax mRNA levels by GHRP in CHF rats. E: Western blot showing inhibition of p38 MAPK activation in GHRP-treated CHF rats.](http://ajpheart.physiology.org/Downloadedfrom/10.220.33.2)
Attenuation of development of cardiac cachexia by GHRP.
At 9 wk after surgery, body weight increased in all animals. Body weight gain was highest in the sham surgery group and lowest in the CHF-saline group. Mean body weight in animals was significantly higher in the four CHF-GHRP groups than in the CHF-saline group but lower than in the sham surgery group (Table 2).

LV weight-to-body weight ratio was higher in the CHF-saline group than in the sham surgery group (Table 2). GHRL did not affect LV weight-to-body weight ratio (Table 2). There was no difference in tibial length (TL) between the CHF-saline group and the sham surgery group. However, TL significantly increased in the CHF-GHRP groups compared with the CHF-saline group (Table 2). LV weight-to-TL ratio showed an insignificant decrease in the GHRP groups compared with the CHF-saline group (Table 2).

Effects of GHRP on stress-related hormones. Circulatory CA, renin, ANG II, aldosterone, ANP, and ET-1 increased significantly in the CHF-saline group compared with the sham-saline group. All these hormones decreased significantly in CHF-GHRP groups compared with the CHF-saline group (Fig. 2).

Suppression of cardiomyocyte apoptosis by GHRP in CHF.
Gel electrophoresis of genomic DNA showed significantly less DNA degradation in the four CHF-GHRP groups than in the CHF-saline group. In addition, the pattern of DNA laddering suggested apoptotic and nonspecific, necrotic-type DNA degradation in cardiomyocytes of CHF rats (Fig. 3A).

In the CHF-saline group, bcl-2 mRNA decreased and bax mRNA increased (Fig. 3, C and D), whereas the changes were partially reversed by GHRP (Fig. 3, C and D).

The 32-kDa procaspase-3 was found in normal rat heart (Fig. 3B). When activated, the 32-kDa procaspase-3 cleaved into 17- and 12-kDa active subunits. The presence of the 17-kDa subunit indicated the activation of caspase-3. The cleavage of procaspase-3 increased in the CHF-saline group but significantly decreased in the CHF-GHRP groups (Fig. 3B).

The protein expression of p38 MAPK significantly increased in the CHF-saline group compared with the sham-saline group and significantly decreased in the CHF-GHRP groups compared with the CHF-saline group (Fig. 3E).

In Western blots of p38 MAPK and caspase-3 in three to four animals of each group, the differences were similar to those in Fig. 3.
The numbers of TUNEL-positive nuclei of cardiomyocytes significantly increased in the CHF-saline group compared with the sham surgery group but were significantly decreased in the four CHF-GHRP groups compared with the CHF-saline group (Fig. 4).

**Effects of GHRP on somatotropic function and GHSR mRNA expression.** Serum GH and IGF-I levels were significantly higher in the CHF-GHRP groups than in the CHF-saline or sham surgery group (Fig. 5). There was no significant difference in GH or IGF-I level between the CHF-saline and sham surgery groups (Fig. 5).

GHSR mRNA levels in the heart significantly increased in the four CHF-GHRP groups compared with the CHF-saline and sham groups (Fig. 5). No difference was found in GHSR mRNA levels between the sham and CHF-saline groups (Fig. 5).

**Effect of hexarelin on cardiac CK release in Hys rats subjected to acute global myocardial ischemia.** Measured serum GH was 2.21 ± 0.09 ng/ml in rats before Hys but was undetectable 3 wk after Hys, indicating success of pituitary removal. Body weight of Hys rats decreased 3 wk after surgery (from 203 ± 11 to 176 ± 11 g, P = 0.00527), possibly due to GH withdrawal. During acute global cardiac ischemia in vitro, CK release increased nearly fourfold in Hys-saline rats: from 4.37 ± 0.68 U/ml before to 12.41 ± 0.88 U/ml 40 min after onset of ischemia (P = 0.00326, n = 6). However, CK release increased by only about twofold in the Hys-GHRP group (from 3.46 ± 0.87 U/ml before to 7.62 ± 1.69 U/ml 40 min after onset of ischemia, P = 0.0248, n = 6), suggesting that hexarelin has a protective role in acute myocardial ischemia in the absence of GH.

**DISCUSSION**

In the present study, CHF was induced by suprarenal abdominal aortic banding. Hypertension was observed in this model (10) mainly because of pressure overload and stress-induced neurohormonal activation. We found that chronic GHRP treatment of pressure-overloaded CHF rats improved LV systolic and diastolic dysfunctions and LV structural remodeling, 2) attenuated cardiac cachexia, 3) suppressed stress-related hormonal activations and improved hemodynamics, 4) alleviated cardiomyocyte apoptosis, and 5) increased serum GH and IGF-I levels and myocardial GHSR expression. We also found that hexarelin reduced myocardial injury in Hys rats subjected to acute myocardial ischemia in vitro. These findings support the potential beneficial effects of GHRP in cachexic CHF.

**Improvement of LV remodeling and dysfunction by GHRP in CHF.** We found that long-term GHRP treatment improved cardiac geometry remodeling and dysfunction. We postulate that the cardioprotective effects of GHRP in vivo are due to its direct and GH/IGF-I-dependent components. The direct cardioprotective effects of GHRP include the cardiotropic and Ca$^{2+}$-mobilizing effects and the suppression of cardiomyocyte apoptosis, as we showed previously (36, 48). Studies by other investigators also suggest that the cardioprotective effect of GHRP is independent of GH (6, 11, 35, 39). A Hys rat model similar to that used in the present study has also been used to demonstrate the protective effects of ghrelin and hexarelin on acute myocardial ischemia-reperfusion without involvement of GH (6). However, it is well known that GHRP stimulates pituitary GH release in vivo (39) and that GH has a beneficial cardiac effect in CHF (2, 3); therefore, a GH-dependent component of the cardioprotective effect cannot be excluded. The present Hys study suggests only that hexarelin shows GH-independent cardioprotective action on acute myocardial ischemia, not on CHF. It appears that the direct action is more important than the GH-dependent action, but strong evidence is needed to clarify this point.

**Suppression of stress-related neurohormonal activations by GHRP in CHF.** In the present study, we showed for the first time that chronic treatment with GHRP attenuated stress-related hormonal activations in CHF rats. The activations of sympathoadrenal and renin-angiotensin systems are important

![Graphs and figures](http://ajpheart.physiology.org/)

*Fig. 5. Effects of GHRP on serum GH and insulin-like growth factor I (IGF-I) levels and cardiac GH secretagogue receptor (GHSR) mRNA expression in rats with CHF. *P < 0.05 vs. sham-saline. #P < 0.05 vs. CHF-saline.*
factors in the development of heart failure (7, 31, 36). NE and ANG II increase peripheral arterial resistance and stimulate myocardial hypertrophy and apoptosis, thereby worsening cardiac performance (16, 18). NE has been found to desensitize the myocardial β-adrenoceptor, thereby impairing the cardiac response to CA (22). Aldosterone retains water and, thereby, increases cardiac preload (15, 24, 26). ET-1 increases arterial pressure and stimulates myocardial hypertrophy and apoptosis in CHF (25). GHRP significantly decreased the circulatory levels of CA, renin, ANG II, aldosterone, and ET-1 in this study. These effects may contribute to improvement of CHF by GHRP. Here we showed that GHRP also decreased plasma ANP, which increases in CHF and is considered an indicator of the severity of CHF (49). The reduction of ANP may also contribute to improvement of cardiac function by GHRP. However, we cannot determine from this study whether decreases in the circulatory levels of these stress hormones represent a direct effect of GHRP, an indirect stress hormone response to stimulation of the anterior pituitary hormones by GHRP, or a consequence of improvement of cardiac dysfunction by GHRP. GHRP-6 could decrease circulatory stress hormones in rats subjected to water immersion stress (unpublished data), suggesting that suppression of stress hormones by GHRP is not necessarily related to cardiac function. The mechanism(s) underlying GHRP suppression of stress hormones needs further investigation.

Improvement of cardiac cachexia by GHRP. Cardiac cachexia, a catabolic state characterized by weight loss and muscle wasting, occurs frequently in patients with CHF and is a strong independent risk factor for mortality (2, 3). CHF rats here showed an impaired body weight gain, suggesting the presence of cardiac cachexia. GHRP increased body weight and TL in CHF rats, suggesting an attenuation of cardiac cachexia. This effect may be due to 1) the orexigenic property of GHRP (23, 43), 2) improvements of cardiac function, 3) the increase in GHSR expression in cardiomyocytes (36), and 4) the increase in GH levels by GHRP. However, the relative importance of each of these pathways needs to be determined, because other studies show that GH secretion and body growth become refractory to repeated GHRP administration. Other mechanisms underlying the cachexia-improving effect of GHRP cannot be excluded.

Suppression of cardiomyocyte apoptosis by GHRP. We have reported that hexarelin attenuates ANG II-induced cardiomyocyte apoptosis in vitro (36). GHRP suppressed cardiomyocyte apoptosis in CHF rats. The bcl-2-to-bax ratio increased after GHRP treatment. The bcl-2 gene is the first of a family with pro- and antiapoptotic activity to be identified (14). Expression of the bcl-2 gene in cardiomyocytes decreases in pathological states such as increased stretching of cardiomyocytes in vitro (21) and myocardial ischemia in vivo (32). Because an increase in bax expression has been reported in CHF (21, 32), the bcl-2-to-bax ratio is an important marker of cardiomyocyte survival probability. The number of TUNEL-positive cardiomyocytes, DNA fragmentation, caspase-3 activity, and p38 MAPK levels were reduced by GHRP treatment in this study. Caspase-3 is a key downstream effector of apoptosis (20). GHRP treatment improved the imbalance of bax and bcl-2 and decreased the activation of caspase-3. These findings are consistent with the results obtained by morphological and DNA fragmentation analysis.

The MAPK family, including ERK1/2, JNK1/2, and p38 MAPK, plays an important role in cell survival (9, 47). Activation of p38 MAPK can induce apoptosis (9, 47), and the p38 MAPK pathway is activated in cardiac tissue of murine hearts subjected to chronic transverse aortic constriction (45). The levels of p38 MAPK decreased in GHRP-treated CHF rats in this experiment. This effect may be augmented by the increase in GHSR synthesis by GHRP (36).

Conclusion. Chronic subcutaneous administration of GHRP improved LV systolic and diastolic function, structural remodeling, and hemodynamics, attenuated development of cardiac cachexia, and suppressed stress-related neurohormonal activations and cardiomyocyte apoptotic death in rats with pressure-overload CHF. These beneficial actions may be mediated by direct and GH/IGF-I-dependent signaling pathways.

Clinical implications. GHRP possesses several promising characteristics in the treatment of CHF, including improvements of LV contractile and dilating dysfunctions, remodeling, and cardiac cachexia and the attenuation of stress-related hormonal activation and cardiomyocyte apoptosis. Because they are small peptides (i.e., are stable and can be administered orally) and have minimal side effects, GHRP potentially have a therapeutic role in CHF management.

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