Ghrelin Protects Against Experimental Sepsis; Relation to Sympathetic Excito-Toxicity and Role of Vagus

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Abstract

Background: Sepsis is a life-threatening complication of infections. Despite improvement in the management of septic patients, none of the current therapies are entirely effective. Ghrelin has been shown to possess anti-inflammatory properties together with prokinetic activity. The aim of this work was to study the effect of administration of ghrelin on outcome of sepsis induced by cecal ligation and puncture (CLP) in rats and to investigate the effect of vagotomy on ghrelin effect. Also, to test whether administration of exogenous ghrelin affects norepinephrine (NE) release, this may contribute to its anti-inflammatory and cardiac protective effects in sepsis.

Methods: Fifty male rats (200–250g) were divided into 5 groups, 10 rats each. Group 1: Normal control rats, sham operated, Group 2: (CLP), rats underwent CLP, Group 3: (CLP+GR) group, rats underwent CLP and received ghrelin at dose of 10nmol/kg at the time of operation and 60nmol/kg BW 5hrs after surgery, Group 4: Rats underwent CLP and followed after 5 hours by vagotomy operation, Group 5: Rats underwent CLP, followed after 5hrs by vagotomy operation and received ghrelin at and 5hrs after the CLP. In all rats, TNF-$\alpha$, IL-6 and NE levels were measured. Systolic blood pressure and the cardiac contractile functions were assessed by measuring the left ventricular developed pressure (LVDP) and The maximum rate of pressure rise (dp/dt).

Results: CLP induced a significant increase in serum levels of TNF-$\alpha$, IL-6 and NE level in vehicle-treated animals as compared with sham-operated animals ($p<0.05$). Administration of ghrelin significantly attenuated serum levels of TNF-$\alpha$, IL-6 and NE. Vagotomy performed 5 hours after CLP had an insignificant effect on TNF-$\alpha$, IL-6 or NE as compared to CLP group values ($p>0.05$). Vagotomy partially abolished the inhibitory effects of ghrelin on serum levels of TNF-$\alpha$, IL-6 and NE to become significantly higher than values recorded in ghrelin treated group ($p<0.05$). SBP, LVDP and dp/dt of rats in ghrelin-treated group was significantly increased compared with CLP group ($p<0.05$). Vagotomy partially blocked the beneficial effect of ghrelin on hemodynamic parameters in CLP+GR+VagX group compared to CLP+GR group.

Conclusion: Ghrelin represents a feasible therapeutic agent for sepsis and other inflammatory disorders. Ghrelin’s anti-inflammatory action may be mediated partially by its direct action on the immune cells, and partially by acting as a modulator that restores the dysregulated balance of sympathetic/parasympathetic nervous system during sepsis. Ghrelin improves cardiac functions and restores blood pressure in septic rats.

Key Words: Ghrelin – Sepsis – CLP – Vagotomy – Norepinephrine – Inflammatory mediators – Blood pressure – Cardiac function.

Introduction

SEPSIS, a life-threatening complication of infections, is characterized by a hyperactive and out-of-balance network of endogenous proinflammatory cytokines. The pathophysiologic sequelae of sepsis are caused by an overreaction of the immune system to microorganisms and their products [1]. During sepsis, bacterial toxins activate macrophages/Kupffer cells to release proinflammatory cytokines and other mediators that initiate specific immune responses [2]. A growing collection of experimental and clinical data has indicated that proinflammatory cytokines play a prominent role in sepsis-induced tissue injury [3,4]. The kinetics and magnitude of cytokine release influence the development of sepsis [3,5].

Despite improvement in the management of septic patients with systemic antibiotics, surgical intervention, aggressive fluid resuscitation, and careful monitoring, none of the current therapies are entirely effective and sepsis continues to be one of the leading causes of death in intensive care units, and a large number of septic patients die of ensuing septic shock and multiple organ failure [6,7], illustrating the need for novel therapeutic approaches.

Ghrelin, a peptide hormone that serves as the endogenous ligand of the growth hormone secretagogue receptor, is secreted mainly by P/D1 cells.
lining the fundus of the human stomach, and the epsilon cells of the pancreas that stimulate hunger [8]. Ghrelin is also secreted from the small intestine and the colon. Ghrelin receptors are expressed in the hypothalamus, pituitary, and several peripheral tissues suggesting that it could have diverse physiological functions [9]. Ghrelin regulates growth hormone secretion, and plays an important role in the regulation of appetite, energy balance and glucose homeostasis. It regulates gastrointestinal, cardiovascular, immune functions, and bone physiology [10,11,12].

Ghrelin (GHR) is an orexigenic peptide that has emerged as a potential endogenous anti-inflammatory factor. Ghrelin has been shown to possess anti-inflammatory properties together with prokinetic activity [13]. The ghrelin receptor is a G protein-coupled receptor. Ghrelin and the ghrelin receptors are expressed by lymphocytes, monocytes and dendritic cells. Activation of the ghrelin receptor results in an inhibition of proinflammatory cytokine expression and an increase in survival in various inflammatory disease models [14].

The interaction between the central nervous system and immune system under various inflammatory diseases has found considerable interest in the past several decades. Sympathetic influence on immune function was initially demonstrated by showing that epinephrine and norepinephrine (NE) inhibited histamine secretion from mast cells [15]. Along with the discovery of cytokines in the late 1970s, greater research efforts were undertaken to investigate neuro-immune interaction.

Recent studies have indicated that intraportal injection of NE, at concentrations found under septic conditions (>20nM), produced an increase of circulating levels of tumor necrosis factor (TNF)-a, interleukin (IL)-1β and IL-6, similar to that found in sepsis [16]. Moreover, NE upregulates TNF-a and IL-1β production in Kupffer cells through an α2-adrenergic pathway [17]. This appears to be, in part, responsible for the increased proinflammatory cytokines in the circulation under such conditions. Therefore, modulation of the sympathetic nervous system represents a novel strategy for sepsis treatment.

The vagus nerve is an important link between the involuntary nervous system and proinflammation, which has been suggested for more than 70 years [18]. This “parasympathetic” nerve is composed of both sensory (input) and motor (output) fibers, suggesting that the vagus nerve can sense continuing inflammation and subsequently suppresses it. This mechanism is more efficient and rapid than other anti-inflammatory pathways. In addition, the vagus nerve has been shown to convey the immunologic state of the gastrointestinal tract to the hypothalamus and the vagus mediator, acetylcholine, has potent anti-inflammatory actions and suppresses TNF-a, IL-6 production by stimulating the alpha7 subunit-containing nicotinic [11] acetylcholine receptor alpha7nACHR [19]. Thus, the vagus nerve provides the endogenous mechanism to regulate the magnitude of innate immune responses and attenuate inflammation. However, it remains unknown whether the novel peptide ghrelin plays any role in such an anti-inflammatory pathways [20].

Recently, ghrelin receptors have been detected in cardiovascular tissue [11], indicating that ghrelin may play an important regulating role in regulating cardiovascular function. Septic shock was accompanied with critical cardiovascular dysfunction. However, there has not been any report on the change in ghrelin and its effects on cardiovascular function in septic shock [21].

**Aim of work:** The aim of this work was to study the effect of administration of ghrelin on the outcome of sepsis and septic shock induced by cecal ligation and puncture in rats and to investigate the effect of vagotomy on ghrelin effect. Also, to test whether administration of exogenous ghrelin affects norepinephrine release, this may contribute to its anti-inflammatory and cardiac protective effects in sepsis.

**Material and Methods**

Fifty male albino rats (180-200g) were supplied by the Animal House Unit of Kasr Al-Ainy, Faculty of Medicine, Cairo University and housed in separate cages at room temperature from Sep. 2011-Dec. 2011 on a 12-hour light/dark cycle and fed a standard rat chow diet and had free access to water. Prior to the induction of sepsis, rats were fasted overnight but allowed water, and were divided into 5 groups, 10 rats each.

Group 1: Sham operated group, rats of this group underwent all the steps of cecal ligation and puncture (CLP) except that their ceca were not ligated nor punctured (sham operated).

Group 2: CLP, rats underwent CLP [22] and received normal saline 10mL/kg through femoral vein at the moment of surgical completion, and sc
injection of 10ml/kg of normal saline again 5h after surgery.

Group 3: CLP+GR group, rats underwent CLP and received ghrelin at a dose of 1 0nmol/kg through femoral vein at the time of operation and sc injection of 60nmol/kg BW 5h after surgery [8].

Group 4: CLP+VagX, rats underwent CLP and 5hrs after the CLP, vagotomy operation was conducted [23] and received saline at the time of CLP and 5h after (CLP+ vagotomy).

Group 5: CLP+GR+VagX, rats underwent CLP, followed after 5h by vagotomy operation and received ghrelin at dose of 10nmol/ kg at the time of operation and 60nmol/ kg BW at 5h after surgery.

Cecal ligation and puncture:
Rats were anesthetized with isoflurane inhalation and abdomen, and groin were shaved and washed with Betadine. CLP was performed as according to Yang et al. [22]. Briefly, 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 then returned to the abdominal cavity. The abdominal incision was closed in 2 layers by a surgical suture. The animals were then returned to their cages.

The rats were singly housed at room temperature after the operation. The animals recovered quickly (within 10 minutes) after the operation. They moved around and drank water.

Administration of ghrelin:
Rats were injected with normal saline 10ml/kg through femoral vein at the moment of surgical completion, and with sc injection of 40ml/kg of normal saline again at 5h after surgery. The administration of saline for rats in ghrelin-treated group was as same as that of the untreated groups, except that the saline for injection contained ghrelin 10nmol/kg at the time of operation and 60nmol/kg BW at 5h after surgery [8].

Vagotomy operation:
The trunks of the subdiaphragmatic vagus were transected according to Wu et al. [23]. Briefly, the rats were re-anesthetized with isoflurane inhalation at 5h after CLP or sham operation. Midline abdominal incision was made and the dorsal and ventral branches of the vagus nerve were dissected from the esophagus. Ghrelin or vehicle (normal saline) was administrated subcutaneously immediately following vagotomy as described above in the vagus nerve intact animals.

After 8 hours of CLP or sham operation rat blood pressure was measured and blood samples were collected from rat tail vein 8h after CLP or sham operation. Blood samples were centrifuged at 3,000g for 10min at 4°C, and the serum samples were stored at −80°C until required for determination of serum concentrations of NE, TNF- α and IL-6. Animals were sacrificed and hearts were excised for measurement of cardiac functional parameters.

Measurement of arterial blood pressure:
Rat blood pressure was measured by Harvard 50-9331 Rectilinear Recording System which is a rat tail blood pressure monitor.

Measurement of cardiac functional parameters:
In all the study groups, left ventricular developed pressure [LVDP] and dp/dt were measured after 8 hours of CLP or sham operation:

After taking blood samples, all animals were heparinized with 1 000U of heparin (i.p.). Animals were then anesthetized with 40mg/Kg sodium pentobarbitone (i.p.). The hearts were excised and placed in ice-cold Krebs-Henselit Bicarbonate (KHB) buffer. The aorta was cannulated with an 18-gauge plastic cannula, in a non circulating Langendorff apparatus, with modified Krebs Henseleit solution at a constant flow rate of 12ml/min. The perfusate solution consists of the following (mmol/l): NaCl (116), NaHCO3 (25), CaCl2 (205), MgSO4 (102), KCl (407), KH2PO4 (102) and glucose (5.5).

The perfusate was oxygenated with 95% O2 and 5% CO2 gas mixture to maintain a PO2 of >40mmHg. A latex balloon-tipped catheter was inserted into the left ventricle through the mitral annulus and inflated with distilled water (0.15-0.3ml) to set an end diastolic pressure of 2mmHg during the initial equilibration.

The distal end of the catheter was connected to a polygraph (san-ei, made in Japan) for recording the different haemodynamic parameters, via pressure transducer, according to the experimental design [24].
Contractile function was assessed by measuring the following parameters:

1. Left ventricular developed pressure (LVDP) which is defined as peak systolic minus end-diastolic pressure.
2. The maximum rate of pressure rise (dp/dt).

Determination of levels of Norepinephrine (NE):

The concentrations of NE in the serum were quantified by the use of commercially obtained ELISA kit specific for NE (IBL-America Inc., Minneapolis, MN, USA). The assay was carried out according to the instructions provided by the manufacturer as we described previously [25].

Determination of Levels of TNF-α and IL-6:

The concentrations of TNF-α and IL-6 in the serum were quantified by using commercially obtained enzyme-linked immunosorbent assay (ELISA) kits specific for rat-TNF-α and IL-6 (BioSource International, Camarillo, CA) [26,27].

Statistical analysis:

Data were analyzed using the statistical package SPSS version 15. Values were expressed as mean ± standard deviation (SD). Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test in normally distributed quantitative variables while non-parametrical Mann-Whitney test was used for non normally distributed quantitative variables. p-values less than 0.05 were considered as statistically significant.

Results

Effects of ghrelin administration on TNF-α and IL-6 release after CLP:

As shown in Table (1) and Figs. (1,2), CLP induced a significant increase in serum levels of TNF-α and IL-6 in vehicle-treated animals as compared with sham-operated animals (p<0.05). TNF-α and IL-6 increased by 10 and 12 fold, respectively, at 8 hours after CLP in vehicle-treated animals as compared with sham-operated animals (p<0.05). Administration of ghrelin significantly attenuated serum levels of TNF-α and IL-6 as compared to CLP (p<0.05); however, they were still significantly higher (2 folds and 5 folds respectively) as compared with sham-operated animals (p<0.05).

Effects of vagotomy on serum TNF-α and IL-6 levels after CLP:

Vagotomy performed 5 hours after CLP insignificantly increased TNF-α and IL-6 as compared to CLP group values (p>0.05) (Table 1, Figs. 1,2). Vagotomy partially abolished the inhibitory effects of ghrelin on serum levels of TNF-α and IL-6 to become significantly higher than values recorded in ghrelin treated group (p<0.05), however, these values were still significantly lower than values recorded in CLP group (p<0.05).

Effects of ghrelin administration on NE level:

Table (2) and Fig. (3), show that CLP significantly increased NE level in CLP group (2.7 fold) as compared to sham operated rats. Ghrelin administration at and 5 hours after the operation significantly decrease NE level as compared with CLP group (p<0.05), however, the NE levels after ghrelin treatment were significantly higher than sham operated group (1.5 folds) (p<0.05). Vagotomy performed 5 hours after CLP had no significant effect on NE level. Vagotomy partially inhibited the effect of ghrelin on NE levels. It can be shown that vagotomy significantly increased NE level in CLP+GR+VagX group as compared with CLP+GR group (p<0.05).

Effects of ghrelin administration on hemodynamic parameters:

Compared to that CLP group, SBP of rats in ghrelin-treated group was significantly increased (1.4 folds) (p<0.05). The values of LVDP and dp/dt significantly increased (2.4 folds) and (2.1 folds) respectively compared with CLP group (p<0.05). Vagotomy partially blocked the beneficial effect of ghrelin on hemodynamic parameters in CLP+GR+VagX group, however values are still significantly higher than CLP group values Figs. (4,5,6).

Table (1): Alterations in serum levels of tumor necrosis factor (TNF)-α and IL-6 in sham-operated animals, CLP treated with vehicle or ghrelin at and 5h after CLP and the effect of vagotomy on CLP(CLП+VagX) and on CLP treated with ghrelin (CLП+GR+VagX).

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham</th>
<th>CLP</th>
<th>CLP+GR</th>
<th>CLP+VagX</th>
<th>CLP+VagX+GR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF pg/ml</td>
<td>6.2± 1.8⁣¹</td>
<td>61.6±13.1⁣⁶</td>
<td>12.3±2.9⁣⁴</td>
<td>75.7± 10.4⁣⁸</td>
<td>46.6±5.6⁣⁹</td>
</tr>
<tr>
<td>IL-6 pg/ml</td>
<td>21.4±4.2⁣⁹</td>
<td>246.8±30.3⁣⁶</td>
<td>101.0±11.8⁣⁴</td>
<td>269.5±15.1⁣⁶</td>
<td>209.0±8.7⁣⁷</td>
</tr>
</tbody>
</table>

Results are expressed in means±SD. (n=10). Results with different letters in the same raw showed significant changes (p<0.05). Results with the same letters in the same raw are insignificant.
Table (2): Norepinephrine level and hemodynamic parameters [systolic blood pressure (SBP), left ventricular developed pressure (LVDP) and dp/dt] in sham operated, CLP treated with vehicle (CLP) or ghrelin (CLP+GR) and the effect of vagotomy on CLP (CLP+VagX) and on CLP treated with ghrelin (CLP+GR+VagX).

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>CLP</th>
<th>CLP+GR</th>
<th>CLP+VagX</th>
<th>CLP+VagX+GR</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE ng/ml</td>
<td>0.35±0.1 a</td>
<td>0.95±0.2 b</td>
<td>0.54±0.05 c</td>
<td>0.97±0.05 b</td>
<td>0.63±0.1 d</td>
</tr>
<tr>
<td>SBP mmHg</td>
<td>120.7±17.0 a</td>
<td>70.1±3.5 b</td>
<td>95.3±5.0 c</td>
<td>71.2±5.6 b</td>
<td>83.5±9.0 d</td>
</tr>
<tr>
<td>LVDP mmHg</td>
<td>92.2±10.5 a</td>
<td>35.9±4.8 b</td>
<td>87±5.7 c</td>
<td>37.1±6.0 b</td>
<td>49.3±9.2 d</td>
</tr>
<tr>
<td>dp/dt mmHg/sec</td>
<td>744.0±69.9 a</td>
<td>320.8±15.0 b</td>
<td>680.7±43.8 c</td>
<td>268.2±15.6 b</td>
<td>301.4±28.6 d</td>
</tr>
</tbody>
</table>

Results are expressed in means±SD. (n=10). Results with different letters in the same raw showed significant changes ( p<0.05). Results with the same letters in the same raw are insignificant.

![Fig. (1): Serum levels of TNFα in Sham, CLP, CLP+GR, CLP+VagX and CLP+GR+VagX groups.](image1)

![Fig. (2): Serum levels of IL-6 in Sham, CLP, CLP+GR, CLP+VagX and CLP+GR+VagX groups.](image2)

![Fig. (3): Serum levels of norepinephrine (NE) in Sham, CLP, CLP+GR, CLP+VagX and CLP+GR+VagX groups.](image3)

![Fig. (4): Systolic blood pressure (SBP mmHg), in Sham, CLP, CLP+GR, CLP+VagX and CLP+GR+VagX groups.](image4)

![Fig. (5): Left ventricular developed pressure (LVDP mmHg) in Sham, CLP, CLP+GR, CLP+VagX and CLP+GR+VagX groups.](image5)

![Fig. (6): dp/dt (mmHg/sec) in Sham, CLP, CLP+GR, CLP+VagX and CLP+GR+VagX groups.](image6)
Discussion

In the present work, it has been documented that CLP sepsis upregulates pro-inflammatory cytokines TNF-α and IL-6 in the blood of the rats. Circulating levels of NE increased significantly during sepsis.

A cross-talk takes place between different immune cells including macrophages, dendritic cells and CD4+ T cells, leading to either a proinflammatory or anti-inflammatory cytokine reaction [3]. Generally accepted is the theory that cells of the innate immune system recognize microorganisms and initiate responses through pattern recognition receptors (PRRs), pathogen-associated molecular patterns (PAMPs) and Toll-like receptors (TLRs) [28].

The bacterial molecules are presented to the innate immune system resulting in the production of proinflammatory cytokines and chemokines [29]. Some reports support a role for the residential macrophages as the first responders and conductors which would orchestrate the inflammatory events after surgical manipulation or endotoxin exposure [30].

The gastrointestinal tract contains a dense population of mucosal macrophages which play a crucial role in tissue homeostasis on the one hand and in the initiation, propagation and resolution of inflammation on the other hand. Mucosal macrophages are conditioned towards an anti-inflammatory role under normal circumstances and switch towards a pro-inflammatory modus during inflammation [31,32] and the major sources of circulating inflammatory cytokines in sepsis are macrophages/Kupffer cells [33,34].

More importantly, an interplay between these initiating cells and the nervous system is suggested, as the mediators released from residential macrophages are able to affect neuronal signaling within and from the gastrointestinal wall [35].

Catecholamines possess both anti-inflammatory and proinflammatory activities. The adrenergic anti-inflammatory effects are mostly mediated by β2-adrenoceptors, which are expressed on lymphocytes and monocytes [36,37]. Only supraphysiological levels of NE can inhibit cytokine release from monocytes/macrophages [38]. Many studies focusing on the immunomodulation by NE used high concentrations of NE (i.e., 10^{-7} M) and thus were more likely to activate β2-receptors that override β2-receptor-mediated proinflammatory responses [39,40]. Alpha2-adrenoceptors are Gi- and G0-protein coupled receptors that decrease intracellular cAMP, open K+ channels, and inhibit voltage gated Ca2+ channels, all of which lead to hyperpolarization of neurons and activation of immune cells [38].

Stimulation of Kupffer cell α2-adrenoceptors regulates TNF-α production [41]. In addition, α2-adrenergic antagonist improved the survival rate following a lethal bolus injection of endotoxin [16,42].

In 2000, Yang et al. [43] discovered that the gut is a major source of the sustained elevation of NE in sepsis. Zhou et al. [44] discovered a sepsis-induced elevation of the tyrosine hydroxylase (TH) in the gut, the rate-limiting enzymatic reaction in the synthesis of NE [45]. In addition to an increased production of NE, they found several mechanisms involved in the increased NE level in the circulation. Increased release of NE-vesicles from the sympathetic varicosities and inhibition of the uptake of released NE, leading to the massive spillover of NE into the circulation [39].

The vagus nerve provides another endogenous mechanism to regulate the magnitude of innate immune responses and attenuate inflammation. Activation of parasympathetic effenter nerves during systemic stress confers an additional protective advantage to the host by restraining a potentially adverse peripheral immune response. Borovikova et al. [45] and Wang et al. [46] described the novel concept of a cholinergic anti-inflammatory pathway mediated by the activation of the vagus nerve and nicotinic α7-cholinergic receptor on macrophages [47]. It has also been shown that electrical stimulation of the vagus nerve during endotoxemia can inhibit synthesis of TNF-α in the liver, spleen, and heart [46].

The present study clearly demonstrates that ghrelin possesses anti-inflammatory effect as shown by decreasing inflammatory mediators, TNF alpha and IL-6.

In agreement with this finding, decreased levels of ghrelin in some rodent models of polymicrobial sepsis have been demonstrated [48,49] and treatment with ghrelin in these sepsis models reduced serum concentrations of proinflammatory cytokines like TNF-α and IL-6 [23]. In contrast to these results, Koch et al. [50] could not reproduce this possible link of inflammatory markers to ghrelin in critically ill patients and they failed to correlate, TNF-α, IL-6, white cell blood count, C-reactive protein or
procalcitonin with serum ghrelin concentrations. Moreover, Wu et al. [23] demonstrated that ghrelin’s direct effect on inflammatory cytokine release from macrophages is negligible [49] and they reported that ghrelin has no direct beneficial effects on cytokine release from either Kupffer cells or peritoneal macrophages isolated from normal rats. This would suggest that an indirect mechanism may be involved in ghrelin’s effects on inflammatory cytokines.

In a rat model of polymicrobial sepsis induced by cecal ligation and puncture, though ghrelin levels decreased after ligation and cecal puncture, its receptor was markedly elevated in early sepsis. Ghrelin and the ghrelin receptor are expressed by lymphocytes, monocytes and dendritic cells. Activation of the ghrelin receptor results in an inhibition of proinflammatory cytokine expression and an increase in survival in various inflammatory disease models [12,51].

In this study, it was observed that ghrelin has an inhibitory effect on NE release as shown by the significant lower levels of NE in ghrelin treated rats as compared to CLP rats. Norepinephrine release during sepsis is crucial in causing upregulation of inflammatory cytokines [43].

In this regard, a close correlation of the increased NE and reduced ghrelin levels in sepsis was reported [43,48,52] suggesting that downregulation of the novel peptide ghrelin in sepsis plays a role in activating sympathostimulatory nuclei in the brain and increasing NE release from the sympathetic nerve fibers. Recent results demonstrated that ghrelin possesses sympathoinhibitory properties as evidenced by the finding that ghrelin reduces NE levels and the downregulatory effect of ghrelin on proinflammatory cytokines can be reversed by coadministration of NE [24,53].

Wu et al. [23] showed that diminished level of NE after intravenous injection of ghrelin was completely blocked by intracerebroventricular injection of the ghrelin receptor antagonist, whereas its downregulatory effect on TNF-a release was only partially diminished by intracerebroventricular injection of the ghrelin receptor antagonist. This would suggest that ghrelin’s effect on TNF-a release was only partially mediated through the sympathetic nervous system. It was reported that ghrelin has sympatho-inhibitory properties that are mediated by central ghrelin receptors, involving a NPY/Y1 receptor-dependent pathway. It is thought that, similar to cytokines, ghrelin enters the brain through the “loopholes” of the blood-brain barrier, which are closely related with the hypothalamic region: The circumventricular organs (CVO) [38].

It should be pointed out that anti-inflammatory properties of ghrelin may also be partially mediated through stimulation of the vagus nerve [47,54]. Some authors hypothesized that the antiinflammatory effects of ghrelin are mediated both by the anti-inflammatory cholinergic pathway and by interactions with immune cells [51,55].

Wu and Kral [20] suggested that ghrelin might be the mediator of vagal signaling to the immune system, and they recommended future investigations to define its functional role in the parasympathetic anti-inflammatory pathway.

In our study, surgical dissection of the nerve (vagotomy) after CLP, showed that the anti-inflammatory effects induced by intravenous injection of ghrelin was diminished. This indicates that ghrelin has the ability to activate the cholinergic anti-inflammatory pathway.

Wu et al. [23] and Das [8] also reported that vagotomy, but not sham vagotomy, prevented ghrelin’s down-regulatory effect on TNF-alpha and IL-6 production, thus confirming that ghrelin down-regulates proinflammatory cytokines in sepsis through activation of the vagus nerve. Also, Shah et al. [56] reported that vagotomy prevented ghrelin’s beneficial effects. Thus ghrelin appears to be a potential modulator to rebalance the dysregulated sympathetic/parasympathetic nervous system during sepsis.

On the other hand, Quan [57] reported that vagal stimulation is responsible for much of the hyperalgesia, fever, anorexia, taste aversions, increased levels of plasma corticosteroid, and brain norepinephrine changes produced by intraperitoneal injections of IL-1 beta and LPS.

Up to now, the pathogenesis of septic shock has not been fully understood. Although great progress has been made by antibiotics and therapeutics with vascular function regulators, high mortality rate is still the most important issue of sepsis, particularly in cardiac function impairment and multiple system organ failure [22]. In this study, it was shown that ghrelin improves cardiac performance during sepsis as evidenced by elevation of the rat systolic blood pressure, left ventricular developed pressure and contractility.

Chang et al. [22] reported that, compared to septic shock group, MABP, LVdp/dtmax of rats in
ghrelin-treated group increased. The plasma glucose concentration and myocardial ATP content increase, but plasma lactate concentration decreased in ghrelin-treated rats. In another protocol, Chang et al. [58] reported that administration of ghrelin either at the same time as lipopolysaccharide injection (early treatment) or 12 h after lipopolysaccharide injection (late treatment) significantly decreased the mortality rate and ameliorated the hypotension seen in rats with endotoxic shock.

Also, Wu et al. [48] stated that administration of ghrelin is a possible therapy to maintain cardiovascular stability and reduce mortality. On the other hand, Wang et al. [59] reported that although ghrelin protects mice against endotoxemia-induced acute kidney injury, they found that ghrelin had no effect on MAP.

Improvement of the cardiac muscle performance during sepsis may be related to the effect of ghrelin on enhancing plasma glucose levels preventing hypoglycemia that is detrimental. Furthermore, ghrelin and insulin seem to have both positive and negative feedback control over each other [60,61], suggesting that ghrelin may be involved in maintaining glucose homeostasis, under both normal conditions and sepsis. Chang et al. [59] reported that early treatment with ghrelin significantly attenuated the deficiency in myocardial ATP content, but late treatment with ghrelin had no effect on myocardial ATP content.

Also, the anti-inflammatory actions of ghrelin can explain why it is able to ameliorate the hemodynamic and metabolic disturbances in septic shock [8].

Investigations on the role of ghrelin in chronic circulatory insufficiency have shown that its intravenous administration significantly affects haemodynamic parameters. The following have been observed: Increase of cardiac index and left ventricular stroke volume, and decrease of peripheral resistance and mean blood pressure [62].

Recent studies of Nagaya et al. [63] also revealed positive effect of ghrelin administration on left ventricular function and exercise capacity in patients with chronic heart failure. Nagaya et al. [64] reported that human ghrelin elicited a potent, long-lasting GH release and had beneficial hemodynamic effects. GH possesses potent myocardial inotropic effects, and has significantly therapeutic effects on serious heart failure and ischemic heart disease [64].

Since ghrelin receptors were detected in cardiovascular tissues [65], ghrelin seemed likely to posses direct effects on cardiovascular system. Hexarelin, another endogenous natural ligand of GHS-R, could protect heart directly in a GH-independent manner on both isolated myocardial cells and myocardial ischemia-reperfusion model [66].

Furthermore, it has been shown that ghrelin inhibits apoptosis of cardiomyocytes and endothelial cells [67]. It is interesting to note that although cardiomyocytes bind ghrelin with high affinity, they do not express GHSR1a. Taken together, these findings imply the existence of other unknown GHSR subtype(s) distinct from the classic GHSR1a in the cardiovascular system [68].

In conclusion, by regulating crucial processes of sepsis, such as the production of inflammatory mediators by macrophages, ghrelin represents a feasible therapeutic agent for this disease and other inflammatory disorders. Ghrelin’s anti-inflammatory action may be mediated partially by its direct action on the immune cells, and partially through being a modulator that restores the dysregulated balance of sympathetic/parasympathetic nervous system during sepsis. Its anti-inflammatory action may be due to vagal stimulation, and decreasing NE release. An important role of ghrelin in sepsis, is its improvement of the cardiac muscle performance and protection against septic shock; may be through a direct action on its receptors in the heart or indirectly through its anti-inflammatory effect or through GH release and metabolic modulation.

References
Ghrelin Protects Against Experimental Sepsis


