LPS inhibits fasted plasma ghrelin levels in rats: role of IL-1 and PGs and functional implications

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Wang, Lixin, Nicole R. Basa, Almaas Shaikh, Andrew Luckey, David Heber, David H. St-Pierre, and Yvette Taché. LPS inhibits fasted plasma ghrelin levels in rats: role of IL-1 and PGs and functional implications. Am J Physiol Gastrointest Liver Physiol 291: G611–G620, 2006; doi:10.1152/ajpgi.00533.2005.—LPS injected intraperitoneally decreases fasted plasma levels of ghrelin at 3 h postinjection in rats. We characterized the inhibitory action of LPS on plasma ghrelin and whether exogenous ghrelin restores LPS-induced suppression of food intake and gastric emptying in fasted rats. Plasma ghrelin and insulin and blood glucose were measured after intraperitoneal injection of LPS, intravenous injection of IL-1β and urocortin 1, and in response to LPS under conditions of blockade of IL-1 or CRF receptors by subcutaneous injection of IL-1 receptor antagonist (IL-1Ra) or astressin B, respectively, and prostaglandin (PG) synthesis by intraperitoneal indomethacin. Food intake and gastric emptying were measured after intravenous injection of ghrelin at 5 h postintraperitoneal LPS injection. LPS inhibited the elevated fasted plasma ghrelin levels by 47.6 ± 4.9%, 58.9 ± 3.3%, 74.4 ± 2.7%, and 48.9 ± 8.7% at 2, 3, 5, and 7 h postinjection, respectively, and values returned to preinjection levels at 24 h. Insulin levels were negatively correlated to those of ghrelin, whereas there was no significant correlation between glucose and ghrelin. IL-1Ra and indomethacin prevented the first 3-h decline in ghrelin levels induced by LPS, whereas astressin B did not. IL-1β inhibited plasma ghrelin levels, whereas urocortin 1 had no influence. Ghrelin injected intravenously prevented an LPS-induced 87% reduction of gastric emptying and 61% reduction of food intake. These data showed that IL-1 and PG pathways are part of the early mechanisms by which LPS suppresses fasted plasma ghrelin and that exogenous ghrelin can normalize LPS-induced-altered digestive functions.

insulin; food intake; gastric emptying; urocortin 1; prostaglandins; interleukin-1; lipopolysaccharide

Ghrelin is a 28-amino acid peptide produced primarily from X/A-like cells found in the gastric oxyntic mucosa, which is the endogenous ligand for the growth hormone (GH) secretagogue receptors (GHS-R) and stimulates GH release (27, 51). The rise in circulating ghrelin before the onset of a meal and its abrupt fall postprandially are consistent with a role in triggering premeal hunger and promoting initiation of food intake in animals and humans (11, 17, 47). Conditions of negative energy balance, including restriction of caloric intake during fasting, result in elevated levels of plasma ghrelin that return to basal levels after refeeding (20, 26, 54, 55). Ghrelin is also involved in the regulation of digestive processes as shown by the stimulation of gastric acid secretion and motility (27, 34, 41). Bacterial LPS is widely used to study the innate defense response to gram-negative bacteria infection (30). In particular, LPS administered systemically at low doses induces anorexia, rise in body temperature, and delayed gastric emptying, which are parts of the manifold systemic symptoms of infection related to the “acute-phase responses” (31, 52). We previously provided the first evidence that LPS, injected intraperitoneally at a low dose (100 μg/kg), reducing food intake and inducing a mild thermogenic response, lowered the elevated circulating levels of ghrelin induced by fasting as monitored 3 h after LPS injection in rats (5). Some clinical studies reported that humans chronically infected with the gram-negative bacteria Helicobacter pylori display lower plasma ghrelin levels than H. pylori-negative subjects of comparable body mass index (23, 39). Conversely, H. pylori eradication results in a rise of integrated levels of ghrelin (38). Mechanisms responsible for LPS-induced suppression of circulating levels of ghrelin are still unknown. The elucidation of transmitters involved in LPS-induced alterations of plasma ghrelin levels may have relevance to the understanding of immune-related suppression of food intake and digestive function and ghrelin regulation during activation of the immune system.

In the present study, we investigated the underlying inhibitory mechanisms contributing to the reduced plasma ghrelin levels in response to a single intraperitoneal injection of LPS at 100 μg/kg that mimics some of the clinical features of acute gram-negative bacterial infection, namely, the reduction of food intake and gastric emptying, without the apparent signs of severe sickness found in endotoxin shock in conscious rats (5, 29, 31). Several reports have indicated that plasma levels of ghrelin can be inhibited in part by increased plasma glucose and insulin levels (59). Because glucose flux and insulin secretion are elevated by LPS injected peripherally at similar doses (29, 33), we concomitantly examined the temporal progression of changes in circulating ghrelin, insulin, and glucose over the 24-h period after LPS injection. LPS stimulates the production of cytokines, including IL-1β and prostaglandins (PGs), that have been involved in the alterations of behavioral and gastric function induced by LPS (12, 53). The contribution of peripheral IL-1 and PGs in LPS-induced inhibition of fasted plasma ghrelin levels was investigated using peripheral injec-

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tion of receptor agonist (IL-1β) and antagonist (IL-1Ra) as well as blockade of PG synthesis by indomethacin. The immune system is well interconnected with the neuroendocrine regulatory system, which results in the activation of the pituitary-adrenocortical (PA) axis after LPS injection (19, 57). We investigated the influence of the long-acting corticotropin-releasing factor (CRF) receptor 1 (CRF1)/CRF2 antagonist, astressin B (45), on the ghrelin response to intraperitoneal LPS and that of activation of the PA axis by peripheral injection of urocortin 1 (60) on circulating levels of ghrelin. Lastly, we examined whether systemic ghrelin administration could restore food intake and gastric emptying inhibited by LPS treatment in fasted rats.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (Harlan, San Diego, CA) weighing 260–350 g were maintained under controlled temperature (21–23°C) and lighting (0600–1800), with ad libitum access to standard rodent chow (Prolab RMH 2500; LabDiet, PMI Nutrition, Brentwood, MO) and tap water. One day before the experiment, rats were kept in individual cages and deprived of food but had free access to tap water. All experimental protocols were conducted at the same time of the day in conscious rats fasted for 18–20 h under the Veterans Administration Animal Component of Research Protocol 99-086-06.

Substances and Treatments

LPS (Escherichia coli, serotype 055:B5; Sigma, St. Louis, MO), IL-1β and IL-1Ra (Amgen, Thousand Oaks, CA), and human octanoylated ghrelin (University of Montréal, QC, Canada) were dissolved in sterile isotonic saline (Sigma). Indomethacin (Sigma) was dissolved in 1% sodium bicarbonate-saline solution. Rat urocortin 1 and astressin B (Clayton Foundation Laboratories, Salk Institute, La Jolla, CA) were stored in powder form at −80°C and dissolved in double-distilled water immediately before administration. Doses of IL-1β, IL-1Ra, urocortin 1, astressin B, and indomethacin were selected based on our previous studies showing biological actions on the stomach or blockade of inhibited gastric responses to IL-1β or CRF (53, 61). Substances were injected in conscious rats either intravenously through a chronic intrajugular catheter, intraperitoneally, or subcutaneously in 0.1, 0.3, and 0.5 ml, respectively.

Blood Sampling

The right external jugular vein was cannulated in nonfasted rats anesthetized with a mixture of ketamine (75 mg/kg ip; Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (5 mg/kg ip; Mobay, Shawnee, KS). The catheter was closed and exteriorized to the back of the neck via subcutaneous tunneling and secured to the skin. Three days afterward, the catheter was opened and blood (0.5 ml) was withdrawn into a syringe while the rat was lightly hand restrained or withdrawn from the heart after rats were anesthetized by CO2 at the end of the experiment. In all the experiments, the initial blood sampling time started between 0900–0930. Blood was collected in chilled Eppendorf tubes containing EDTA (7.5%, 10 µl; Sigma) and aprotinin (0.6 TIU; ICN Pharmaceuticals, Costa Mesa, CA). Plasma was separated from red blood cells that were resuspended in sterile saline and administered back to each rat after each subsequent blood sampling. Plasma was aliquoted and kept at −80°C until RIA.

Hormone Assays and Glucose Measurements

Plasma immunoreactive insulin levels were measured in duplicate with commercially available RIA kits from Linco Research (St. Charles, MI). Plasma levels of total ghrelin were determined by a ghrelin (rat/mouse) RIA kit from Phoenix Pharmaceuticals (Belmont, CA) that detects both acylated and des-acyl-ghrelin. Previous studies (1, 36) using two different RIA kits to measure the active form of the peptide (acylated ghrelin) (37) and total ghrelin showed that plasma levels of total ghrelin and acetylated ghrelin are positively correlated and that the ratio of the two remains constant under a variety of physiological conditions. Therefore, levels of total ghrelin are considered a good surrogate for those of acylated ghrelin. The limit of the assay sensitivity was 54 pg/ml, and the intra- and interassay variations were <5% and <14%, respectively. Blood glucose was measured using a commercially available glucose monitor (One Touch Ultra; LifeScan, Milpitas, CA).

Food Intake and Gastric Emptying Measurements

Food intake was monitored as in our previous studies (61). Fasted rats were given preweighed food, and the remaining food, including spills, was measured 30 min and 1 and 2 h later. Food intake/period was calculated as the difference between the food weights before and after food exposure, and cumulative food intake was calculated by adding the values for each time period.

For gastric emptying, a semiliquid nonnutrient test meal (1.5 ml), consisting of 1.5% methylcellulose (Sigma) and 0.05% phenol red (Sigma), was administered via an intragastric stainless steel cannula to conscious rats. After a period of 20 min, animals were euthanized by CO2 inhalation and the gastric emptying was determined as reported previously (53).

Experimental Protocols

LPS effects on plasma ghrelin and insulin and blood glucose levels: time-course studies. Fasted conscious rats with a chronic intrajugular vein catheter implanted 3 days prior were injected intraperitoneally with either LPS (100 µg/kg) or vehicle (saline), and blood (0.5 ml) was collected through the jugular vein catheter at 0, 1, 2, 3, 5, and 7 h after the intraperitoneal injection of LPS or vehicle for the measurement of plasma ghrelin levels. Blood levels of glucose and plasma levels of ghrelin and insulin were measured.

Effects of intraperitoneal indomethacin on intraperitoneal LPS-induced decrease in plasma ghrelin levels. Fasted rats with the chronically implanted jugular vein catheter were injected intraperitoneally with either indomethacin (10 mg/kg) or vehicle (1% sodium bicarbonate solution) and, 1 h later, with intraperitoneal LPS (100 µg/kg) or saline. Blood was collected at −1, 0, 1, 2, 3, and 5 h after intraperitoneal injection of LPS or vehicle for the measurement of plasma ghrelin by RIA.

Effects of intravenous IL-1β on ghrelin plasma levels and subcutaneous IL-1Ra on intraperitoneal LPS-induced suppression of ghrelin plasma levels. Fasted rats with a chronically implanted catheter in the jugular vein were injected intravenously with IL-1β (1.5 µg/kg) or saline, and blood samples were collected before and at 1, 3, and 5 h after the intravenous injection. IL-1Ra (1.5 mg/kg) was injected subcutaneously in conscious rats without intravenous catheter at 30 min before and 2 h after the intraperitoneal injection of LPS (100 µg/kg) or vehicle (saline). Blood was collected directly from the right jugular vein under brief isoflurane anesthesia before any treatments and from the heart at 3 h after LPS injection. Plasma ghrelin levels were monitored.

Effects of subcutaneous astressin B on intraperitoneal LPS-induced decrease in plasma ghrelin levels and intraperitoneal urocortin 1 on plasma ghrelin levels. Fasted rats with a chronically implanted intravenous catheter were injected with intravenous urocortin 1 (10 µg/kg) or vehicle (saline), and blood was collected at 0, 1, 3, and 5 h after the intraperitoneal injection of LPS (100 µg/kg) or vehicle (saline). Blood was collected before the...
subcutaneous injection and at 1, 2, 3, and 5 h after the intraperitoneal injection. Blood glucose and plasma ghrelin levels were monitored.

Effect of intravenous injection of ghrelin on LPS-induced inhibition of food intake and gastric emptying. Rats with a chronically implanted intravenous catheter and fasted overnight were injected intraperitoneally with LPS (100 μg/kg) or vehicle (saline, 0.3 ml), and, 5 h later, ghrelin (30, 100, and 300 μg/kg) or saline (0.2 ml) was injected intravenously. Cumulative food intake was monitored for 30 min, 1 h, and 2 h after ghrelin or saline intravenous injection. For gastric emptying studies, fasted rats were injected intraperitoneally with vehicle or LPS (100 μg/kg), and, 5 h later, ghrelin (30 or 300 μg/kg) or saline was injected intravenously followed by the oral gavage of an intragastric nonnutrient viscous meal. At 20 min after the meal delivery, rats were euthanized to monitor gastric emptying. Ghrelin doses were based on previous reports showing dose-related orexigenic response upon intraperitoneal injection from 40 to 400 μg/kg in freely fed rats (64).

Statistical Analysis

Values are means ± SE. Data were analyzed using ANOVA followed by the Student-Newman-Keuls test. In the time-course experiments, comparison between two groups was performed with a Student’s t-test or paired Student’s t-test. The correlations between plasma levels of ghrelin and insulin or glucose were determined by univariate linear regression. P values of <0.05 were considered statistically significant.

RESULTS

LPS decreases circulating levels of ghrelin and increases insulin and glucose: time course. LPS (100 μg/kg) injected intraperitoneally in fasted conscious rats induced a time-related and sustained drop in total ghrelin plasma levels from 1 to 7 h postinjection with the nadir at 5 h, followed by a return to preinjection levels at 24 h after injection (Fig. 1A). The percentages of significant decrease in ghrelin plasma levels compared with preinjection levels were 22.6 ± 6.3%, 47.6 ± 4.9%, 58.6 ± 3.3%, 74.4 ± 2.7%, and 48.9 ± 8.7% at 1, 2, 3, 5, and 7 h post-LPS injection, respectively. The vehicle did not significantly alter fasted plasma levels of ghrelin throughout the experimental period (Fig. 1A).

In contrast, plasma insulin levels rose significantly at 2, 5, and 7 h after intraperitoneal LPS injection with values of 0.51 ± 0.09, 0.45 ± 0.06, and 0.41 ± 0.04 ng/ml (P < 0.05) compared with basal levels (0.25 ± 0.03 ng/ml) or intraperitoneal saline at the corresponding time point (0.24 ± 0.03, 0.22 ± 0.05, and 0.20 ± 0.03 ng/ml, respectively; Fig. 1B). There was no significant change in intraperitoneal vehicle-injected rats throughout the 24-h experimental period, although there was a trend to a rise at 24 h.

Blood glucose rose significantly at 1, 2, 5, and 7 h after intraperitoneal LPS injection (130 ± 11, 118 ± 9, 131 ± 6, and 128 ± 5 compared with 99 ± 10 mg/dl before injection, respectively, P < 0.05; Fig. 1C). The differences were significant between LPS and saline intraperitoneal injections at 2 and 5 h (118 ± 9 vs. 94 ± 3 and 131 ± 6 vs. 100 ± 4 mg/dl, respectively; P < 0.05) but no longer at 7 h (Fig. 1C). There were no significant changes in glucose levels during the 0- to 7-h period after the intraperitoneal injection of vehicle (Fig. 1C).

In LPS-treated animals, there was a negative correlation between plasma levels of insulin and ghrelin over the 24-h period (r = −0.90, P < 0.05; Fig. 2A). No correlation was observed between plasma ghrelin and blood glucose levels (r = −0.60, P > 0.05; Fig. 2B) or between insulin and glucose levels (r = 0.58, P > 0.05; Fig. 2C) from 0 to 7 h postinjection.

Indomethacin prevents the early phase of intraperitoneal LPS-induced reduction of ghrelin plasma levels. Fasted rats injected intraperitoneally with vehicle or indomethacin plus

Fig. 1. Time course of changes in circulating levels of ghrelin (A), insulin (B), and glucose (C) after intraperitoneal injection of LPS (n = 7) or vehicle (n = 6) in overnight-fasted rats implanted with an intrajugular catheter 3 days before. Repeated blood samplings were performed through the catheter during the experiment and from the heart at 24 h. Each point represents the mean ± SE of 6–7 rats.

*P < 0.05 vs. basal (0 h) and **P < 0.05 vs. vehicle at the same time point.
vehicle had similar levels of plasma ghrelin levels compared with preinjection values during the 5-h experimental period (Fig. 3). In vehicle-pretreated rats, LPS (100 μg/kg ip) induced a linear time-related decrease in ghrelin plasma levels from 1–5 h postinjection. Indomethacin (10 mg/kg) injected intraperitoneally 1 h before LPS blocked LPS-induced suppression of fasting ghrelin levels by 100% at 2 h and 80% at 3 h but no longer at 5 h after LPS injection (Fig. 3).

IL-1β reduces ghrelin plasma levels, and IL-1Ra blocks LPS-induced decrease in plasma ghrelin levels. The intravenous injection of IL-1β (1.5 μg/kg) significantly suppressed preinjection fasting levels of total ghrelin by 67 ± 10% and 63 ± 11% at 3 and 5 h after injection, respectively, whereas intravenous vehicle did not alter plasma values throughout the 5-h period. The decreases in total ghrelin levels after LPS injection were significantly different compared with the vehicle group at each corresponding time point (P < 0.05; Fig. 4A). IL-1Ra, injected at 1.5 mg/kg sc, twice at 30 min before and 2 h after LPS, blocked LPS-induced reduction of fasting plasma ghrelin levels at 3 h after LPS injection (Fig. 4B). IL-1Ra alone did not modify plasma ghrelin levels (Fig. 4B).

Peripheral injection of CRF receptor antagonist and urocortin 1 did not influence LPS-induced changes in circulating levels of ghrelin. Fasted rats injected subcutaneously with vehicle and intraperitoneal saline had no significant change in plasma levels of ghrelin for the 5-h observation period from preinjection levels (Fig. 5). Astressin B (100 μg/kg), injected subcutaneously before intraperitoneal saline, induced a rise in ghrelin levels at 3 h that reached a significant difference at 5 h compared with subcutaneous vehicle plus intraperitoneal vehicle (20.8 ± 6.2% vs. −24.9 ± 5.4% of basal levels as zero, P < 0.05; Fig. 5). Astressin B pretreatment had no significant effect on the LPS (100 μg/kg ip)-induced time-related drop in plasma ghrelin levels throughout the 5-h experimental period (Fig. 5).

Fig. 2. Correlations between plasma ghrelin and insulin from 0–24 h (A), between plasma ghrelin and blood glucose from 0–7 h (B), and between plasma insulin and blood glucose from 0–7 h (C) after LPS injected intraperitoneally in overnight-fasted rats (each solid circle represents the mean from 7 animals). Values of r and P are indicated in each graph.
Urocortin 1 (10 μg/kg iv) did not significantly alter fasted plasma levels of ghrelin from the 1- to 5-h period after injection (Fig. 6A). However, intravenous urocortin 1 induced a rapid and sustained hyperglycemic response with a significant rise at 30 min and a peak response at 1 h postinjection, reaching 159.4 ± 7.6 mg/dl compared with 106.2 ± 6.5 mg/dl in the subcutaneous vehicle group (Fig. 6B). The subcutaneous injection of vehicle did not significantly alter blood glucose levels throughout the 5-h period (Fig. 6B).

Ghrelin injected intravenously prevents intraperitoneal LPS-induced reduction of food intake and gastric emptying. At 5 h after LPS (100 μg/kg ip) injection, fasted rats exposed ad libitum to food for 2 h had a significant 61%, 41%, and 33% reduction in the cumulative food consumption at 30 min, 1 h, and 2 h, respectively, compared with the intraperitoneal vehicle-treated group at the same time interval (P < 0.05; Fig. 7A). Ghrelin (30, 100, or 300 μg/kg iv) injected at 5 h after LPS injection prevented the significant reduction of food intake induced by LPS monitored at the 30-min period postghrelin intravenous injection at the three doses (2.3 ± 0.2, 2.6 ± 0.4, and 2.6 ± 0.2 g, respectively, vs. 1.2 ± 0.2 g in vehicle + LPS; P < 0.05) and at 1 h with the 100 and 300 μg/kg doses (3.4 ± 0.3 and 3.2 ± 0.2 g, respectively, vs. 2.3 ± 0.4 g; P < 0.05; Fig. 7A). Ghrelin (30, 100, or 300 μg/kg iv) did not further significantly enhance the cumulative food intake in overnight-fasted rats injected intraperitoneally with vehicle at all the time points.

At 5 h after LPS (100 μg/kg ip) injection, the 20-min gastric emptying of a nonnutrient liquid meal was reduced to 8.6 ± 3.0% (P < 0.05) compared with 65.3 ± 4.9% in the intraperitoneal vehicle group (Fig. 7B), corresponding to an 87% inhibition of transit. Ghrelin (30 or 300 μg/kg iv) injected 5 h after LPS completely prevented LPS inhibitory effect, and gastric emptying values were restored to 74.4 ± 2.1% and 79.0 ± 3.4%, respectively (P > 0.05; Fig. 7B). In intraperitoneal vehicle-pretreated rats, ghrelin (30 or 300 μg/kg iv) did not significantly increase the gastric emptying of viscous non-nutrient meal (74.7 ± 2.7% or 74.8 ± 6.0%) compared with intravenous vehicle (65.3 ± 4.9%).

At the dose injected, LPS did not produce apparent signs of sickness or endotoxin shock in conscious rats in all experiments.

DISCUSSION

The present study shows that LPS injected at a low dose (100 μg/kg ip) triggers clinical features of gram-negative bacterial infection, including reduced food intake and gastric emptying but not endotoxemic shock, which inhibits circulat-
levels induced by the intravenous injection of urocortin 1 (10 μg/kg, iv) or vehicle in overnight-fasted rats implanted with an intrajugular catheter 3 days before. Repeated blood samplings were performed in conscious, lightly hand-restrained rats. Each point represents the mean ± SE of 6–7 rats/group *P < 0.05 vs. basal (0 h) and #P < 0.05 vs. vehicle at the same time point.

Fig. 6. Time course of changes in plasma ghrelin (A) and blood glucose (B) levels induced by the intravenous injection of urocortin 1 (10 μg/kg) or vehicle in overnight-fasted rats implanted with an intrajugular catheter 3 days before. Repeated blood samplings were performed in conscious, lightly hand-restrained rats. Each point represents the mean ± SE of 6–7 rats/group *P < 0.05 vs. basal (0 h) and #P < 0.05 vs. vehicle at the same time point.

ing levels of ghrelin for over 7 h in conscious fasted rats with a chronic intravenous catheter. Time-course studies revealed a linear decline from 1–5 h after injection with a nadir of 74% from basal levels at 5 h, followed by a return to basal levels with a 49% suppression at 7 h and values reaching preinjection levels at 24 h after LPS injection. These results expand our previous report showing that LPS injected under the same conditions shifted fasted levels of ghrelin to postprandial levels at 3 h (5). Another study showed that LPS, injected intraperitoneally at a high dose (10 mg/kg), inducing endotoxemic shock in fed rats, blocked the rise in plasma ghrelin levels induced by subsequent food deprivation for 6 and 12 h (22). LPS action is specific, because the intraperitoneal injection of saline did not significantly alter fasted plasma ghrelin values during the same time intervals. The lack of further increase in ghrelin levels throughout the additional 24-h fasting period suggests that mechanisms increasing ghrelin release are already maximally stimulated after the initial 18- to 20-h period of food deprivation. Other reports indicate that ghrelin plasma levels start to increase 6 h after food deprivation and reach similar levels after a 24- or 48-h fasting period (2, 48). Taken together, these observations showed that an acute injection of LPS at a low dose induces, within 1 h onset, a time-related decline in plasma levels of ghrelin with a peak inhibitory effect at 5 h that starts receding at 7 h to return to fasted levels after 24 h. The pattern of changes in gastric ghrelin store and production under these conditions is still to be investigated and cannot be inferred from alterations in circulating levels of peptide, because under conditions of decreased plasma levels of ghrelin, either no change or a reduction in gastric ghrelin levels has been reported (43, 47).

Although a negative correlation between plasma levels of ghrelin and feeding status or body mass index has been extensively demonstrated (51, 63), these factors cannot account for the observed suppression of ghrelin levels under our acute and fasted experimental conditions. It is also unlikely that the decreases in plasma levels of ghrelin result from a direct effect of LPS on the gastric mucosa. In a gastric fundus mucosal strip incubated with LPS at doses of 1–100 μg/ml, there is a dose-related and time-dependent increase in ghrelin levels in the media (9). The opposite alterations of ghrelin release by LPS in vivo and in vitro indicate that LPS inhibitory action is mediated by integrated systems that are recruited by LPS.

The production of PGs and cytokines are among the major mediators that orchestrate the biological acute phase response to peripheral injection of LPS (21, 56). The present data provide evidence that the inhibition of circulating ghrelin observed during the first 3 h is linked with the production of PGs and IL-1β. Indomethacin, which inhibits cyclooxygenase (COX)-1 and COX-2 (involved in the synthesis of PGs from arachidonic acid), suppressed the intraperitoneal LPS-induced drop in ghrelin levels by 100% and 80% at 2 and 3 h postinjection, respectively. Indomethacin did not influence fasted levels of circulating ghrelin in intraperitoneal vehicle-treated rats throughout the same experimental period. These findings indicate that PGs are not involved in modulating fasted levels of ghrelin, consistent with the low PG production under fasting conditions (58). However, under stimulated conditions by LPS injection (18, 24, 58), PGs mediate the early phase of ghrelin inhibition. The time-course study revealed that at 5 h postinjection, LPS decreased ghrelin plasma values to the same extent in both vehicle- and indomethacin-pretreated rats. The blocking action of indomethacin during the first 3 h, which is no longer observed at 5 h, is unlikely to be related to the vanishing of indomethacin inhibitory action on COX activity during the time of the experimental period. Other studies (58) have shown that indomethacin injected peripherally at a similar dose as used in the present study (10 mg/kg) completely suppressed the large rise in PGE2 production in the rat gastric mucosa 6 h after LPS injected intravenously at 3 mg/kg, a 30-fold higher dose than used in the present study. The PG-independent mechanisms through which LPS maintains inhibited ghrelin levels in the later phase may be linked with cytokines and/or nitric oxide production still present at this time post-LPS injection (58). For instance, the acute febrile response to LPS has been delineated to include two phases within the first 2 h and a third one at 5 h that are brought about by a sequential burst of regulatory effectors and transcription factors (6, 24).

The role of IL-1β in the alterations of circulating ghrelin in response to LPS is supported by the demonstration that the
subcutaneous injection of the receptor antagonist IL-1Ra blocks the intraperitoneal LPS inhibitory action as monitored 3 h later while not influencing fasted ghrelin levels in intraperitoneal vehicle-treated group. Moreover, an intravenous bolus injection of IL-1β resulted in a maximal 67% inhibition of fasting plasma levels of ghrelin at 3 h postinjection that was still maintained 2 h later. Another study (4) showed that intraperitoneal injection of IL-1β also decreases gastric preproghrelin mRNA expression in mice. Lastly, LPS (25 or 1,000 μg/kg) was characterized to elevate plasma levels of IL-1β between 2 and 5 h after intravenous injection, whereas the surge of circulating TNF-α occurs 1 h post-LPS injection followed by a rapid decline (19), which is consistent with our time-course study.

These results, to our knowledge, are the first to demonstrate that the production of PGs and IL-1β play a pivotal role in inhibiting the fasted levels of plasma ghrelin during the early hours (2–3 h) after peripheral LPS injection. These findings suggest a link between immune mediators, such as IL-1 and PGs, and acute suppression of ghrelin secretion, although their mechanisms of action (central and/or peripheral) remain to be determined. These data may also open new venues to understand effectors that regulate ghrelin release. For instance, feeding was reported to increase gastric PGE2 release (8) and to decrease plasma levels of ghrelin (47). Whether the food-related gastric PGE2 release also contributes to suppressing the acute drop in ghrelin after feeding is worth investigation based on the present data.

LPS is well established to stimulate the PA axis (19, 57), which may have an influence on ghrelin release (40). However, several studies rule out a role of PA axis in LPS-induced inhibition of ghrelin release. First, the mammalian CRF1/CRF2 agonist urocortin 1 (60), injected intravenously at a dose reported to induce a sustained increase in ACTH and corticosterone plasma levels for over 6 h (3, 60), did not alter the fasted levels of ghrelin throughout the 5-h experimental period. The influence of urocortin 1 on circulating ghrelin levels has not been previously documented in rodents, although in humans, a decline or no change in ghrelin was observed 2 h after the end of an intravenous urocortin 1 infusion (14). Second, the long-acting CRF1 and CRF2 antagonist astressin B (45) did not influence the suppression of plasma ghrelin levels when ad-
ministered subcutaneously at a threefold higher dose reported to block the plasma ACTH response to a low dose of LPS (44). Lastly, other nonimmune stressors, namely fasting or water avoidance stress, which activates the PA axis (16, 28), are well-established to increase, not decrease, circulating levels of ghrelin (5, 28, 63). Collectively, these findings indicate that the reduction of fasting ghrelin levels in rats induced by LPS is not secondary to the acute rise in ACTH and corticosterone induced by LPS and does not involve the activation of peripheral CRF receptors.

A well-recognized metabolic response of peripheral injection of LPS is the stimulation of carbohydrate metabolism (characterized by hyperglycemia, hepatic glucose production, and glucose utilization in peripheral tissues) and rise in glucagon and insulin plasma levels (29, 50). In the present study, the rise in blood glucose at 1 h post-LPS injection preceded the increase in plasma insulin occurring at 2 h, and, thereafter, a sustained elevation of blood glucose and insulin was maintained for 7 h postinjection, except a decline at 3 h. It is, however, unlikely that ghrelin changes are secondary to the hyperglycemic response to LPS. There was a lack of correlation between circulating levels of ghrelin and glucose after intraperitoneal injection of LPS. In addition, the intravenous injection of urocortin 1 in fasted rats, which resulted in a sustained hyperglycemic response similar to that induced by LPS, did not induce a drop in ghrelin levels. The hyperglycemic response to urocortin 1 expands the recent demonstration that the selective CRF2 agonist urocortin 3 injected intraperitoneally response to LPS is the stimulation of carbohydrate metabolism induced reduction of food intake was achieved by an intravenous injection of ghrelin at intravenous doses that did not significantly change food intake over the 30-min to 2-h experimental period in intraperitoneal vehicle-treated rats fasted for 24 h. These results suggest that the orexigenic response to systemic injection of ghrelin is increased under conditions of low circulating levels of ghrelin induced by intraperitoneal LPS (5) (present study). Similarly, because ghrelin injected intravenously or intraperitoneally at a similar dose range increased food intake at 1 h postinjection in freely fed rats (13, 64), the lack of orexigenic response to ghrelin in fasted rats pretreated with intraperitoneal vehicle may be related to low vs. high levels of endogenous ghrelin in freely fed vs. fasted rats (5, 47, 55). Alternatively, the potent orexigenic response to fasting in the vehicle group may not be further enhanced by ghrelin.

In addition to the reduction in food intake, at 5 h after LPS injection, the 20-min gastric emptying of a nonnutrient meal was inhibited by 87%, consistent with a previous time-course study (10). We showed that ghrelin injected at either 30 or 300 μg/kg iv, 5 h after LPS injection, completely normalized gastric emptying in rats. Other studies in mice showed that ghrelin injected at 20 μg/kg ip had no effect on basal transit but prevented septic gastric ileus induced by LPS given at 20 mg/kg (15). Ghrelin is the natural ligand of the GHS-R and displays a strong GH releasing activity in animals and humans (59). The decrease in circulating ghrelin may also play a role in the abolition of the pulsatile release of GH induced by LPS injected at 100 μg/kg or higher doses in conscious rats (25, 42, 49). Taken together, these findings suggest that the long-lasting inhibition of ghrelin, which synchronizes physiological processes regulating nutrition, digestion, and growth (59), may have a bearing on the symptoms of acute infections.

In summary, the time-course study clearly established that a single injection of LPS, at a low dose that inhibits gastric emptying and food intake, induces a sustained suppression of fasting circulating levels of ghrelin in awake rats that is reversible after 24 h. There is a correlation between LPS-induced rise in insulin and inhibition of ghrelin, whereas the ghrelin response is not correlated with the rise in glucose. The inhibition of fasting levels of ghrelin induced by LPS during the first 3-h postinjection is brought about by the production of PGs and IL-1β. This is supported by the blocking action of peripheral administration of indomethacin and IL-1Ra and by the drop in fasting plasma levels of ghrelin induced by IL-1β injected intravenously. In contrast, the activation of the PA axis and peripheral CRF receptors do not play a role because intravenously urocortin 1 does not influence fasting plasma ghrelin levels, while producing a hyperglycemic response, and subcutaneous injection of CRF receptor antagonist does not alter LPS action. In LPS-treated rats, intravenous injection of ghrelin dose-dependently restored LPS-induced decrease in food intake and potently prevented the slowing of gastric emptying. These data suggest that the sustained decline in circulating ghrelin may be an important factor in the development of systemic symptoms of infections such as the suppression of appetite and gastric transit under conditions of bacterial infection and serves as an effector that contributes to the adaptive response of the first line of host defense.
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