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Human Ghrelin Protects Animals from Renal Ischemia-Reperfusion Injury through the Vagus Nerve

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Abstract

BACKGROUND—Acute kidney injury (AKI) secondary to renal ischemia and reperfusion (I/R) injury is widely prevalent. Ghrelin, a stomach-derived peptide, has been shown to be anti-inflammatory. The purpose of this study was to examine whether human ghrelin has any beneficial effects after renal I/R injury and if so, whether ghrelin's action in renal I/R injury is mediated by the vagus nerve.

METHODS—Male adult rats were subjected to renal I/R by bilateral renal pedicle clamping for 60 min, treated intravenously with human ghrelin (4 nmol/rat) or normal saline (vehicle) immediately following reperfusion. After 24 h, the animals were euthanized, and samples were harvested. In separate groups, subdiaphragmatic vagotomy prior to renal I/R was performed, treated with human ghrelin or vehicle, and at 24 h, blood and organs were harvested.

RESULTS—Renal I/R injury caused significant increases in the serum levels of tissue injury markers as compared to sham operation. Human ghrelin treatment significantly attenuated serum creatinine and BUN, by 55% and 53%, and liver enzymes (AST and ALT) by 20% and 24%, respectively as compared to vehicle-treated groups. Tissue water contents, plasma and kidney IL-6 and kidney MPO activity were reduced. Bcl-2/Bax ratio was increased, and histology of the kidneys was improved. More importantly, prior vagotomy abolished ghrelin's protective effect in tissue injury markers and tissue water contents in renal I/R injured animals.

CONCLUSION—Human ghrelin treatment in renal I/R injured rats attenuated systemic and kidney specific inflammatory responses. The protection of human ghrelin in renal I/R injury was mediated by the vagus nerve. These data suggest ghrelin can be developed as a novel treatment for patients with AKI induced by renal I/R injury.

Keywords

acute kidney injury; renal ischemia-reperfusion; ghrelin; inflammation

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INTRODUCTION

Acute kidney injury (AKI) is a critical clinical problem which poses a significant economic and financial burden on the society.^{1–3} Acute kidney injury occurs in various clinical settings with manifestations ranging from a minimal but sustained elevation in serum creatinine to anuric renal failure. Although the incidence of AKI in the community is very low, it is quite common in hospitalized patients, affecting 3–7% of general admissions, and as much as 25–30% of patients in intensive care units.^{4,5} Current strategies used to prevent AKI consist mainly of fluid resuscitation and diuretics, and/or the prevention of the insinuating factor. Despite these efforts, the mortality remains unacceptably high and has not improved in more than 40 years.⁶ There is an urgent need to develop therapeutic agents to fight this disease.

During renal ischemia and reperfusion (I/R) injury, renal damage begins immediately from the onset of ischemia. Upon restoration of perfusion, however, the tissues undergo further injury. Reperfusion injury involves the accumulation of neutrophils, generation of free oxygen radicals, and cytokine activation. These changes may also be seen histopathologically, as demonstrated by the loss of the brush border, tubular disruption, and cast formation.⁷ Renal damage due to ischemia/reperfusion injury occurs as early as 5 h following injury as evidenced by a rising serum lactate, TNF- α , IL-6 and TGF- β levels, as well as decreasing systemic venous oxygen levels.⁸ In the clinical setting, serum markers such as blood urea nitrogen and creatinine, are regarded as gold standards for renal compromise, but these markers may not become elevated until 24h after the initial injury. Studies looking at early biomarkers, such as keratinocyte-derived chemokine (KC) and neutrophil gelatinase-associated lipocalin (NGAL), demonstrate that increases in these markers are associated with the development of AKI^{9,10}. With a better understanding of the pathophysiology of AKI as well as the identification of new biomarkers, one is able to determine the actual time point in the evolution of renal compromise pharmacological or hormonal therapy would be beneficial.

Ghrelin is an orexigenic hormone identified as an endogenous ligand for the growth hormone secretagogue receptor (GHSR).¹¹ Human ghrelin is a 28-amino acid peptide and has an n-octanoyl group at Ser3, a modification essential for its activity and not previously reported in a human peptide.^{12,13} Rat ghrelin differs from human ghrelin by only two amino acids.¹³ Ghrelin is produced predominantly by the stomach, with substantially lower amounts derived from other central and peripheral tissues.^{14,15} The biological effects of ghrelin are mediated through the ghrelin receptor GHSR. Although a group of synthetic molecules featuring a growth hormone secretagogue can bind to GHSR, ghrelin is the only identified endogenous ligand for this receptor. GHSR is found in the pituitary, hypothalamus, stomach, heart, blood vessels, lungs, pancreas, intestines, kidneys, adipose tissue, and immune system (B and T cells, neutrophils).^{16–18} The wide distribution of the ghrelin receptor indicates multiple paracrine, autocrine and endocrine roles for ghrelin. The presence of appreciable amounts of GHS binding sites and mRNA in the cardiovascular system suggests that ghrelin has direct cardiovascular effects through growth hormone-independent mechanisms.^{19,20} Exogenous ghrelin injections stimulate prolactin, ACTH, and cortisol secretion. Ghrelin also induce hyperglycemia, decrease insulin levels, and exert orexigenic effects in rodents and humans.^{21,22} In addition to its growth hormone-releasing properties²³, ghrelin possesses other endocrine and nonendocrine activities reflecting central and peripheral GHSR-1a distribution.^{24,25} Administration of ghrelin has been shown to be protective following heart failure and in cardiac ischemia/reperfusion injury.^{26,27} Additionally, we have shown ghrelin is beneficial in other disease conditions, including sepsis and intestinal I/R injury.^{28,29}

A large body of evidence indicated that the physiological function of ghrelin is mediated by the central and peripheral receptor distributions and the wide array of GHSR presence suggests diverse pathways for ghrelin action.²⁴ One of these studies demonstrates the presence of such GHSR in afferent neurons of nodose ganglia suggesting that ghrelin signals are transmitted to the brain by the vagal afferent nerves.³⁰ Bernik et al³¹ have shown that electrical stimulation of the vagus nerve suppresses serum and organ TNF levels in rats with ischemia/reperfusion injury caused by cardiac occlusion. Vagus nerve stimulation also significantly protects animals against the development of hypotension and shock.³¹ Studies have also demonstrated that electrical stimulation of the vagus nerve subsequent to LPS administration in rats prevented the release of TNF- α from splenic macrophages through the activation of the nicotinic acetylcholine receptor subunit $\alpha 7$, $\alpha 7nAChR$.^{32,33} We have previously shown that ghrelin downregulates pro-inflammatory cytokines in sepsis and gut I/R injury through the activation of the vagus nerve.^{28,29} However, the effects of ghrelin's action, through the vagus nerve, in renal I/R injury has not been elucidated. Therefore, we have investigated if treatment with ghrelin in renal I/R injury in a rat model produces any beneficial effects, and if those effects are mediated through the vagus nerve.

METHODS

Experimental animals

Male Sprague-Dawley rats (250–300g), purchased from Charles River Laboratories (Wilmington, MA), were used for this study. The rats were housed in a temperature controlled room and on a 12 h light/dark cycle. The rats were fed a standard Purina rat chow diet and allowed water *ad libitum* and were acclimated for one week after arrival. The animal experiments carried out were in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources). This project was approved by the Institutional Animal Care and Use Committee (IACUC) of The Feinstein Institute for Medical Research.

Animal model of renal I/R injury

Rats were fasted overnight but given water *ad libitum*. Isoflurane inhalation was used for induction and maintenance of anesthesia. Renal I/R was performed as previously described^{34,35}. Briefly, a midline laparotomy incision was made to expose the abdomen, and the intestines were retracted to expose the renal pedicles. Microvascular clamps were placed around first the left and then the right renal pedicles. The total clamp time was 60 min, during which the intestines were covered in warm and moist gauze. Afterwards, the clamps were removed and the restoration of blood flow into the kidneys was visually confirmed. The intestines were then returned into the abdominal cavity and the incision was then closed in layers. Hence, both renal ischemia and reperfusion are components of this experimental injury. Sham-operated animals underwent the same procedure with the exception that the renal pedicles were not exposed or occluded. The animals were allowed to recover in their cages with food and water. At 24 h post surgery, the animals were euthanized, and blood and tissue samples were harvested for analyses.

Subdiaphragmatic vagotomy prior to renal I/R injury

In additional groups of animals, the trunks of the subdiaphragmatic vagus nerve were transected as previously described.²⁸ Briefly, before renal I/R injury, a 2-cm ventral midline abdominal incision was made. The dorsal and ventral branches of the vagus nerve were carefully exposed and dissected away from the esophagus. Each branch of the vagus nerve was tied with surgical sutures at two points separated by 1 cm, and then severed between the sutures. Sham-vagotomized animals underwent the same surgical procedure with the exception that their vagus nerves were neither tied nor severed. After the surgery, the

animals then underwent renal I/R injury followed by human ghrelin treatment as described above.

Experimental groups

Prior to surgery, animals were randomly selected into three groups; Sham group (sham operation), Ghrelin group (renal I/R rats treated with human ghrelin at the end of) and Vehicle group (renal I/R rats treated with normal saline). In additional groups, rats were divided into three groups; Ghrelin Vagotomy (rats treated with human ghrelin in those that underwent vagotomy just prior to renal I/R injury), Vehicle Vagotomy (rats treated with normal saline in those that underwent vagotomy just prior to renal I/R injury), and Sham Vagotomy (rats that were neither subjected to vagotomy nor renal I/R injury).

Administration of human ghrelin

Human ghrelin (4 nmol/rat) was slowly infused over 30 min in a volume of 1 ml normal saline immediately after removal of the microvascular clamps, (i.e., at the beginning of reperfusion). This dose of human ghrelin was determined from a previous model of injury.²⁹ Vehicle treated animals received 1 ml of normal saline infused over a period of 30 min.

Determination of serum levels of organ injury markers

Blood samples were centrifuged for 15 min at 2,000 g to collect serum, and stored at -80°C for determination of serum levels of creatinine, blood urea nitrogen (BUN), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The levels were measured using commercially available assay kits according to manufacturer's specifications (Pointe Scientific, Canton, MI).

Determination of water content

The water content in the kidney, liver, lung and gut was determined by drying freshly harvested organs in 70°C for 48 h. Water content was calculated as $\% \text{H}_2\text{O} = (1 - \text{dry wt/wet wt}) \times 100\%$.

Determination of IL-6 levels

IL-6 levels in serum and renal tissues were quantified using an enzyme-linked immunosorbent assay kit specifically for rat IL-6 (BD Biosciences Pharmingen, San Diego, CA). The assay was carried out according to the instructions provided by the manufacturer. Renal IL-6 levels were normalized to the protein concentration in the sample as determined by using Bio-Rad DC Protein Assay Kit (Bio-Rad, Hercules, CA).

Granulocyte myeloperoxidase (MPO) assessment

Neutrophil accumulation in the kidneys was estimated using the MPO activity assay as previously described.³⁶ Briefly, 100 mg samples of tissue were resuspended in phosphate buffer, sonicated and cleared by centrifugation. The protein concentration of supernatants was determined by using Bio-Rad DC Protein Assay Kit (Bio-Rad, Hercules, CA). MPO assay was carried out as previously described and MPO activity (1 unit defined as change in absorbance of 1 per min) was expressed as units per gram of tissue.

Determination of protein levels of Bcl-2 and Bax

Protein was extracted from renal tissues and fractionated on 4–12% Bis-Tris gels and transferred to 0.45 μm nitrocellulose membranes. The membrane was blocked and then incubated with 1:1000 dilution of anti-Bcl-2 or anti-Bax monoclonal antibody (Santa Cruz

Biotechnology, Santa Cruz, CA) overnight at 4°C, followed by incubation in 1:10,000 dilution of HRP-linked anti-mouse IgG for 1 hour at room temperature. To reveal the reaction bands, the membrane was treated with a chemiluminiscent peroxidase substrate (ECL; Amersham, Piscataway, NJ) and exposed to radiography film. The band densities were determined with the use of a Bio-Rad Laboratories image system and the densitometric measurements were used to calculate the ratio between Bcl-2 and Bax proteins.

Determination of tubular damage by histology

Renal tissues were fixed in 10% formalin and paraffin-embedded. The tissue blocks were then sectioned to 5 µm in thickness and stained with hematoxylin and eosin, dehydrated, and coverslipped. Morphological examinations were performed with the use of a light microscope and documented by photographs. Changes in the cortex and in the outer stripe of the outer medulla (OSOM) were assessed by quantitative measurements of tissue damage³⁷. Tubular damage was defined as tubular epithelial swelling, loss of brush border, vacuolar degeneration, necrotic tubules, cast formation, and desquamation. The degree of kidney damage was estimated by the following criteria: 0, normal; 1, area of damage <25% of tubules; 2, damage involving 25–50% of tubules; 3, damage involving 50–75% of tubules; and 4, 75–100% of the area being affected.

Statistical analysis

All data are expressed as mean ± SE and compared by one-way analysis of variance (ANOVA) and Student-Newman-Keuls test. Differences in values were considered significant at $P < 0.05$.

RESULTS

Effect of ghrelin on serum creatinine and BUN after renal I/R injury

To determine the effect of human ghrelin in renal I/R injury, rats were either treated with human ghrelin or normal saline (vehicle) for 24 h after reperfusion. We examined the alterations in serum creatinine (Figure 1A) and BUN (Figure 1B), which are specific systemic markers of renal injury. Rats subjected to renal I/R injury had a significant increase in serum creatinine and BUN by 302% and 368% in vehicle treated animals as compared to sham, respectively ($P < 0.05$). Human ghrelin treatment significantly decreased serum creatinine and BUN levels by 55% and 53% as compared to vehicle group, respectively ($P < 0.05$).

Effect of ghrelin on systemic markers of organ injury after renal I/R injury

To examine whether other organ injury markers are altered due to renal I/R injury, serum levels of AST (Figure 1C) and ALT (Figure 1D) were measured. Rats subjected to renal I/R injury had a significant increase in serum AST and ALT by 199% and 65% as compared to sham, respectively ($P < 0.05$). Human ghrelin treatment significantly decreased serum AST and ALT by 20% and 24% as compared to vehicle in renal I/R injured rats, respectively ($P < 0.05$).

Effect of ghrelin on tissue water content after renal I/R injury

Tissue water content reflected by tissue edema is an indirect measure of tissue injury. To determine the changes in tissue water content, organs were harvested for analysis. The water content in the kidneys significantly increased from $75.0 \pm 0.3\%$ in sham animals to $78.7 \pm 0.4\%$ in vehicle treated animals (Figure 2A). Following treatment with ghrelin, kidney water content significantly decreased to $77.43 \pm 0.4\%$ (Figure 2A; $P < 0.05$). Water content in the liver, gut and lungs were significantly increased from $68.9 \pm 0.3\%$, $75.4 \pm 0.5\%$ and $75.2 \pm$

0.2% in sham-operated animals to $70.27 \pm 0.1\%$, $77.0 \pm 0.2\%$ and $78.6 \pm 0.2\%$, respectively, in vehicle treated animals (Figures 2B–D). Treatment with human ghrelin significantly decreased the amount of edema in these organs. In addition, there were no statistical differences between sham-operated and human ghrelin treated renal I/R animals in the liver and gut water contents but there were statistically significant differences between these groups in the kidney and lung water contents.

Effect of ghrelin in serum and kidney IL-6 levels after renal I/R injury

To examine whether human ghrelin affects renal I/R induced proinflammatory cytokine levels, serum and kidney levels of IL-6 were measured. Rats subjected to renal I/R injury had a significant increase in serum and kidney IL-6 by 208% and 49% as compared to sham respectively ($P < 0.05$). Human ghrelin significantly decreased the levels by 30% and 18%, respectively ($p < 0.05$).

Effect of ghrelin on kidney MPO activity after renal I/R injury

An increase in MPO activity denotes increased neutrophil infiltration. Figure 4A shows alterations in MPO activity in the kidney in sham, vehicle, and human ghrelin treated animals. Rats subjected to renal I/R injury had a significant increase in MPO activity in the kidney from 0.71 ± 0.4 in sham-operated animals to 2.8 ± 0.2 in vehicle treated animals. Human ghrelin treatment significantly reduced MPO activity in the kidney to 1.7 ± 0.2 in renal I/R injured rats ($P < 0.05$).

Effect of ghrelin on Bcl-2/BAX ratio in the kidney after renal I/R injury

To assess whether human ghrelin alters renal I/R induced apoptosis, alterations in Bcl-2/BAX ratio in renal tissues were assessed (Figure 4B). Rats subjected to renal I/R injury had a significant decrease in Bcl-2/BAX ratio from 0.12 ± 0.02 in sham-operated animals to 0.04 ± 0.02 in vehicle treated animals. Human ghrelin treatment significantly increased Bcl-2/BAX ratio (0.14 ± 0.03) after renal I/R injury ($P < 0.05$).

Effect of ghrelin on kidney morphology after renal I/R injury

In terms of the histopathological changes, tubular destruction, and loss of brush border, casts were observed microscopically in the rat kidney after I/R injury. The tubular injury in outer stripe of the outer medulla (Figure 5A) and cortical areas (Figure 5B) was significantly increased at 24 hours after reperfusion in vehicle treated group as compared with that in sham-operated animal. Administration of human ghrelin at the onset of reperfusion reduced the injury score by 31% in outer stripe of the outer medulla and 33% in cortical areas ($P < 0.05$).

Effects of ghrelin on organ injury and tissue water content after renal I/R injury in vagotomized animals

To examine whether ghrelin's beneficial effects in renal I/R injury are mediated by the vagus nerve, a subdiaphragmatic vagotomy was performed in rats prior to renal I/R injury. As shown in Table 1, vagotomized rats demonstrated increased circulating levels of creatinine, BUN, AST and ALT compared to sham vagotomized rats ($P < 0.05$). Ghrelin treatment in vagotomized rats did not alter the circulating levels of the above parameters from that of vehicle in vagotomized rats. Likewise, ghrelin treatment did not reduce the water content in the vagotomized rats.

DISCUSSION

Acute kidney injury [is] a protean syndrome of varied severity and manifestations, ranging from a minimal but sustained elevation in serum creatinine to anuric renal failure. Acute renal ischemia with reperfusion is likely to be a component of the clinical syndrome but rarely its sole cause. For purposes of diagnosis and management, AKI has been characterized as diseases associated with pre-, intra- and post-renal states. Pre-renal AKI which accounts for 55–60% cases is a state when renal hypoperfusion occurs due to a mild or moderate insult in which the integrity of renal parenchymal tissue is still preserved. Intra-renal AKI is a state in which renal parenchymal tissue is involved and it accounts for 35–40%, and post-renal AKI refers to diseases due to acute obstruction of the urinary tract and it constitutes <5% of the cases. Acute injury to the renal tubules, either mediated by ischemia or toxins, account for greater than 90% of cases of intra-renal AKI. Renal I/R injury, which leads eventually to AKI, is a serious condition also affecting intensive care patients. There is a rising trend in all-cause renal injury with a case fatality rate that has approached 50% among patients requiring dialysis. Current therapy is geared towards supportive treatment, ranging from intravenous fluid boluses to temporary dialysis. In view of the very limited treatment options, the high morbidity and mortality of the disease, and the significant financial burden, there is an obvious unmet medical need for a specific therapeutic agent.

In the present study, we show that human ghrelin administered as a single dose immediately following ischemia, (i.e., at the beginning of reperfusion), protects the kidneys from I/R injury by attenuating tissue injury indicators (creatinine, BUN, AST and ALT), downregulating systemic and local IL-6 levels, decreasing neutrophil infiltration to the kidneys, and improving histological integrity. Thus, we demonstrated that ghrelin attenuates renal tissue damage and improves renal function following renal I/R injury.

There is strong evidence that I/R and/or inflammation lead to a significant increase in the expression of IL-6 in many organs including the brain,³⁸ myocardium,³⁹ hind limb,⁴⁰ and gut.⁴¹ Recently it was shown that renal I/R injury was associated with a significantly lower level of inflammation in IL-6 knockout mice. Additionally, endogenous IL-6 enhances the degree of renal injury, dysfunction, and inflammation caused by I/R of the kidney by promoting the expression of adhesion molecules and subsequent oxidative and nitrosative stress.⁴² Systemic IL-6 levels have been shown to be predictors of mortality in end-stage renal disease patients.⁴³ In this study, we have shown that ghrelin significantly reduces IL-6 levels in the kidneys as well as in serum. The attenuation of the IL-6 production may also be responsible for the attenuation of tissue edema in the kidney as well in remote organs, i.e., the liver, gut and lungs, as well as an improvement of liver function. This finding is consistent with our previous observation that ghrelin has anti-inflammatory properties, by attenuating IL-6 levels in sepsis and gut I/R injury.^{29,44}

It is believed that the Bcl-2/Bax ratio is a determining factor for the cell's fate for apoptosis.⁴⁵ Bcl-2 inhibits apoptosis by preventing mitochondrial membrane depolarization⁴⁶ whereas Bax promotes apoptosis by inducing mitochondrial membrane depolarization and cytochrome *c* release.⁴⁷ Our study showed that the Bcl-2/Bax ratio is decreased by renal I/R insult, whereas ghrelin treatment results in complete restoration of the Bcl-2/Bax ratio to normal levels. Similar findings were observed in simian virus 40 murine mesangial cells, in which high glucose decreased the Bcl-2/Bax ratio, whereas ligand activation of the IGF-1 receptor increased it⁴⁸. Ghrelin's beneficial effect on renal I/R injury could be mediated in part by attenuating I/R induced apoptosis.

Our results also showed that ghrelin induced protection in renal I/R injury is most likely due to the central effect of ghrelin leading to vagus nerve activation and subsequent

downregulation of inflammatory responses. The stimulation of the cholinergic pathway has been shown to be beneficial in reducing inflammatory responses^{32,33}. The cholinergic anti-inflammatory pathway is described as a physiological mechanism in which local inflammation activates the afferent (sensory) fibers of the vagus nerve to trigger anti-inflammatory brain signals that transmit through the efferent (motor) vagus nerve. Several studies indicate that action potentials transmitted in the vagus nerve traverse the subdiaphragmatic vagus nerve, the celiac ganglion and the splenic nerve^{49,50}. The neural signals in the spleen suppress the immune activation of the splenic macrophages through a molecular mechanism that requires signal transduction through the nicotinic acetylcholine receptor, $\alpha 7$ nAChR^{50,51}. Recent studies indicate that $\alpha 7$ nAChR has been expressed in the kidneys⁵². However, it is not known whether the $\alpha 7$ nAChR associated immune suppression can be a possible mechanism of ghrelin induced benefits in renal I/R injury. Further studies are needed to investigate this concept.

Physiological functions of ghrelin are mediated by the central and peripheral GHSR receptors. Date et al reported that ghrelin activates the vagus nerve and that blockade of the gastric vagal afferent abolishes ghrelin-induced feeding and growth hormone secretion⁵³. Central administration of ghrelin stimulates the vagal efferent nerve in anesthetized rats⁵⁴. In addition, intravenous and intracerebroventricular injection of ghrelin has been shown to induce an increase in vagal efferent discharge^{55,56}. The mechanism of ghrelin's protection towards inflammatory responses in various pathological conditions has been unraveling over the past few years. In this regard, we have previously shown that subdiaphragmatic vagotomy in rats prevents the anti-inflammatory effects of ghrelin in sepsis, radiation combined injury and gut I/R injury^{28,29,57}. In the present study, we showed that ghrelin's protective effect in renal I/R injury is indeed mediated by the vagus nerve. The vagus nerve is an important link between the involuntary nervous system and pro-inflammation. It has been widely reported that the sympathetic and parasympathetic nervous systems regulate multiple inflammatory responses.

In addition to vagus nerve activation, ghrelin has been reported to inhibit sympathetic activity. Centrally administered ghrelin inhibits lipolysis in adipose tissues, which is mediated by a decrease in sympathetic activity⁵⁸. Recent studies have shown a decrease in sympathetic activity in the kidneys after intravenous or intracerebroventricular injection of ghrelin⁵⁸⁻⁶⁰. Since ghrelin can pass the blood brain barrier⁶¹, it is possible that the observed anti-inflammatory effects of ghrelin in renal I/R injured rats is due to its sympathoinhibitory features. Taken together, this data suggests that the protective effect of ghrelin in renal I/R injury can be attributed to possible rebalance of the dysregulated sympathetic and the parasympathetic nervous system in renal I/R injury.

In summary, we show that a single dose of ghrelin²⁹ administered immediately following ischemia, at the beginning of reperfusion, attenuates renal I/R injury in rats. The protective effect of ghrelin is mediated via the vagus nerve. Future studies are needed to show if delayed administration, and various doses of human ghrelin, have any effect in a model of renal I/R injury. Nevertheless, these data suggest human ghrelin can be developed as a novel treatment for renal I/R injury.

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LIST OF ABBREVIATIONS

AKI	acute kidney injury
I/R	ischemia-reperfusion
IL-6	interleukin-6
BUN	blood urea nitrogen
AST	aspartate aminotransferase
ALT	alanine aminotransferase
GHSR	Growth hormone secretagogue receptor
ACTH	adrenocorticotrophic hormone
TNF	tumor necrosis factor
MPO	myeloperoxidase
OSOM	outer stripe of the outer medulla
NO/cGMP	nitric oxide/cyclic guanosine monophosphate

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Figure 1.

Alterations in serum levels of systemic injury markers after renal I/R injury. Data are presented as mean $\pm$ SEM (n = 5–8) and compared with one-way ANOVA and Student-Newman-Keuls method: * p < 0.05 versus Sham group; # p < 0.05 versus Vehicle group.

Figure 2.

Alterations in water content of systemic organs after renal I/R injury. Data are represented as mean $\pm$ SEM (n =5–8) and compared by one-way ANOVA and Student-Newman-Keuls method: * p<0.05 versus Sham group, # p<0.05 versus Vehicle group.

Figure 3. Alterations in serum (A) and kidney (B) levels of IL-6 after renal I/R injury. Data are represented as mean $\pm$ SEM (n =5–8) and compared by one-way ANOVA and Student-Newman-Keuls method: * p<0.05 versus Sham group, # p<0.05 versus Vehicle group.

Figure 4.

Alterations in kidney MPO levels (A) and Bcl-2 BAX ratio (B) after renal I/R injury. Data are represented as mean $\pm$ SEM (n = 5–8) and compared by one-way ANOVA and Student-Newman-Keuls method: * p < 0.05 versus Sham group, # p < 0.05 versus Vehicle group.

Figure 5A

Figure 5B

Figure 5.

Figure 5A. Alterations in the morphology of the kidney outer stripe of the outer medulla after renal I/R injury. Representative photomicrographs of the renal outer stripe of the outer medulla section from sham operated (A), vehicle treated (B), and ghrelin treated (C) renal I/R injured rats, and histopathologic scoring of tubular injury in OSOM (D). Data are represented as mean $\pm$ SEM and compared by one-way ANOVA and Student-Newman-Keuls method: * $p<0.05$ versus Sham group, # $p<0.05$ versus Vehicle group.

Figure 5B. Alterations in the morphology of the kidney cortex after renal I/R injury. Representative photomicrograph of the renal cortex section from a sham-operated (A) vehicle treated (B) and ghrelin treated (C) renal I/R injured rats, and histopathologic scoring of tubular injury in cortex of the kidney (D). Data are represented as mean $\pm$ SEM and compared by one-way ANOVA and Student-Newman-Keuls method: * $p<0.05$ versus Sham group, # $p<0.05$ versus Vehicle group.

Table 1

Effect of subdiaphragmatic vagotomy on serum levels of organ injury markers at 24 h post renal I/R injury.

	Sham Vagotomy	Renal I/R	
		Vehicle Vagotomy	Ghrelin Vagotomy
Creatinine (mg/dL)	0.357 ± 0.12	2.14 ± 0.40 [*]	1.86 ± 0.27 [*]
BUN (mg/dL)	20.79 ± 0.41	47.42 ± 11.76 [*]	53.10 ± 7.21 [*]
AST (IU/L)	18.04 ± 1.22	56.56 ± 1.93 [*]	54.10 ± 3.37 [*]
ALT (IU/L)	17.90 ± 1.99	31.86 ± 1.83 [*]	31.33 ± 3.66 [*]
Kidney water content (%)	75.36 ± 0.17	77.91 ± 0.13 [*]	77.45 ± 0.20 [*]
Liver water content (%)	68.32 ± 0.35	70.06 ± 0.32 [*]	70.49 ± 0.34 [*]
Gut water content (%)	75.39 ± 0.28	76.99 ± 0.18 [*]	76.65 ± 0.25 [*]
Lung water content (%)	75.65 ± 0.31	78.09 ± 0.27 [*]	77.59 ± 0.38 [*]

Data are presented as mean ± SE (n =4–5) and compared by one-way ANOVA and Student-Newman-Keuls method:

^{*} p < 0.05 versus Sham group.