Upregulation of cardiovascular ghrelin receptor occurs in the hyperdynamic phase of sepsis

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Wu, Rongqian, Mian Zhou, Xiaoxuan Cui, H. Hank Simms, and Ping Wang. Upregulation of cardiovascular ghrelin receptor occurs in the hyperdynamic phase of sepsis. Am J Physiol Heart Circ Physiol 287: H1296–H1302, 2004. First published May 20, 2004; 10.1152/ajpheart.00852.2003.—Ghrelin, a newly identified endogenous ligand for growth hormone secretagogue receptor 1a (GHSR-1a, i.e., ghrelin receptor), was recently demonstrated to be a potent vasoactive peptide. Although sepsis is characterized by an early, hyperdynamic phase, it remains unknown whether ghrelin or GHSR-1a plays a role in the cardiovascular response to sepsis. To determine this, polymicrobial sepsis was induced by cecal ligation and puncture in male adult rats. At 5 h (i.e., early sepsis) or 20 h (i.e., late sepsis) after cecal ligation and puncture, blood and tissue samples were collected. Ghrelin levels and ghrelin and GHSR-1a mRNA expression were assessed by RIA and RT-PCR, respectively. In addition, GHSR-1a protein levels in aorta, heart, and small intestine were determined by Western blotting. The vascular response to ghrelin was determined by using an isolated gut preparation. A primary rat aortic smooth muscle cell culture was used to determine the effects of LPS on GHSR-1a expression. The results indicate that although ghrelin levels decreased at early and late sepsis, its receptor was markedly elevated in early sepsis. Moreover, ghrelin-induced relaxation in resistance blood vessels of the isolated small intestine increased significantly during early sepsis but was not altered in late sepsis. Furthermore, GHSR-1a expression in smooth muscle cells was significantly increased at mRNA and protein levels with stimulation by LPS at 10 ng/ml. These results demonstrate that GHSR-1a expression is upregulated and vascular sensitivity to ghrelin stimulation is increased in the hyperdynamic phase of sepsis.

vamositive peptide; growth hormone secretagogue receptor; polymicrobial sepsis; aorta; gut

SEPSIS AND SEPTIC SHOCK are the most common causes of death in intensive care units (1, 6, 10). Approximately 450,000 cases of sepsis occur annually in the United States; 50% of them develop septic shock, causing >100,000 deaths (1). The cardiovascular response to polymicrobial sepsis is characterized by an early, hyperdynamic phase, followed by a late, hypodynamic phase (35, 41). One difficulty in the management of septic patients is prevention of the transition from the early, hyperdynamic to the late, hypodynamic phase. Elucidation of the mediators could ultimately allow prevention of the hypodynamic phase.

Ghrelin, a novel octanoylated peptide, was recently identified in the rat stomach as an endogenous ligand for the growth hormone secretagogue receptor (GHSR) by Kojima et al. (19). Ghrelin has a strong stimulatory effect on growth hormone secretion in the rat and in the human (3, 11, 22). GHSR-1a, a seven-transmembrane domain G protein-coupled receptor, transduces the growth hormone-releasing effect of the synthetic growth hormone secretagogues as well as ghrelin (24). A nonspliced, nonfunctional receptor mRNA variant that was also identified (i.e., GHSR-1b) contains all the exon 1 and an additional 74 bases from the intron (13); however, it lacks transmembrane domains 6 and 7 and is apparently devoid of high-affinity ligand binding and signal transduction capacity (12). The presence of appreciable amounts of growth hormone secretagogue binding sites and mRNA in the cardiovascular system suggests that ghrelin may have direct cardiovascular effects through growth hormone-independent mechanisms (4, 16). Wiley and Davenport (39) showed that, in addition to its effects on growth hormone release and energy homeostasis, ghrelin is a potent vasodilatory peptide. The cardiovascular response to ghrelin appears to be similar to the hyperdynamic response observed in the early stage of sepsis. In healthy human volunteers, intravenous administration of exogenous ghrelin significantly reduced peripheral vascular resistance and increased cardiac output without a significant change in heart rate (21). Despite these vasodilatory properties of ghrelin under normal physiological conditions, it remains unknown whether ghrelin or its receptor plays a role in the cardiovascular response to sepsis. This study was conducted to test the hypothesis that cardiovascular responsiveness to ghrelin is enhanced in the hyperdynamic phase of sepsis.

MATERIALS AND METHODS

Animal model of sepsis. Male Sprague-Dawley rats (275–325 g) were housed in a temperature-controlled room on a 12:12-h light-dark cycle and fed a standard Purina rat chow. Before induction of sepsis, the rats were fasted overnight but allowed water ad libitum. The rats were anesthetized with isoflurane inhalation, and the ventral neck, abdomen, and groin were shaved and washed with 10% povidone iodine. A 2-cm midline abdominal incision was performed. The cecum was exposed, ligated just distal to the ileocecal valve to avoid intestinal obstruction, punctured twice with an 18-gauge needle, squeezed slightly to allow a small amount of fecal matter to flow from the holes, and returned to the abdominal cavity; the abdominal incision was then closed in layers. Sham-operated (control) animals underwent the same procedure, except the cecum was neither ligated nor punctured. The animals were resuscitated with normal saline (3 ml/100 g body wt sc) immediately after surgery. Animals were then anesthetized at various intervals after cecal ligation and puncture (CLP) or sham operation for collection of blood and tissue samples. All experiments were performed in accordance with the National
Institutes of Health guidelines for the use of experimental animals. This project was approved by the Institutional Animal Care and Use Committee of the North Shore-Long Island Jewish Research Institute.

**Measurement of plasma ghrelin levels.** At 5 or 20 h after CLP, blood and gastric tissue were collected. Ghrelin levels were measured using an RIA kit (Phoenix Pharmaceuticals, Belmont, CA). A portion of the stomach was homogenized at 4°C with 50 mM Tris·HCl and 5 mM MgCl₂ (pH 7.4) containing aprotinin (500 kallikrein-inactivating units/ml). The homogenate was centrifuged at 2,500 × g for 15 min at 4°C. The collected supernatant was used for ghrelin extraction by a C₁₈ Sep-Pak column. The eluate was precipitated with 99% ethanol or stored at -20°C. Protein was then dissolved in RIA buffer [50 mM sodium phosphate buffer (pH 7.4), 0.5% BSA, 0.5% Triton X-100, 80 mM NaCl, 25 mM EDTA, Na₂, and 0.05% NaN₃]. Plasma samples were used for ghrelin measurement as recommended by the manufacturer. Each RIA incubation mixture was composed of 100 μl of standard ghrelin or an unknown sample and 200 μl of antisera diluted with RIA buffer containing 0.5% normal rabbit serum. After 16 h of incubation, 100 μl of 125I-labeled tracer (15,000 cpm) were added. After an additional 24 h of incubation, 100 μl of anti-rabbit IgG goat serum were added. Free and bound tracers were separated after 90 min of incubation by centrifugation at 3,000 rpm for 30 min. After aspiration of the supernatant, radioactivity in the pellet was counted with a gamma counter. Ghrelin levels were assessed using the manufacturer's instructions.

**RNA extraction and determination of ghrelin and GHSR-1α genes.** Total RNA was extracted from various tissues (i.e., stomach, aorta, heart, and small intestine) by TriReagent (Molecular Research Center, Cincinnati, OH). Tissues (100–150 mg) were homogenized in 1.5 ml of TriReagent, and the homogenate was separated into aqueous and organic phases by chloroform addition and centrifugation. RNA was precipitated from the aqueous phase by addition of isopropanol and washed with ethanol. The pellet was dissolved in 0.1% diethyl pyrocarbonate-treated, deionized distilled water. RNA concentration and purity were determined by measuring the absorbance at 260 and 280 nm. Five micrograms of RNA from each tissue were reverse transcribed in a 20-μl reaction volume containing 50 mM KCl, 10 mM Tris·HCl, 5 mM MgCl₂, 1 mM dNTP, 20 units of RNase inhibitor, 2.5 mM oligo d(T)₁₆ primer, and 50 units of reverse transcriptase. The reverse transcription reaction solution was incubated at 42°C for 1 h and then heated at 95°C for 5 min. One microliter of cDNA was amplified with 3’ and 5’ primers (0.15 μM each) specific for rat ghrelin (19) (347 bp, 5’-CAC CAG AAA-3’, 5’-TTG AGC CCA GAG-3’), rat GHSR-1α (21) (314 bp, 5’-GAG ATC GTG CAG ATC AGC CAG CAC TAC-3’, 5’-TAA TCC CCC AAG TAC GGT GTT GC C-3’), and rat G3PDH (30) (983 bp, 5’-TGA AGG TCG GTG TCA ACG GAT TGG GC-3’ and 5’-CAT GAA GCC GAT CAT GAG TGC CAC CAC-3’) in 25 μl of PCR mixture containing 50 mM KCl, 10 mM Tris·HCl, 2 mM MgCl₂, 0.2 mM dNTP, and 0.7 units of AmpliTaq DNA polymerase. PCR was carried out in a thermal cycler (Bio-Rad, Hercules, CA). After RT-PCR, 5 μl of the reaction mixture were electrophoresed in 1.2% Tris-boric acid-EDTA agarose gel containing ethidium bromide (0.22 μg/μl). The gel was then developed, and band intensities were normalized by G3PDH using the Bio-Rad Image System.

**Western blotting analysis of GHSR-1α.** Tissue samples (0.1 g) or cells (10⁶) were lysed and homogenized in 1 ml of lysis buffer (10 mM Tris-buffered saline, 1 mM EDTA, 1 mM EGTA, 2 mM sodium orthovanadate, 0.2 mM PMSF, 2 μg/ml leupeptin, 2 μg/ml aprotinin, and 1% Triton X-100) for 30 min on ice and cleared by centrifugation at 14,000 rpm for 15 min at 4°C. Fifty micrometers of protein were fractionated on 4–12% Bis-Tris gel and transferred to 0.2-μm nitrocellulose membrane. Nitrocellulose blots were blocked by incubation in Tris·HCl (pH 7.5) 150 mM NaCl, 2.5 mM MgCl₂, 0.25% Tween 20 containing 5% milk for 1 h. Blots were incubated with rabbit anti-rat GHSR-1α IgG (1:5,000; Alpha Diagnostic International, San Antonio, TX) overnight at 4°C. The blots were then washed five times in TBST for 10 min. Blots were incubated with horseradish peroxidase-labeled goat anti-rabbit IgG for 1 h at room temperature and then washed five times in TBST for 10 min. A chemiluminescent peroxidase substrate (ECL, Amersham Biosciences) was applied according to the manufacturer’s instructions, and the membranes were exposed briefly to X-ray film. The band densities were normalized by β-actin with the use of the Bio-Rad Image System.

**Ghrelin immunohistochemistry.** Portions of the aorta, heart, and small intestine (jejunum) were harvested immediately after the animals were euthanized by an overdose of pentobarbital sodium at 5 and 14 h after CLP or sham operation in additional groups of animals (3 rats/group). The tissue was fixed overnight in 10% buffered formalin phosphate. A Vibrotome was used to cut the tissue into 30-μm sections. Preembedding immunohistochemistry was performed as previously described by us (42). Briefly, the paraffin sections were dewaxed and rehydrated and then subjected to a microwave antigen retrieval procedure. Slides were soaked in 20% citric acid buffer, pH 6.0 (Vector Laboratories, Burlingame, CA), and heated in the microwave oven and maintained at 95°C for 15 min. The slides were cooled at room temperature for 5 min and then rinsed with Tris-buffered saline (pH 7.5). Endogenous peroxidase was blocked by 2% H₂O₂ in 60% methanol for 20 min. Normal goat serum (2%) was used to block the nonspecific binding sites. The sections were then incubated in a 1:400 dilution of anti-rat ghrelin polyclonal antibodies (Phoenix Pharmaceuticals, Belmont, CA) for 1.5 h at room temperature. After they were washed with TBS, the sections were reacted in 1:200 biotinylated anti-rabbit IgG (Vector Laboratories) for 0.5 h. Vectastain ABC reagent and a diaminobenzidine kit (Vector Laboratories) were used to reveal the immunohistochemical reaction. For the negative control, the primary antibody was substituted by nonspecific rabbit IgG.

**Isolation of the small intestine and determination of ghrelin-induced vascular relaxation.** An isolated intestinal preparation was used as previously described (20, 33). The abdomen was opened, and a section (~12 cm) of the jejunum and ileum was ligated on either side with a 4-0 silk suture. The duodenum was ligated adjacent to the pyloric sphincter, and the sigmoid colon was ligated adjacent to the rectum. The intestinal vessels on either side of the small intestine were then ligated, with only the vessels sustaining the isolated small intestine left unobstructed. The large intestine and the small intestine proximal to the isolated segment were removed. Animals were given 0.5 ml of heparin sodium solution (500 U) intravenously. The superior mesenteric arterial cannula was connected with PE-50 tubing and perfused with aerated Krebs-Ringer bicarbonate buffer (in mM: 118.3 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KHPO₄, 25.0 NaHCO₃, 0.026 Ca-EDTA, and 11.1 glucose), which was aerated with 95% O₂-5% CO₂ at 37°C. A constant perfusion rate (2.5 ml/min) was maintained by a pump. Vascular resistance was measured with a blood pressure analyzer (Micro-Med, Louisville, KY) coupled to the mesenteric arterial catheter. After 15 min of equilibration and pressure stabilization at the 2.5 ml/min perfusion rate, blood vessels in the isolated gut were constricted with 2 × 10⁻³ M norepinephrine (NE) added to the buffer reservoir of the organ chamber. When pressure was stabilized, synthetic rat ghrelin was applied, and the vascular response was measured by determination of the change in perfusion pressure to the isolated small intestine. The intestinal preparation remained viable throughout the experiment, inasmuch as there was no significant change in NE-induced increase in perfusion pressure. To determine the optimal dose of ghrelin for gut perfusion, dose-dependent vascular responsiveness to ghrelin (5 × 10⁻⁹, 5 × 10⁻⁸, 5 × 10⁻⁷, and 5 × 10⁻⁶ M) was determined in an additional group of sham animals (n = 3). Ghrelin at 5 × 10⁻⁸ M was chosen to assess the alterations in intestinal vascular responsiveness to ghrelin stimulation after CLP.

**Rat aortic smooth muscle cell isolation and culture.** Rat aortic smooth muscle cells (SMCs) were prepared as described by Rasch (25). Male Sprague-Dawley rats (175–185 g) were euthanized with pentobarbital sodium, and the thoracic aortas were harvested and denuded of endothelium and adventitia. The media were sectioned (3 mm²) and...
Table 1. Alterations in ghrelin levels 5 and 20 h after CLP or sham operation

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<tr>
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<th>5 h</th>
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<tr>
<td></td>
<td>Sham (pg/ml)</td>
<td>CLP (pg/ml)</td>
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<td></td>
<td>Stomach (pg/g)</td>
<td>Stomach (pg/g)</td>
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<tr>
<td>Plasma</td>
<td>102.0±20.7</td>
<td>28.4±3.5*</td>
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<tr>
<td>Stomach</td>
<td>82.2±12.8</td>
<td>29.2±4.8*</td>
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<tr>
<td>Tissue</td>
<td>337.0±20.0</td>
<td>251.5±20.0*</td>
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<td>314.9±20.4</td>
<td>239.0±10.5*</td>
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Values are means ± SE (n = 5/group). CLP, cecal ligation and puncture. *P < 0.05 vs. respective sham (1-way ANOVA and Tukey’s test).

ALTERATIONS IN GHR ELIN LEVELS AFTER CLP. Plasma levels of ghrelin at 5 and 20 h after sham operation were 102 ± 21 and 82 ± 13 pg/ml, respectively (Table 1). At 5 and 20 h after CLP, plasma levels of ghrelin significantly decreased by 72% and 64% (P < 0.05; Table 1). Ghrelin levels in the gastric tissue were 337 ± 20 and 315 ± 20 pg/g tissue in the 5- and 20-h sham-operated animals and decreased by 25% and 24% at 5 and 20 h after CLP, respectively (P < 0.05; Table 1). Ghrelin mRNA expression in the stomach also decreased by 45–59% after CLP (Fig. 1); however, there was no significant difference in ghrelin levels and mRNA expression between 5 and 20 h in the CLP groups (Table 1, Fig. 1).

ALTERATIONS IN GHR ELIN RECEPTOR AFTER CLP. GHSR-1a mRNA expression in the aorta increased by 33-fold (P < 0.05) at 5 h after CLP (Fig. 2); however, there was no significant difference at 20 h between the CLP and sham-operated animals. GHSR-1a protein levels in the aorta were also significantly higher after 5 h in the CLP than in the sham animals; there was no significant difference after 20 h in the CLP and sham-operated animals (P < 0.05; Fig. 2). Although the increase of GHSR-1a mRNA expression and protein levels in the heart and small intestine after 5 h of sepsis was not as dramatic as that in the aorta, these differences were statistically significant (P < 0.05; Figs. 3 and 4).

Fig. 1. Alterations in ghrelin mRNA expression in stomach 5 and 20 h after cecal ligation and puncture (CLP) or sham operation (Sham). G3PDH, glyceraldehyde 3-phosphate dehydrogenase. A representative blot is also presented. Values are means ± SE (n = 4/group). *P < 0.05 vs. sham (1-way ANOVA and Tukey’s test).

Fig. 2. Alterations in growth hormone secretagogue receptor (GHSR)-1a mRNA expression (A) and protein level (B) in aorta 5 and 20 h after CLP or sham operation. Representative blots are also presented. Values are means ± SE (n = 4–6/group). *P < 0.05 vs. sham; #P < 0.05 vs. 20 h CLP (1-way ANOVA and Tukey’s test).

Fig. 3. Alterations in GHSR-1a mRNA expression (A) and protein level (B) in heart 5 and 20 h after CLP or sham operation. Representative blots are also presented. Values are means ± SE (n = 4–6/group). *P < 0.05 vs. sham (1-way ANOVA and Tukey’s test).
Ghrelin immunohistochemistry. Strong ghrelin immunostaining was detected in aorta, heart, and small intestine in sham-operated animals (Fig. 5, A1, B1, and C1). In positive cells, the cytoplasm was uniformly stained, whereas cell nuclei remained negative. The ghrelin immunostainings decreased at 5 h (Fig. 5, A2, B2, and C2) and 20 h (Fig. 5, A3, B3, and C3) after the onset of sepsis in all three tested tissues, but the decrease does not appear to be as dramatic as its decrease in the plasma (Table 1). As a control for specificity, substitution of the primary antibody with a nonimmunized rabbit IgG resulted in negative staining (Fig. 5, A4, B4, and C4).

Alterations in intestinal vascular responsiveness to ghrelin stimulation after CLP. The resistance blood vessels of the isolated intestinal preparation were preconstricted with 2 × 10^{-6} M NE. Ghrelin (5 × 10^{-8} M) was applied, and the percentage of ghrelin-induced vascular relaxation was determined. Ghrelin at 5 × 10^{-8} M induced 22.0 ± 3.8% and 22.4 ± 6.2% relaxation in the NE-preconstricted resistance blood vessels in the isolated small intestine after 5 h and 20 h, respectively, in the sham-operated animals (Fig. 6). Ghrelin-induced vascular relaxation significantly increased to 49.1 ± 7.8% at 5 h after CLP (P < 0.05), which was even higher than the vascular relaxation induced by 5 × 10^{-6} M ghrelin in sham-operated animals (Table 2). Although there were some increases in ghrelin-induced vascular relaxation from CLP animals examined after 20 h, these increases were not significantly different (Fig. 6).
Alterations in ghrelin receptor mRNA expression in SMC after LPS stimulation. Incubation of primarily cultured SMC with 1 ng/ml LPS for 4 h resulted in a threefold increase in GHSR-1a gene expression (Fig. 7); this was not statistically significant. LPS at 10 and 100 ng/ml significantly upregulated GHSR-1a mRNA expressions in the SMC by 6-fold ($P < 0.05$) and 5.9-fold ($P < 0.05$), respectively. Similarly, GHSR-1a protein levels in the SMC were also significantly increased by stimulation with 10–100 ng/ml LPS ($P < 0.05$; Fig. 7).

**DISCUSSION**

Ghrelin is a novel peptide recently identified as an endogenous ligand for the GHSR (19). It is produced predominantly by the stomach, with substantially lower amounts derived from other central and peripheral tissues (26, 27). Human ghrelin is a 28-amino acid peptide and has an $n$-octanoyl group at Ser$^3$, a modification essential for its activity and not previously reported in a human peptide (7, 31). Rat ghrelin differs from human ghrelin by only two amino acids (31). Ghrelin was thought to be exclusively expressed in several nuclei of the brain, particularly in hypothalamus and anterior pituitary gland, and at very low levels in pancreas (14); however, recent studies have suggested that the GHSR is expressed in many tissues. GHSR is found in pituitary, hypothalamus, stomach, heart, blood vessels, lung, pancreas, intestine, kidney, adipose tissue, and immune system (B and T cells and neutrophils) (15, 23, 28). Although a group of synthetic molecules featuring growth hormone secretagogue can bind with GHSR, ghrelin is the only identified endogenous ligand for this receptor. The wide distribution of ghrelin receptor suggests multiple paracrine, autocrine, and endocrine roles for ghrelin. Ghrelin stimulates growth hormone release and plays a role in feeding regulation, energy homeostasis, and cardiovascular regulation (31). In addition to growth hormone-releasing properties, ghrelin possesses other endocrine and nonendocrine activities affecting central and peripheral GHSR distribution. Exogenous ghrelin injections stimulate prolactin, ACTH, and cortisol secretion. They also induce hyperglycemia, decrease insulin levels, and exert orexigenic effects in rodents and humans (2, 8).

The discovery of vasodilatory properties of ghrelin and the presence of appreciable amounts of GHSR binding sites and mRNA in the cardiovascular system are important in the pathophysiology of sepsis. Our recent studies have shown that kidneys are the primary site of ghrelin clearance, which is significantly diminished in late sepsis (40). Basa et al. (5) found that plasma levels of ghrelin significantly decreased after LPS administration; however, the role of ghrelin in the progression of sepsis remains unknown.

Our previous studies demonstrated that the biphasic hemodynamic response occurs during the progression of polymicrobial sepsis (34, 35, 37, 38). The early, hyperdynamic response to sepsis (i.e., 2–10 h after CLP) is characterized by an increase in cardiac output and regional perfusion, increased oxygen delivery and consumption, and decreased systemic vascular resistance. In contrast, the late, hypodynamic response (i.e., $\geq 16$ h after CLP) is characterized by decreased cardiac output, blood flow, and oxygen delivery and increased vascular resistance. The changes in ghrelin levels after CLP did not appear to be responsible for the biphasic cardiovascular response to sepsis.

Here we measured the alterations in ghrelin levels in the plasma and stomach during sepsis. We found that plasma levels of ghrelin decreased significantly at early and late stages of sepsis, despite the decreased clearance in late sepsis. Moreover, ghrelin levels and ghrelin gene expression in gastric tissue also decreased significantly at early and late stages of sepsis. Immunohistochemistry results show that strong ghrelin immunostaining in the sham-operated animals also decreased at 5 and 20 h after the onset of sepsis in aorta, heart, and small intestine. Although the mechanism responsible for regulating

### Table 2. Dose-dependent vascular responsiveness to ghrelin in sham-operated animals

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<tr>
<th>Ghrelin</th>
<th>5 x $10^{-9}$ M</th>
<th>5 x $10^{-8}$ M</th>
<th>5 x $10^{-7}$ M</th>
<th>5 x $10^{-6}$ M</th>
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<tr>
<td>Vascular relaxation, %</td>
<td>15.1±2.8</td>
<td>24.7±4.5</td>
<td>33.4±5.0</td>
<td>36.0±5.2</td>
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Values are means ± SE ($n = 3$ / group).
ghrelin biosynthesis and release in sepsis has not been elucidated, the recently reported presence of Toll-like receptor 4 in gastric cells (17, 18) suggests that endotoxin might have some direct effects on ghrelin production. Inasmuch as endogenous ghrelin levels appear directly related to nutritional state (29), the deceased plasma levels of ghrelin may contribute to the reduction of food intake observed in sepsis.

To determine the factors responsible for the transition from the hyperdynamic response to the hypodynamic response, we assessed the alterations in ghrelin receptor and the vascular responsiveness to ghrelin stimulation during sepsis. The results showed that expression of the ghrelin receptor in aorta, heart, and small intestine was markedly upregulated only in early sepsis; ghrelin-induced relaxation in resistance blood vessels of small intestine increased significantly in the early stage of sepsis (5 h after CLP) but was not altered in the late stage of sepsis (20 h after CLP). The dose response to ghrelin showed that vascular relaxation induced by 5 × 10⁻⁶ M ghrelin in the 5-h-CLP animals (49% relaxation) was higher than that induced by 5 × 10⁻⁸ M ghrelin (i.e., 100-fold higher concentrations of ghrelin) in sham-operated animals (36% relaxation). Because vascular ghrelin sensitivity increased ≥100-fold in the early stage of sepsis, the reduced levels of plasma and tissue ghrelin can indeed induce greater vascular relaxation under such conditions. This demonstrates that ghrelin receptor expression is upregulated and vascular sensitivity to ghrelin stimulation is increased in the hyperdynamic phase of sepsis. Because ghrelin receptor levels and vascular responsiveness to ghrelin were not reduced in the late stage of sepsis, administration of ghrelin is a possible therapy to maintain cardiovascular stability and reduce mortality. Chang et al. (9) found that administration of ghrelin could partially correct the hemodynamic and metabolic disturbances in a rat septic shock model.

It can be argued that the responses of the isolated small intestine preparation to other vasodilators also increased via a nitric oxide (NO)-dependent pathway in sepsis. In this regard, we have also shown that acetylcholine-induced vascular relaxation (via NO) decreased significantly at 10–20 h after CLP (32). Moreover, endothelial NO synthase is downregulated in sepsis (43). Thus there is a decrease in NO release in sepsis. This would suggest that increased sensitivity to ghrelin observed in early sepsis is not mediated by NO but by the increased expression of ghrelin receptors. Furthermore, our previous studies have shown that vascular responsiveness to another potent vasodilatory peptide, adrenomedullin, decreased significantly after sepsis (36).

Ghrelin receptor is expressed within the cardiovascular system; however, the mechanism responsible for upregulation of ghrelin receptor in early sepsis remains unknown. Wiley and Davenport (39) showed that ghrelin could induce vascular relaxation in human endothelium-denuded internal mammary artery, which indicated that ghrelin might have direct effects on SMC. Because plasma levels of LPS increase soon after the onset of sepsis, we assessed its role in GHSR-1a expression. With an aortic SMC culture system, LPS at 10 ng/ml significantly upregulated GHSR-1a expression at mRNA and protein levels in isolated aortic SMC, indicating that LPS upregulates GHSR-1a expression. The effects of proinflammatory cytokines with and without LPS on ghrelin receptor expression need to be studied.

Although ghrelin levels decreased at early and late stages of sepsis, its receptor gene expression and protein levels were markedly elevated in early sepsis. Ghrelin-induced relaxation in resistance blood vessels of the small intestine increased significantly in the early stage of sepsis but was not altered in the late stage of sepsis. This demonstrates that cardiovascular responsiveness to ghrelin is increased in the hyperdynamic phase of sepsis. However, molecular mechanisms that regulate ghrelin receptor expression in the cardiovascular system, as well as the effects of ghrelin administration in clinical cases of sepsis, warrant further investigation.

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