Ghrelin-mediated sympathoinhibition and suppression of inflammation in sepsis

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First published November 8, 2011; doi:10.1152/ajpendo.00508.2011.—Sepsis, a systemic inflammatory response to infection, continues to carry a high mortality despite advances in critical care medicine. Elevated sympathetic nerve activity in sepsis has been shown to contribute to early hepatic cell dysfunction and subsequently multiple organ failure, resulting in a poor prognosis, especially in the elderly. Thus, suppression of sympathetic nerve activity represents a novel therapeutic option for sepsis. Ghrelin is a 28-amino acid peptide shown to inhibit sympathetic nerve activity and inflammation in animal models of tissue injury. Age-related ghrelin hyporesponsiveness has also been shown to exacerbate sepsis. However, the mechanistic relationship between ghrelin-mediated sympathoinhibition and suppression of inflammation remains poorly understood. This review assesses the therapeutic potential of ghrelin in sepsis in the context of the neuroanatomical and molecular basis of ghrelin-mediated suppression of inflammation through inhibition of central sympathetic outflow.

sympathoinhibition; ventrolateral medulla; α2A-adrenergic receptor; norepinephrine

DESPITE STRIDES IN CRITICAL CARE, the 751,000 annual cases of sepsis in the United States continue to be costly, causing 215,000 deaths, with an estimated economic burden of 16.7 billion dollars on the United States annually. Multiple organ failure, resulting in part from the deleterious effects of proinflammatory cytokines, is responsible for most deaths in sepsis (5, 70). We discovered that norepinephrine released from the gut causes early hepatic cell dysfunction in sepsis by stimulating α2A-adrenoceptors on Kupffer cells, potentiating lipopolysaccharide (LPS)-stimulated NF-κB-mediated cytokine release (56). The proinflammatory cytokines released by Kupffer cells contribute to multiple organ failure. Thus, suppression of sympathetic nerve activity and norepinephrine release represents a promising novel therapeutic approach to sepsis.

Ghrelin is a stomach-derived 28-amino acid peptide that exerts anti-inflammatory effects in sepsis. Serum ghrelin levels are reduced during sepsis, and administration of exogenous ghrelin improves survival in sepsis (69, 85, 86). Ghrelin can act centrally to inhibit peripheral sympathetic nerve activity following tissue injury (67, 73, 87). However, the mechanisms of central ghrelin-mediated sympathoinhibition and how this translates into suppression of systemic inflammation in sepsis remain poorly understood. This review assesses the therapeutic potential of ghrelin in sepsis by discussing the neuroanatomical and molecular bases of ghrelin-mediated inhibition of central sympathetic outflow and the relation to suppression of inflammation and attenuation of tissue injury in sepsis. Guided by the current recommendations of the surviving sepsis campaign (19), we make suggestions for further studies aimed at developing human ghrelin as a novel treatment for sepsis.

Control of Sympathetic Outflow

To understand the mechanism of sepsis-induced increase in sympathetic nerve activity and how ghrelin treatment suppresses inflammation through sympathoinhibition, it is important to understand the relevant anatomy involved in the regulation of central sympathetic outflow. The sympathetic nervous system consists of a central network of neurons that respond to cardiovascular, metabolic, immune, and endocrine stress signals to maintain homeostasis in the body. There is a cross-interaction between the sympathetic nervous system and the functionally opposite parasympathetic nervous system in the maintenance of homeostasis (60).

Central sympathetic network. The rostral ventrolateral medulla (RVLM) is the major source of central sympathetic outflow to the periphery. The RVLM consists of predominantly glutaminergic neurons (70%), which project excitatory output to sympathetic preganglionic neurons in the intermediolateral column of the spinal cord. The RVLM serves as a point for integration of information from many structures in the brain. Among the structures modulating the output of RVLM are the paraventricular nucleus (PVN), caudal ventrolateral medulla (CVLM), and nucleus tractus solitarius (NTS). The PVN stimulates sympathetic outflow by making excitatory connection with RVLM or through direct excitatory output to sympathetic preganglionic neurons in the intermediolateral column of the spinal cord. The CVLM, which consists of interneurons, serves as the main negative regulator of RVLM through the release of
the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (29) (Fig. 1A). The NTS, which receives excitatory afferent vagal input (61), can suppress sympathetic outflow by a direct excitatory glutaminergic input to CVLM (4). The CVLM interneurons then cause sympathoinhibition through the release of GABA at RVLM (1, 3, 11). The NTS may also inhibit RVLM sympathetic discharge via excitatory glutaminergic projection to the nucleus ambiguus (NA) (2). The NA in turn stimulates the CVLM (55), which in turn inhibits the RVLM through GABA release (1). Thus, the vagus nerve-mediated inhibition of sympathetic nerve activity, which has been demonstrated in several studies (9, 33, 34, 78), may be explained by the NTS-CVLM-RVLM inhibitory pathway.

**Sympathetic efferents.** Sympathetic efferents are classified into three main groups: thermosensitive, glucosensitive, and barosensitive efferents. The main neurotransmitters of sympathetic efferents at the target organs are norepinephrine and epinephrine. Thermosensitive efferents innervate cutaneous vasoconstrictors and result from the response of the rostral ventromedial medulla to hyperventilation, hypothermia, and emotional stimuli (29). Glucosensitive efferents are regulated in part by the RVLM in response to hypoglycemia and mediate epinephrine release from the adrenal medulla (58). Epinephrine then elevates blood glucose levels by stimulating gluconeogenesis in the liver (20). Barosensitive efferents are controlled by the RVLM and are activated by carotid and aortic baroreceptors, visceral nociceptors, and central chemoreceptors, as well as disease states such as sepsis (32, 82). The primary and secondary lymphoid organs, which are involved in the immune functions of the body, are extensively innervated and regulated by the sympathetic nervous system (49).

Electrophysiological and histochemical studies indicate that RVLM neurons are organized topographically into separate groups that control specific organs and vascular beds (organotypic arrangement) (53). Based on these studies, the muscle vasoconstrictor (MVC), cutaneous vasoconstrictor (CVC), vis-
ceral vasoconstrictor (VVC), and renal sympathetic (RSN) groups of RVLM neurons have been identified (53). The neurotransmitters of sympathetic efferents act on adrenergic receptors on target cells. Adrenergic receptors are subdivided into α₁A, α₁B, α₁C, α₂A, α₂B, α₂C, β₁, β₂, and β₃ receptors, with different affinities for norepinephrine and epinephrine (10). The physiological effects of adrenergic receptors are mediated through the G protein intracellular signaling system (35).

Sepsis and Sympathetic Activation

The pathogenesis of sepsis involves a systemic inflammatory response to an infection. Severe sepsis, which has a high mortality, is associated with multiple organ failure. Hepatocellular dysfunction occurs early in sepsis (13, 83, 84) and subsequently contributes to the failure of other vital organs such as the lungs. Sepsis-associated hepatocellular dysfunction is mediated by proinflammatory cytokines (41), which cause hepatocyte injury evidenced by elevations of aminotransferases, and dysfunction of the cytochrome P-450 enzymatic metabolic pathway (44). In addition, the release of cytokines by Kupffer cells leads to overproduction of nitric oxide. Curran et al. (17) found that the specific combination of TNF-α, IL-1β, interferon-γ, and endotoxin resulted in upregulation of nitric oxide production through the L-arginine-dependent biochemical pathway within hepatocytes. Increased production of nitric oxide resulted in decreased hepatocyte protein synthesis. Nitric oxide overproduction suppresses hepatic glucose output (75), which may explain the hypoglycemia observed in early bacterial sepsis.

We have previously shown that increased norepinephrine secretion during sepsis aggravated hepatocellular dysfunction by driving the production of cytokines through α₂A-adrenergic receptor-mediated activation of p38 MAP kinase pathway in Kupffer cells (90, 94) (Fig. 1A). The sympathoexcitation associated with sepsis results from stimulation of the central sympathetic network by a variety of stimuli. Pyrogenic cytokines IL-1β and IL-6, produced by Kupffer cells and other macrophages, enter the brain through the circumventricular organs and stimulate vascular endothelial cells to produce prostaglandin E₂ (PGE₂). PGE₂ stimulates its receptor subtype 3 (EP3R) on neurons in the preoptic area of the hypothalamus. EP3R stimulation leads to excitatory signals to the PVN and subsequently to the RVLM, leading to increased sympathetic outflow (23, 66, 92). LPS-induced hypoglycemia, resulting from nitric oxide overproduction (44), also activates the central sympathetic network, leading to glucosensitive sympathetic efferent output, which stimulates epinephrine release from the adrenal medulla (58). Sepsis may also be complicated by metabolic acidosis and respiratory acidosis (59). Decreased pH is sensed by chemoreceptors in the brain, leading to stimulation of barosensitive sympathetic efferent output from the RVLM (40) (Fig. 1A). Sepsis-induced activation of the RVLM has been confirmed by the finding that intravenous injection of LPS increased c-fos immunoactivity at the RVLM (22). Thus, a variety of sepsis-associated stimuli cause sympathoexcitation and systemic catecholamine release. In addition to centrally mediated stimulation of catecholamine release, the increase in circulating catecholamine levels in sepsis could also be partly due to decreased clearance. The liver and the gut are responsible for metabolizing 86–93% of catecholamines on first pass (14). Thus, hepatocellular dysfunction and gut dysfunction in sepsis will decrease catecholamine metabolism, resulting in increased circulating levels of norepinephrine and epinephrine. The increased levels of catecholamines will then cause further hepatic damage. This may account, in part, for the finding of Beloeil et al. (8), who in the assessment of norepinephrine kinetics in septic shock found a negative correlation between norepinephrine clearance and sepsis severity.

Proinflammatory Role of Catecholamines in Sepsis

We have previously established a predominantly deleterious proinflammatory role of norepinephrine in sepsis, mediated by α₂A-adrenergic receptors (56). Using a rat model of polymicrobial sepsis by cecal ligation and puncture (CLP), we found that sepsis resulted in upregulation of α₂A-adrenergic receptor gene expression in Kupffer cells (56). Moreover, in the same study, the binding capacity and affinity of α₂-adrenergic receptor were increased by sepsis (56). Norepinephrine binding to α₂A-adrenergic receptor dramatically potentiated LPS-induced secretion of TNF-α by Kupffer cells. We demonstrated that the potentiation of TNF-α release by norepinephrine was mediated by activation of the p38 MAP kinase pathway. Significantly, suppression of the effects of norepinephrine using BRL-44408 maleate, a specific α₂A-adrenergic receptor antagonist, resulted in attenuation of multiple organ failure and significant improvement in sepsis survival (56). Other investigators have similarly demonstrated deleterious effects of catecholamines in sepsis and other inflammatory conditions. Anнат et al. (6) showed that various catecholamines induced proinflammatory cytokines in human hepatocytes and potentiated the effects of LPS. The augmenting effect of norepinephrine on LPS-induced TNF-α secretion may further be explained by the discovery of Kotlyarov et al. that MAPKAP kinase-2 (MK2), which is a substrate of p38α, is required for TNF-α biosynthesis (42). The p38 MAP kinase pathway activates the TNF-α gene via the AU-rich element (ARE) (93). Not only are catecholamines responsible for elevations in cytokine levels, they have also been shown to suppress macrophage and neutrophil phagocytosis leading to decreased bacterial clearance in sepsis (26, 27, 36). These deleterious effects of catecholamines may be partly responsible for the continuing high mortality rates of sepsis in the face of advances in other aspects of sepsis management such as the use of vasopressors for hemodynamic support.

It is also worth noting that other investigators, focusing mainly on β-adrenergic receptors on leukocytes, have described anti-inflammatory effects of catecholamines (21). One explanation for the seemingly contradictory effects of catecholamines with regards to inflammation may be the differential receptor binding preferences of norepinephrine at different concentrations. At concentrations found in septic conditions (~20 nM), norepinephrine has been shown to selectively bind α₂-adrenergic receptors (30, 57, 74). Our studies have shown that norepinephrine at this concentration upregulates proinflammatory cytokines (89). Other explanations could include the fact that there is downregulation of β-adrenergic receptors with concomitant upregulation of α-adrenergic receptors, with increased binding affinity, under inflammatory conditions (51). Thus, proinflammatory effects, mediated by α₂A-ad-
Ghrelin-Mediated Sympathoinhibition and Anti-Inflammatory Effects

Ghrelin is a 28-amino acid peptide produced mainly in the fundus of the stomach. Ghrelin acts on the growth hormone secretagogue receptor type 1a (GHSR-1a). GHSR-1a is a G protein-coupled receptor that is widely distributed throughout the body, with high expression in parts of the brain (15, 39, 72) (Fig. 1B). We have previously shown (85, 86) that ghrelin suppresses inflammation and sympathetic nervous system activity in sepsis (87). We further found that ghrelin’s anti-inflammatory action depends on its action on receptors in the brain. Other investigators have similarly demonstrated ghrelin’s ability to suppress central sympathetic outflow (47). However, the pathways of ghrelin-mediated sympathoinhibition and their link to ghrelin’s anti-inflammatory effects in sepsis have not been previously explained. To act on its receptors located in the brain, ghrelin must be able to cross or circumvent the blood-brain barrier. Banks et al. (7) demonstrated that human ghrelin is able to cross the blood-brain barrier in mice through a saturable transport mechanism. However, recent evidence suggests that, even if such a transport mechanism existed, it would be too slow, as it was shown that a time lapse of 40–50 min was required after intravenous injection to increase cerebrospinal fluid ghrelin levels twofold (28). Other investigators have suggested that ghrelin may act at the circumventricular organs, where the blood-brain barrier is nonexistent and ghrelin receptors are expressed (63, 95). In the systemic inflammatory condition of sepsis, however, ghrelin is likely to enter the brain easily, since it has been shown that systemic inflammation disrupts the blood-brain barrier (48, 54). In the brain, ghrelin may act on its receptor GHSR-1a, which is densely expressed at the NTS, and agouti-related protein (AgRP)-neuropeptide-Y (NPY) neurons of the arcuate nucleus (ARC) in the hypothalamus (15, 51).

Several studies have demonstrated sympathetic nervous system suppression by centrally acting ghrelin. It has been shown that ghrelin acts at the NTS to suppress renal sympathetic nerve activity (52). Ghrelin has also been shown to suppress cardiac (67, 73) and brown adipose tissue (91) sympathetic nerve activity. Further evidence of ghrelin’s sympathoinhibition comes from the work of Sato et al., who found that blood norepinephrine and epinephrine levels were elevated in rodents after chronic administration of the ghrelin receptor antagonist [d-Lys³]-GHRP-6 (65). The NTS has been shown to make direct and indirect (via NA) excitatory projection to the CVLM (2, 4). Stimulation of CVLM interneurons results in inhibitory GABAAergic output to the RVLM, which is the main source of sympathetic outflow. This may be one of the pathways by which ghrelin decreases sympathetic nerve activity (Fig. 1B).

Another possible pathway by which ghrelin may act at the NTS to cause sympathoinhibition is through the vagus nerve. Vagus nerve stimulation has been shown to decrease sympathetic nerve activity (34). Date et al. (18) showed that the ghrelin receptor GHSR-1a is expressed at afferent vagus nerve terminals and that stimulation of these receptors modulates vagal afferents, leading to increased c-fos activity in the brain. Cui et al. (16) further elucidated the signaling of ghrelin at the NTS when they discovered that ghrelin decreases the presynaptic release of glutamate at the terminals of vagal afferents at the NTS (Fig. 1B). The decreased glutamate release resulted in inhibition of both catecholamine and noncatecholamine neurons at the NTS. The mechanism by which the inhibitory effect of ghrelin at the NTS leads to suppression of peripheral sympathetic nerve activity awaits further research. Ghrelin’s effects may also be mediated by efferent vagal signals. Shimizu et al. (71) provided evidence that centrally administered ghrelin activates the efferent vagus nerve, leading to increased acetylcholine release in the heart. Peripherally released acetylcholine, acting on the α7-subunit of the nicotinic acetylcholine receptor, mediates the cholinergic anti-inflammatory pathway (12). Thus, ghrelin’s anti-inflammatory effect appears to be dependent on ghrelin’s ability to shift the autonomic balance: suppressing sympathetic nerve activity on one hand and increasing parasympathetic nerve activity on the other hand.

The AgRP-NPY neurons of the ARC of the hypothalamus, which express GHSR-1a, have also been shown to make synaptic connections with the PVN (25). Ghrelin stimulates the phosphorylation of 5'-adenosine monophosphate-activated protein kinase (AMPK) in AgRP-NPY neurons, leading to activation of of N-type calcium channels (37, 38). The resulting inward calcium current could stimulate the release of GABA from synaptic vesicles. Tong et al. (80) demonstrated that ghrelin signaling at the ARC results in an inhibitory GABAAergic output. GABAAergic inhibition of the PVN could dampen the excitatory output of the PVN to the RVLM, resulting in suppression of sympathetic outflow (Fig. 1B). Indeed, we have previously demonstrated that ghrelin’s inhibitory effect on norepinephrine release and anti-inflammatory effects were attenuated by GHSR-1a antagonist [d-Arg¹,d-Phe⁵,d-Trp⁷,⁹,Leu¹¹]substance P, or an NPY/Y1 inhibitor (87). We measured norepinephrine and cytokine release after treating septic rodents with intracerebroventricular injection of ghrelin. [d-Arg¹,d-Phe⁵,d-Trp⁷,⁹,Leu¹¹]substance P, or an NPY/Y1 inhibitor and found that ghrelin treatment suppressed sepsis-induced norepinephrine and TNF-α release. The inhibitory effect of ghrelin on norepinephrine and cytokine release was abolished with intracerebroventricular administration of [d-Arg¹,d-Phe⁵,d-Trp⁷,⁹,Leu¹¹]substance P, or an NPY/Y1 inhibitor (87).

In other studies, we had previously linked the proinflammatory effects of norepinephrine to activation of the p38 MAP kinase pathway (56). Ghrelin administration in sepsis was shown to antagonize the p38 MAP kinase pathway by upregulating MAP kinase phosphatase-1 (31). Ghrelin, through its mechanisms of sympathoinhibition and suppression of inflammation, was shown to rescue rodents from sepsis-associated multiple-organ failure by attenuating acute lung injury and intestinal barrier dysfunction (85, 86). Sepsis may also be aggravated in the presence of other kinds of tissue injury that suppress the immune system. Radiation injury suppresses the immune system, and Turai et al. (81) found in their studies that sepsis was the main cause of death in recent incidents of radiation accidents. Human ghrelin was shown to decrease proinflammatory cytokine levels and improve survival in an animal model of radiation combined with sepsis (69).
A notable exception to the sympathoinhibitory effect of ghrelin is the finding of Lambert et al. (43) that intravenous infusion of ghrelin resulted in elevation of muscle sympathetic nerve activity in both healthy lean and overweight individuals. The exact mechanism for the seemingly opposite effects of ghrelin on muscle sympathetic nerve activity compared with other organs, such as kidney, is unknown. However, one may speculate that the organotypically regulated groups of RVLM neurons (53) are probably differentially regulated by ghrelin. Further mechanistic studies are required to determine the possible organotypic regulation of the RVLM by ghrelin.

**Ghrelin and Age-Related Changes in Sympathetic Nerve Activity**

Sepsis in the elderly constitutes 65% of all cases of sepsis, with a higher case fatality rate than in younger patients (50). The increased severity of sepsis with age is partly due to an exaggerated inflammatory response. Tateda et al. (79) showed that aged rodents were more sensitive to LPS and produced greater amounts of TNF-α, IL-1α, and IL-6 than younger rods after intraperitoneal LPS challenge. Roubenoff et al. (64) reported similar findings in human subjects, with IL-6, IL-1Ra, and C-reactive protein observed to be higher in elderly rodents after intraperitoneal LPS challenge. Roubenoff et al. (64) reported similar findings in human subjects, with IL-6, IL-1Ra, and C-reactive protein observed to be higher in elderly patients than in their younger counterparts. The greater cytokine release in the elderly during sepsis may be due to an age-related increase in sympathetic nerve activity. Norepinephrine release driven by the central sympathetic network is increased in older men, whereas epinephrine release from the adrenal medulla has been shown to decrease with age (24, 68). Nonetheless, epinephrine levels in the elderly are still higher due to markedly decreased epinephrine clearance (68). Leong et al. (45) have linked the hyperinflammatory response in the elderly to α2A-adrenoceptor-mediated activation of the CD14/TLR4 pathway. Starr et al. also discovered that the increased mortality of sepsis in the elderly is related to a hypercoagulable state mediated by age-related suppression of the protein C anticoagulant pathway. This hypercoagulable state exacerbates microvascular thrombosis associated with disseminated intravascular coagulation in late sepsis (76). Increased release of norepinephrine in sepsis may also contribute to disseminated intravascular coagulation, as it has been shown that α2A-adrenergic receptor stimulation promotes platelet activation and aggregation, resulting in thrombosis (62).

The increased sympathetic nerve activity and exaggerated cytokine release associated with sepsis in the elderly may be explained by an age-related decrease in ghrelin receptor GHSR-1a, in the brain. In our previous study (88), comparing 3-mo-old with 24-mo-old rodents, we found that in the aged rodents GHSR-1a expression was decreased at the dorsal vagal complex, which includes the NTS. We demonstrated that LPS challenge in the aged rodents, with decreased GHSR-1a, resulted in greater cytokine release compared with younger rodents. Moreover, treatment of young rodents with GHSR-1a antagonist resulted in increased cytokine release (88). The effect of decreased GHSR-1a expression in aged rodents on catecholamine release will be similar to chronic GHSR-1a antagonism, which was shown by Sato et al. (65) to result in elevated blood levels of norepinephrine and epinephrine. Treatment of aged rodents with ghrelin, together with growth hormone, restored GHSR-1a levels and attenuated cytokine release (88). Thus, ghrelin is a promising therapeutic agent for the severe sepsis seen in the elderly.

**Perspectives and Future Directions**

The further development of sympathoinhibition as an anti-inflammatory therapy for sepsis should be guided by the current practice recommendations of the surviving sepsis campaign (SSC). Among the SSC recommendations (19), adequate fluid resuscitation is fundamental to the hemodynamic management of the septic patient. However, vasopressor support, preferably norepinephrine or dopamine, is often required as an early emergency measure in severe shock patients to maintain mean arterial pressure. It is important to acknowledge that different pathogenic mechanisms are at play at various stages of the sepsis-septic shock spectrum. Some of the early mechanisms in sepsis have a cascading effect, which leads to other mechanisms in the late stage of septic shock. Sympathoinhibition is one of the early mechanisms of sepsis that contributes to the late stages of the sepsis-septic shock spectrum. Early norepinephrine-mediated hepatocellular dysfunction leads to perturbation of the cytochrome P-450 enzyme-metabolic pathway, which may severely affect antibiotic efficacy, one of the cornerstones of sepsis management. Proinflammatory cytokines, released by norepinephrine stimulation of α2A-adrenergic receptor, lead to overproduction of nitric oxide. Nitric oxide contributes to vasoplegia and loss of mean arterial pressure in the late stages of shock (46), requiring vasopressor support. Thus, sympathoinhibition in early sepsis may be beneficial in arresting some of the late developments of the sepsis-shock cascade.

In summary, ghrelin ameliorates sepsis-associated organ injury and improves survival through suppression of central sympathetic outflow, resulting in suppression of inflammation. The beneficial effects of ghrelin through sympathoinhibitory suppression of inflammation have been demonstrated so far in the rodent hypodynamic sepsis model. Some investigators also advocate for experimental studies using the hyperdynamic sepsis seen in higher-order mammals and humans (77), where ghrelin’s beneficial effects on sepsis may be further tested. In conclusion, ghrelin-mediated sympathoinhibition is a promising therapy for sepsis that needs further development. Future preclinical research into ghrelin-mediated sympathoinhibition may include more neurophysiological studies as well as dose-response and time-course studies in animal models of sepsis.

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