Histamine H₃ Receptors Are Involved in the Protective Effect of Ghrelin against HCl-Induced Gastric Damage in Rats

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Key Words

Ghrelin · Histamine · Histamine H₃ receptor · Gastric mucosal defense · HCl

Abstract

In the present study, the effects of ghrelin against the gastric damage induced by intragastric administration of 0.6 N HCl and the involvement of histamine H₃ receptors (H₃Rs) were investigated in conscious rats with selective H₃R ligands. Intraperitoneal (i.p.) injection of ghrelin (40 µg/kg) significantly reduced (43%) the gastric lesions caused by concentrated acid. The effect of ghrelin was prevented by prior administration of the ghrelin receptor antagonist [D-Lys³]-GHRP-6 (100 µg/kg i.p.) and by subcutaneous (s.c.) injection of the non-imidazole H₃R antagonist UCL2138 (30 mg/kg). The selective H₃R agonist immethridine (30 mg/kg s.c.) significantly inhibited (64.60%) the gastric lesions induced by 0.6 N HCl. The effect of immethridine was prevented by prior administration of UCL2138 (30 mg/kg s.c.), but not by [D-Lys³]-GHRP-6 (100 µg/kg i.p.). Neither [D-Lys³]-GHRP-6 nor UCL2138 modified HCl-induced gastric damage per se. These data enlarge previous studies showing protective effects of ghrelin against ulcerogenic stimuli; in addition, they clearly indicate

that ghrelin-induced gastroprotection involves the release of histamine, which enhances gastric mucosal defense through the activation of histamine H₃Rs.

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Introduction

Ghrelin is a 28-amino-acid acylated peptide, which was recently identified in the rat and human gastrointestinal tract as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R) [1]. Ghrelin is predominantly produced in the stomach [2, 3] by endocrine cells of the fundic mucosa, formerly known as X/A-like cells [4]; substantially lower amounts of the peptide were detected in other peripheral tissues, such as the intestine, pancreas, kidney, lung and immune system [5, 6]. The secretion of ghrelin is regulated by several factors, including the autonomic nervous system, blood glucose, insulin and leptin [7, 8]; since the most important factor that increases plasma ghrelin levels is fasting [9], this peptide has been recognized as a peripheral 'orexigenic' signal which communicates with the hypothalamus to increase appetite and food intake [3, 6, 8, 10].

Fig. 1. Schematic protocol of compound administration. Antagonists (ghrelin receptor antagonist, histamine H₃R antagonist or atropine) were administered 30 min before ghrelin.

Several studies have suggested that ghrelin displays a variety of biological activities influencing neuroendocrine, cardiovascular and gastrointestinal functions [11, 12]; in the gastrointestinal tract, ghrelin has been shown to affect gastric acid secretion, motility and mucosal defense [for a review, see ref. 8]; these effects, however, are the subject of debate and the underlying mechanisms remain to be defined. In particular, the effects of ghrelin on acid secretion are controversial since stimulation [13–17], inhibition [18, 19] or lack of effect [19, 20] has been reported, according to the route of administration (central vs. peripheral) or the experimental assay (conscious vs. anesthetized animals).

The effects of ghrelin on mucosal defense have been unanimously documented after central or peripheral administration and against a variety of ulcerogenic stimuli, including ethanol [13, 21], stress [13] and ischemia-reperfusion [22]. Such effects were correlated with the activation of a specific receptor, namely the growth hormone secretagogue receptor type 1a (GHS-R_{1a}), which mediates most of the actions of ghrelin both in the central nervous system and in the periphery [23–26]. Several mediators are involved in the gastrointestinal effects of ghrelin, including the vagus [13, 14, 16, 27], sensory neuropeptides released by capsaicin-sensitive afferent neurons [22, 28], prostaglandins [22] or nitric oxide [13, 22, 28, 29]. The involvement of histamine in the acid secretory and protective effects of ghrelin is a matter of debate: an increase in histidine decarboxylase expression in the rat gastric mucosa after peripheral administration of ghrelin was not unanimously detected [16, 27, 30]; moreover, the study by Konturek et al. [30] ruled out a role for histamine H₃ receptors (H₃Rs) in the ac-

tion of ghrelin, as the H₃R antagonist clobenpropit did not modify ghrelin-induced gastroprotection. This was rather unexpected since previously published data have clearly shown that the histamine H₃R subtype is predominantly involved in gastric mucosal protection [31–34]. The use of clobenpropit is questionable since this compound, originally described as a highly selective H₃R antagonist [35], displays affinity for histamine H₄ receptors (H₄Rs) [36]; as this last receptor has been recently involved in ulcerogenesis [37], further studies employing more selective H₃R ligands are required in order to identify the histamine receptor subtype involved in the gastric effects of ghrelin.

The primary aim of this study was to investigate whether histamine H₃Rs are involved in the gastroprotection induced by ghrelin. We examined the effect of ghrelin and of the selective GHS-R_{1a} antagonist [D-Lys³]-GHRP-6 [38] against the ulcerogenic effect of concentrated HCl, a necrotizing agent that has never been tested with ghrelin and is sensitive to H₃R-mediated gastroprotection. In the same experimental model, selective H₃R ligands, such as the nonimidazole antagonist UCL2138 [39] and the agonist immethridine [40] were used.

Materials and Methods

Animals

Male Wistar rats (180–250 g), purchased from Harlan Laboratories, Italy, were housed in a restricted-access room with controlled temperature (23°C) and a light/dark cycle of 12 h:12 h, and placed in wire mesh cages with a maximum of 4 rats per cage. Food and water were provided ad libitum. Animals were fasted 24 h before the experiment, but retained free access to tap water. The study received the approval of the local Animal Ethics Committee of the Faculty of Medicine, University of Parma, Italy.

Effect of Ghrelin on the Gastric Lesions Induced by 0.6 N HCl

Gastric lesions were induced in 24-hour-fasted animals by intragastric administration of 0.6 N HCl (5 ml/kg). Rats were randomized to receive intraperitoneally (i.p.), 30 min before HCl, either single doses of ghrelin (20–80 µg/kg) or vehicle (1 ml/kg). In another set of experiments, the ghrelin receptor antagonist [D-Lys³]-GHRP-6 (100 µg/kg) was administered intraperitoneally 30 min before ghrelin (fig. 1). The rats were sacrificed by cervical dislocation 30 min after administration of HCl and the stomachs were immediately removed, opened along the lesser curvature, rinsed, and laid on a flat surface. Macroscopic gastric damage was measured by two observers blinded to the treatment: each individual hemorrhagic lesion was measured along its greatest length (<1 mm: rating of 1; 1–2 mm: rating of 2; >2 mm: rating according to lengths in millimeters) and the overall total was designated as the 'lesion index' [41].

Involvement of H₃Rs in the Protective Effect of Ghrelin against 0.6 N HCl-Induced Damage

In order to investigate a possible involvement of histamine H₃Rs in the protective effect of ghrelin, the selective H₃R antagonist UCL2138 (30 mg/kg) was administered subcutaneously (s.c.) to conscious rats 30 min before ghrelin (see the protocol shown in fig. 1). In parallel experiments, the selective H₃R agonist, immethridine (30 mg/kg s.c.) was tested against 0.6 N HCl-induced gastric damage in the absence or in the presence of UCL2138 or the ghrelin receptor antagonist, [D-Lys³]-GHRP-6 (100 µg/kg i.p.). The dose of immethridine was chosen on the basis of previous experiments [31] as the dose inducing the maximum protective effects against 0.6 N HCl. Stomachs were evaluated for the presence of macroscopic gastric lesions according to the method above described.

Effect of Atropine on the Protective Effect of Ghrelin against 0.6 N HCl-Induced Damage

In order to investigate whether vagal mechanisms were involved in the gastroprotective effects of ghrelin, atropine (3 mg/kg s.c.) was administered to HCl-treated rats 30 min before ghrelin or vehicle. The dose of atropine was based on literature data [16, 42].

Chemicals

Ghrelin, [D-Lys³]-GHRP-6, atropine and other chemicals were purchased from Sigma-Aldrich (St. Louis, Mo., USA); immethridine and UCL2138 were synthesized by Rob Leurs and Holger Stark, respectively. Ghrelin was dissolved in 1% acetic acid and a stock solution (1 mg/ml) was stored at -20°C for a month; dilutions in distilled water were made on the day of the experiment. All the other compounds were dissolved in distilled water.

Statistical Analysis

Data are expressed as the means $\pm$ SEM of 6–8 rats per group. Comparisons between two groups were made by using Student's t test for unpaired data; comparisons among different groups were examined by analysis of variance (ANOVA), followed by Dunnett's test. A value of $p < 0.05$ was considered statistically significant. The software package Prism GraphPad 3.0 (GraphPad Software Inc., San Diego, Calif., USA) was used to process data.

Results

Effect of Ghrelin on 0.6 N HCl-Induced Gastric Lesions

Intragastric administration of 0.6 N HCl to conscious rats induced hemorrhagic lesions in all treated animals; the lesions involved the whole mucosa (fig. 2a) and reached a lesion index of 122.80 ± 15.71 mm (fig. 2b). Ghrelin (40 µg/kg i.p.) significantly reduced the gastric lesions induced by 0.6 N HCl, with a maximum inhibition of 43% (lesion index = 70.00 ± 10.82 vs. 122.80 ± 15.71 mm, $p < 0.05$; fig. 2b). In figure 2b, the gastroprotective effect of immethridine (30 mg/kg s.c.) in HCl-treated rats is reported for comparison (maximum inhibition = 64.60%).

Effect of the Ghrelin Receptor Antagonist D-[Lys³]-GHRP-6 on the Gastroprotection Induced by Ghrelin or Immethridine

The ghrelin receptor antagonist [D-Lys³]-GHRP-6 (100 µg/kg i.p.) prevented the inhibition of HCl-induced gastric damage exerted by ghrelin (40 µg/kg i.p.) (fig. 3a), while being ineffective against the protective effect induced by immethridine (30 mg/kg s.c.) (fig. 3b). [D-Lys³]-GHRP-6 (100 µg/kg i.p.) did not modify 0.6 N HCl-induced mucosal lesions (lesion index = 111.50 ± 18.30 vs. 134.90 ± 15.27 mm, $p > 0.05$, data not shown).

Effect of the H₃R Antagonist UCL2138 on the Gastroprotection Induced by Ghrelin or Immethridine

The selective H₃R antagonist UCL2138 (30 mg/kg s.c.) prevented the protective effects of both ghrelin (40 µg/kg i.p.) and immethridine (30 mg/kg s.c.) against 0.6 N HCl-induced lesions (fig. 4). UCL2138 (30 mg/kg s.c.) was ineffective against HCl-induced gastric damage (lesion index = 141.90 ± 9.56 vs. 149.20 ± 17.81 mm, $p > 0.05$, data not shown).

Effect of Atropine on the Gastroprotective Effect Induced by Ghrelin

In rats pretreated with atropine (3 mg/kg s.c.), 0.6 N HCl-induced lesions were not significantly different from those observed in control rats (table 1) whereas the inhibitory effect of ghrelin (40 µg/kg i.p.) was partially, but significantly, reduced when compared to rats without atropine (about 35% inhibition; table 1).

Discussion

This study demonstrates that peripheral administration of ghrelin to conscious rats reduced the gastric damage induced by concentrated HCl. These findings are in keeping with previous data showing protective effects of ghrelin against other models of gastric injury, including ethanol, stress and ischemia [13, 21, 22, 43], and confirm that ghrelin is a gastroprotective factor in the rat stomach. The partial efficacy (43% inhibition) displayed by the peptide in the present study is in line with literature data, which showed maximal effects of ghrelin in intact animals, ranging from 20 to 70%, according to the experimental assay [13, 18, 19, 22, 29]. This variability is possibly related to the different routes of administration employed; in this connection it has been shown that gastric effects of ghrelin are both centrally and peripherally mediated [19, 29] and gastroprotection against ethanol, in

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Fig. 2. Effect of ghrelin and immethridine against the gastric lesions induced by intragastric administration of 0.6 N HCl (5 ml/kg). **a** Note the hemorrhagic lesions in the HCl-treated stomach, involving the whole gastric mucosa. **b** Protective effects of ghrelin and immethridine. On the ordinate, macroscopic damage reported as lesion index in millimeters. Differences among multiple groups were assessed by one-way ANOVA, followed by Dunnett's test: * $p < 0.05$ and ** $p < 0.01$ compared to the vehicle-treated group. Mean values $\pm$ SEM from 6–8 rats.

Fig. 3. Protective effects of ghrelin (**a**) and immethridine (**b**) against 0.6 N HCl-induced lesions. The effects of ghrelin and immethridine were tested in the presence intraperitoneal injection of the ghrelin receptor antagonist [D-Lys³]-GHRP-6 (100 µg/kg). On the ordinate, macroscopic damage reported as lesion index in millimeters. Differences among multiple groups were assessed by one-way ANOVA, followed by Dunnett's test: * $p < 0.05$ and ** $p < 0.01$ compared to the vehicle-treated group. Mean values $\pm$ SEM from 6–8 rats.

Fig. 4. Protective effects of ghrelin (a) and immethridine (b) against 0.6 N HCl-induced lesions. The effects of ghrelin and immethridine were tested in the presence of the histamine H₃ receptor antagonist, UCL2138 (30 mg/kg s.c.). Differences among multiple groups were assessed by one-way ANOVA, followed by Dunnett's test: * $p < 0.05$ and ** $p < 0.01$ compared to the vehicle-treated group. Mean values $\pm$ SEM from 6–8 rats.

Table 1. Effect of atropine (3 mg/kg s.c.) on the gastroprotection induced by ghrelin (40 µg/kg i.p.) against 0.6 N HCl-induced damage

	No atropine	With atropine
0.6 N HCl	96.00 $\pm$ 8.72	108.50 $\pm$ 15.18
0.6 N HCl + ghrelin	40.01 $\pm$ 10.82*	67.40 $\pm$ 13.69

The macroscopic damage is reported as lesion index (mm). Data are means $\pm$ SEM from 6–8 experiments; * $p < 0.05$ versus 0.6 N HCl alone, without atropine.

particular, was observed after central, but not peripheral, administration of ghrelin [21]. Likewise, it has been shown that peripheral ghrelin does not easily enter the brain in rodents, as opposed to humans [44].

The effect of ghrelin was totally antagonized by [D-Lys³]-GHRP-6, a compound which is considered the reference antagonist of GHS-R_{1a} in functional studies [11, 26, 43, 45], indicating that the GHS-R_{1a} is involved in the gastroprotective activity of ghrelin. The lack of ulcerogenic effects of [D-Lys³]-GHRP-6 by itself in basal conditions or under HCl challenge confirms previous studies [22, 43], which showed that this antagonist by itself did not modify ethanol- or ischemia/reperfusion-induced gastric lesions. These data would suggest that endogenous ghrelin does not play a major role in the maintenance of gastric mucosal defense under physiological conditions or under exposure to ulcerogenic stimuli.

As opposed to this, a delay in gastric emptying and a reduction of food intake was observed after administration of the ghrelin receptor antagonist [10]. The possibility that nonspecific effects of [D-Lys³]-GHRP-6 leading to gastroprotection had masked ulcerogenic effects related to ghrelin receptor blockade cannot be excluded; in line with this, a recent study [46] reported that [D-Lys³]-GHRP-6 may induce smooth-muscle contraction through activation of 5-hydroxytryptamine receptors (5-HT_{2B}). In our model, however, a possible activation of gastric 5-HT receptors induced by [D-Lys³]-GHRP-6 has to be excluded, since, if present, it would have resulted in ulcerogenic rather than protective activity [47].

The major finding of the present study is the involvement of H₃Rs in the gastroprotective effects of ghrelin. In line with previous findings suggesting that histamine participates in the gastric effects of ghrelin [27, 30], the present study using the selective H₃R ligand immethridine [40] and UCL2138 [39], demonstrated, for the first time, that the H₃R subtype is specifically involved in the gastroprotection induced by ghrelin. To the best of our knowledge, an interaction between ghrelin and histamine involving H₃Rs has been obtained in the same species only in one study [48] showing that histaminergic neurotransmission in the brainstem and the activation of H₃Rs in particular may play a role in cardiovascular effects elicited by ghrelin.

Fig. 5. Scheme illustrating the proposed interaction between ghrelin and histamine in the rat gastric mucosa, based on present data. The gastroprotective effect of ghrelin involves a release of histamine from ECL cells, partly mediated by vagal activation. A direct effect of ghrelin on ECL cells cannot be derived from the present data.

Present data concerning the interaction of ghrelin-histamine in the rat stomach are in keeping with previous observations obtained by Konturek et al. [30], showing that the gastroprotective effect of ghrelin is associated with increased histidine decarboxylase mRNA expression in the gastric mucosa and increased plasma histamine levels. In that study, however, a precise definition of the histamine receptor involved in the gastroprotection induced by ghrelin was not obtained. In particular, the involvement of H_3R s was ruled out based on the ineffectiveness of the H_3R antagonist clobenpropit. As opposed to the study by Konturek et al. [30], our data clearly suggest that histamine released by ghrelin activates H_3R s to afford protective effects, in accordance with literature data [31–34]. The reason for this discrepancy may possibly be attributed to the use of clobenpropit; indeed, it has recently been demonstrated that this compound, originally described as a potent and highly selective H_3R antagonist [35], also displays affinity for the newly discovered H_4R , behaving as a partial agonist in functional assays [36]. H_4R s have recently been detected in the rodent gastric mucosa and are functionally related to ulcerogenic effects [37, 49]; interestingly, H_4R expression was specifically detected in ghrelin-producing cells [49], suggesting a further link between histamine and ghrelin in the rat gastric mucosa.

The mechanism underlying histamine release by ghrelin was not investigated in our study; however, a direct effect of ghrelin on gastric enterochromaffin-like (ECL) cells could be hypothesized (fig. 5) since these cells represent the major source of histamine in the oxyntic mucosa of the rat stomach [50] and are under the control of a variety of neuromessengers and hormones, including autonomic and peptide transmitters [51]. Yakabi et al. [27], however, ruled out a direct effect of ghrelin on ECL cells and suggested an indirect ECL cell activation by ghrelin through the central vagal nerves and pituitary adenylate cyclase-activating peptide. In line with previous studies, which showed an involvement of vagal pathways in the gastric effects of ghrelin [13, 14, 16], the present experiments with atropine provide evidence that histamine released by ghrelin is, at least partly, under vagal influence.

In conclusion, the present study confirmed that the peptide ghrelin is endowed with protective activity against gastric damage induced by a necrotizing agent in the rat gastric mucosa; in addition, the involvement of endogenous histamine and of H_3R activation in response to ghrelin is demonstrated by the use of selective H_3R ligands. Our data, however, do not support a release of ghrelin induced by histamine, based on the ineffectiveness of ghrelin receptor antagonism on the immethridine-induced gastroprotection. The question whether histamine H_4R s may be involved in the interaction between ghrelin and histamine needs to be addressed in further studies.

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