Ghrelin selectively reduces mechanosensitivity of upper gastrointestinal vagal afferents

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Page AJ, Slattery JA, Milte C, Laker R, O’Donnell T, Dorian C, Brierley SM, Blackshaw LA. Ghrelin selectively reduces mechanosensitivity of upper gastrointestinal vagal afferents. Am J Physiol Gastrointest Liver Physiol 292: G1376–G1384, 2007. First published February 8, 2007; doi:10.1152/ajpgi.00536.2006.—Ghrelin is a peptide released from gastric endocrine cells that has an orexigenic effect via a vagal pathway. Here we determine the effect of ghrelin on mechanosensitivity of upper-intestinal vagal afferent fibers in ferret and mouse. The responses of gastroesophageal vagal afferents to graded mechanical stimulation were determined in vitro before and during application of ghrelin to their peripheral endings. Three types of vagal afferent were tested: tension receptors responding to circumferential tension, mucosal receptors responding only to mucosal stroking, and tension/mucosal (TM) receptors in ferret esophagus that responded to both stimuli. In the mouse, ghrelin did not significantly affect the response of mucosal receptors to mucosal stroking with calibrated von Frey hairs. However, it significantly reduced responses of tension receptors to circumferential tension (P < 0.005; two-way ANOVA) by up to 40%. This inhibition was reversed by the ghrelin receptor antagonist [D-Lys-3]-growth hormone-releasing peptide (GHRP)-6. In the ferret, ghrelin significantly reduced the response of mucosal and TM receptors to mucosal stroking with calibrated von Frey hairs. Surprisingly, ghrelin did not significantly alter the response to circumferential tension in either tension or TM receptors. RT-PCR analysis indicated that both ghrelin and its receptor are present in vagal afferent cell bodies in mouse nodose ganglia. In conclusion, ghrelin selectively inhibits subpopulations of mechanosensitively sensitive gastroesophageal vagal afferents; there is also potential for ghrelin release from vagal afferents. However, the subpopulation of afferents inhibited differs between species. These data have broad implications for ghrelin’s role in food intake regulation and reflex control of gastrointestinal function.

in vitro gastroesophageal preparation; neuromodulation; mechanoreceptor

Ghrelin is a 28-amino acid peptide (38) originally discovered in rat stomach as an endogenous ligand for growth hormone secretagogue receptor (GHS-R), (38). Ghrelin has been shown to be located in endocrine X/A cells, now designated ghrelin cells, from the stomach to the colon (38). The highest concentrations are found within the acid-producing oxyntic glands of the gastric fundus (21, 38, 39). In the intestine, ghrelin concentration gradually decreases from the rat duodenum to the colon (36), and a similar pattern for ghrelin mRNA expression has been demonstrated in humans (31). The ghrelin cells synthesize round, electron-dense granules containing ghrelin peptide. When administered to rodents or humans, ghrelin rapidly increases food intake (6, 27, 43, 59, 61, 63) in addition to stimulating gastric motility (6, 40, 58). It also increases body weight in rodents (59). Ghrelin expression increases with prolonged fasting (6, 19, 59), and fasting blood levels are suppressed by refeeding or by infusion of nutrients into the stomach (59, 60). On the basis of these findings it has been proposed that ghrelin is a hormone that contributes to the initiation of individual meals.

Ghrelin is secreted from gastric endocrine cells into the blood stream; however, at least part of ghrelin signaling from the stomach is mediated by an ascending neural network through the vagus nerve and brain stem nuclei that ultimately reaches the hypothalamus (6, 22). Recent work indicates that ghrelin can stimulate appetite via the vagus nerve (22), although another study has shown that the acute eating-stimulatory effect of ghrelin does not require vagal afferent signaling (5). However, growth hormone secretagogue 1 receptors are localized to vagal afferents that project to the stomach (54). Ghrelin inhibits the resting discharge of the whole vagal nerve and lesion of vagal afferent fibers inhibits the appetite-stimulating actions of ghrelin (6). Therefore, functional and molecular evidence indicates that there are ghrelin receptors at peripheral vagal afferent endings. In humans and other animals, a major source of satiety signals from the stomach is gastric filling and distention (26, 30). These gastric distention-induced satiety signals are transferred to the central nervous system via vagal afferent mechanisms (55). However, there is a substantial gap in our knowledge of this system because there is no direct information on the effect of ghrelin on signaling of distention by vagal afferent fibers.

We used an in vitro gastroesophageal vagal afferent preparation to study accurately different populations of mechanosensory afferent fibers (45, 47). Using this preparation, we investigated the effect of ghrelin on the sensitivity of gastroesophageal vagal afferents to mechanical stimulation. We studied all of the mechanosensitive gastroesophageal vagal afferent subtypes to determine whether ghrelin actions were selective for a particular type of mechanosensitive afferent fiber. We also investigated the effect of ghrelin in two species, namely mice and ferrets, to determine whether the effects are conserved across species, which may be important since the vast majority of evidence for ghrelin’s role so far originates from rodent studies.

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MATERIALS AND METHODS

All studies were approved and performed in accordance with the guidelines of the Animal Ethics Committees of the Royal Adelaide Hospital and Institute for Medical and Veterinary Science, Adelaide, Australia. These committees followed the Australian code of practice for the care and use of animals for scientific purposes, 6th edition 1997. Every attempt was made to minimize the number of animals required and to minimize their suffering.

In Vitro Ferret and Mouse Gastroesophageal Afferent Preparations

Female ferrets (0.5–1.0 kg; N = 21) fasted overnight were deeply anesthetized with sodium pentobarbitone (50 mg/kg ip), and the thorax and abdomen were opened by a midline incision. The ferrets were then exsanguinated by cardiac puncture. The stomach and esophagus with attached vagal nerves were removed and placed in a modified Krebs solution of the following composition (in mM): 118.1 NaCl, 4.7 KCl, 25.1 NaHCO3, 1.3 NaH2PO4, 1.2 MgSO4, 7H2O, 1.5 CaCl2, 1.0 citric acid and 11.1 glucose, bubbled with 95% O2-5% CO2. The temperature was maintained at 4°C during dissection to prevent metabolic degradation. After further dissection the preparation was opened out longitudinally along the esophagus and greater curve of the stomach. The preparation was then placed mucosa side up in the organ bath. This preparation has been described in detail previously (45). Nifedipine (1 μM) was also added to the Krebs superfusate to prevent smooth muscle contraction. In a previous preliminary study we have shown that nifedipine has no effect on the mechanical sensitivity of gastroesophageal vagal afferents (48).

Female C57 mice fed ad libitum (20–30 g; N = 37) were killed via CO2 inhalation, and the thorax was opened by a midline incision. The stomach and esophagus were then placed in an organ bath in a similar manner to the ferret preparation. This preparation has been described in detail previously (47).

Characterization of Gastroesophageal Vagal Afferent Properties

In the ferret three distinct types of afferent were recorded: those responding to circular tension but not to low intensity mucosal stimuli (tension receptors), those responding only to mucosal stroking (mucosal receptors), and those responding to both mucosal stroking and circular tension (tension/mucosal TM; only found in the esophagus). These three types of afferent have been described in detail previously (44, 45). In the mouse two types of mechanosensitive afferent were studied: those responding to mucosal stroking but not circular tension (mucosal receptors) and those responding to mucosal stroking and circular tension (tension receptors) as reported previously (47).

Location of receptive fields of all types of afferent fiber was determined by mechanical stimulation throughout the preparation with a brush. Accurate quantification of mechanical responses was performed differently according to the primary adequate stimulus for the type of fiber. Mechanical thresholds of all types of fiber were determined by using calibrated von Frey hairs. The most reproducible, stimulus-dependent responses of these afferents to mucosal stimuli were evoked when the probe was moved at a rate of 5 mm/s across the receptive field rather than being static. Because receptive fields are increasingly lower compared with that used for reversal of the effect of exogenous ghrelin. [D-Lys-3]-GHRP-6 had a different effect on gastric and esophageal tension receptors, and so the afferents were compared according to location.

Data Recording and Analysis

Afferent impulses were amplified with a biological amplifier (DAM 50, World Precision Instruments, Sarasota, FL), filtered (bandpass filter 932, CWE, Ardmore, PA) and monitored with an oscilloscope (DL 1200A, Yokogawa, Tokyo). Single units were discriminated on the basis of action potential shape, duration, and ampli-
tude by use of Spike 2 software (Cambridge Electronic Design, Cambridge, UK). All data were recorded and analyzed off-line with a personal computer (IBM Thinkpad). Peristimulus time histograms and discharge traces were displayed by use of Spike 2 software. Data are expressed as means ± SE with n = number of individual afferents in all instances. The pharmacological protocol was performed on a maximum of one afferent fiber in each preparation. Differences between stimulus-response curves were evaluated by two-way ANOVA. Differences were considered significant if P < 0.05.

**Drugs**

Stock solutions of all drugs were kept frozen and diluted to their final concentration in Krebs solution on the day of the experiment. Ghrelin was obtained from AUSPEP (Parkville, Victoria, Australia), and [d-Lys-3]-GHRP-6 was obtained from Sigma (Sydney, Australia).

**Determination of Relative Ghrelin and Ghrelin Receptor Transcript Expression in Nodose Ganglia by Quantitative RT-PCR**

Nodose ganglia were removed bilaterally from five male or female mice (20–30 g) as described previously (46, 57). RNA quantification was determined by measuring the absorbance at 260 nm (A260) via a spectrophotometer (Bio-Rad, Regents Park, New South Wales, Australia). RNA quality was estimated by the A260 and A280 nm ratio.

Quantitative RT-PCR (QRT-PCR) reactions were performed as described in detail previously (37). Briefly, QRT-PCR reactions were performed by using a Chromo4 (MJ Research, Bio-Rad) real-time...
instrument attached to a PTC-200 Peltier thermal cycler (MJ Research) and analyzed with OptiGene Monitor software (MJ Research). QRT-PCR reactions were performed with a Qiagen QuantiTect SYBRgreen RT-PCR one-step RT-PCR kit (Qiagen) according to the manufacturer’s specifications, with specific Quantitect Primer Assays (Qiagen) optimized for the detection of the known sequence of mouse ghrelin, ghrelin receptor, and β-actin transcripts contained in the NCBI reference sequence database (www.ncbi.nlm.nih.gov/RefSeq).

These primer assays were used under the following conditions: reverse transcription, 50°C for 30 min; initial PCR activation, 95°C for 15 min; PCR cycles 94°C for 15 s, 55°C for 30 s, and 72°C for 30 s repeated for 45 cycles. A melting curve program verified the specificity and identity of the RT-PCR products and no primer dimers were observed. Confirmation of the amplified products was resolved by 3% agarose gel electrophoresis and visualized with ethidium bromide staining. Each assay was run in at least triplicate in separate experiments. Control PCRs were performed by substituting RNA template with distilled RNase-free water. All assays were validated for linearity of amplification efficiency and quantitative standard curves obtained by serial dilutions of RNA. Calculations for relative mRNA expression were performed by the comparative CT method, comparing to the internal reference gene β-actin using the equation ΔΔCT (Ct of target transcript − Ct of β-actin). To determine the relative expression of these transcripts in whole nodose ganglia, the ΔΔCT was calculated by using the formula ΔΔCT = ΔCT(ghrelin receptor) − ΔCT(β-actin) and the relative fold differences were calculated by using the modified version of 2^-ΔΔCT (52) correcting for PCR efficiencies. Quantitative data are expressed as means ± SD, and significant differences in transcript expression were determined by a Mann-Whitney test at a significance level of P < 0.05.

RESULTS

Electrophysiology

Effect of ghrelin on the mechanosensitivity of gastroesophageal vagal afferents. Mouse. The effect of ghrelin (1–10 nM) on the mechanosensitivity of mouse gastroesophageal vagal afferents is illustrated in Fig. 1. Ghrelin (1–10 nM) did not significantly affect the response of nine mucosal receptors to mucosal stroking with calibrated von Frey hairs (10–1,000 mg; Fig. 1A). However, ghrelin (3–10 nM) did significantly reduce the response of mouse tension receptors (n = 11) to circular tension (1–5 g; Fig. 1B and D).

Ghrelin (1–10 nM) did not significantly affect the spontaneous activity of mouse mucosal receptors (data not illustrated), the majority of which were silent. The spontaneous activity of mouse tension receptors was 11.54 ± 2.54 impulses/s. In the presence of ghrelin (3 and 10 nM) the spontaneous activity was significantly reduced (Table 1). The spontaneous activity of mouse tension receptors was similar with 1, 3, or 10 nM ghrelin (P > 0.05; one-way ANOVA, Bonferroni post hoc test), suggesting a very steep dose-response relationship.

The effect of ghrelin (3 nM) and the ghrelin receptor antagonist [D-Lys-3]-GHRP-6 (30–100 μM) on mouse tension receptors (n = 7) is illustrated in Fig. 1C. Ghrelin alone significantly reduced the response of mouse tension receptors to circular tension (1–5 g; Fig. 1C). When [D-Lys-3]-GHRP-6 (30–100 μM) was added to the Krebs superfusate along with ghrelin (3 nM), the inhibitory effect of ghrelin was reversed (Fig. 1C). [D-Lys-3]-GHRP-6 (100 μM) completely reversed the inhibition back to the original control values. [D-Lys-3]-GHRP-6 (30–100 μM) did not significantly affect the spontaneous activity of the tension receptors (data not shown).

Ghrelin reduces mechanosensitivity of vagal afferents (G1379)

Table 1. Spontaneous activity of gastroesophageal afferents sensitive to tension

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<tr>
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<th>Mouse</th>
<th>Ferret</th>
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<tr>
<td></td>
<td>Tension receptor</td>
<td>Tension receptor</td>
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<tr>
<td>Control</td>
<td>11.54±2.54</td>
<td>0.31±0.18</td>
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<tr>
<td>Ghrelin (1 nM)</td>
<td>8.42±2.11</td>
<td>0.57±0.35</td>
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<tr>
<td>Ghrelin (3 nM)</td>
<td>7.46±1.88*</td>
<td>0.57±0.25</td>
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<tr>
<td>Ghrelin (10 nM)</td>
<td>8.73±2.59</td>
<td>0.92±0.54</td>
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The spontaneous activity of mouse tension and ferret tension and tension/mucosal (TM) receptors in the absence and presence of ghrelin (1, 3, and 10 nM). Values are means ± SE in impulses/s. Ghrelin significantly reduced the spontaneous activity of mouse tension-sensitive receptors but did not significantly affect the spontaneous activity of ferret tension and TM receptors. *p < 0.05. †p < 0.01 compared with control (paired t-test). The spontaneous activity of mouse tension receptors was similar with 1, 3, or 10 nM ghrelin (P > 0.05).

Ferret. The effect of ghrelin on the mechanosensitivity of ferret gastroesophageal vagal afferents is illustrated in Fig. 2. Ghrelin (3–10 nM) significantly reduced the response of a subpopulation of mucosal receptors to mucosal stroking with calibrated von Frey hairs (10–1,000 mg; Fig. 2A). The mechanosensitivity of three, from a total of eight mucosal receptors studied, was reduced in the presence of ghrelin (3–10 nM). This reduction in mechanosensitivity was reversed upon washout of ghrelin from the Krebs superfusate (Fig. 2D). Ghrelin (1–10 nM) did not significantly alter the response of tension receptors (n = 7) to circular tension (1–5 g; Fig. 2B). The effect of ghrelin (1–10 nM) on the mechanosensitivity of TM receptors (n = 11) is illustrated in Fig. 1C. Ghrelin (1–10 nM) significantly reduced the response of ferret TM receptors to mucosal stroking with calibrated von Frey hairs (10–1,000 mg; Fig. 2CII). However, it did not affect the response of the same TM receptors to circular tension (1–5 g; Fig. 2CII).

Ghrelin (1–10 nM) did not significantly affect the spontaneous activity of ferret mucosal (data not illustrated because of the fact that these receptors are generally silent), TM, or tension receptors (Table 1).

Effect of the ghrelin receptor antagonist [D-Lys-3]-GHRP-6. The effect of the ghrelin receptor antagonist [D-Lys-3]-GHRP-6 (0.1 μM) alone on mouse tension receptors is illustrated in Fig. 3. It significantly increased the response of gastric tension receptors to circular tension (Fig. 3A) but did not significantly affect the response of esophageal tension receptors to circular tension (Fig. 3B). The antagonist did not significantly affect the spontaneous activity of the tension receptors (data not shown).

Quantitative PCR

Using gel electrophoresis we confirmed the size of the amplified products generated by the Quantitect Primer Assays specific for ghrelin, ghrelin receptor, and β-actin, showing intense single bands, corresponding to the predicted sizes of ghrelin, ghrelin receptor, and β-actin transcripts (Fig. 4A). Quantitative RT-PCR analysis of whole nodose ganglia revealed that, with β-actin as a reference Ct value, ghrelin receptor had a significantly greater transcript expression than ghrelin (P < 0.05, Fig. 4B). Specifically, ghrelin receptor transcripts were 2.36-fold more abundant than ghrelin transcripts in whole nodose ganglia.
DISCUSSION

The present study provides the first evidence that activation of GHS-R inhibits mechanotransduction in primary sensory endings. Ghrelin, the endogenous ligand for the GHS-R, inhibited gastroesophageal vagal afferents in mice and ferrets. This was reversed when the ghrelin receptor antagonist [D-Lys3]-GHRP-6 was present. Our data indicate important differences between species in the afferent subtypes influenced by ghrelin. In mice, ghrelin’s effect was confined to tension receptors, with no effect on mucosal afferents. In contrast, in the ferret ghrelin had no effect on the responses of tension and TM receptors to circumferential tension; however, it inhibited responses to mucosal stroking of TM and mucosal receptors with similar potency to that seen in mouse. This suggests there may be specific roles for ghrelin in specific pathways, which are discussed in detail below.

Vagal afferents provide input to pathways in the brain that are involved in triggering of behavioral responses (9, 17), reflexes controlling digestive function (32) and motor patterns that lead to gastroesophageal reflux (8, 41). Thus several physiological and pathophysiological processes can be triggered by upper gastrointestinal vagal afferents. These include
retching and vomiting, transient lower esophageal relaxations (which may allow gastroesophageal acid reflux), secondary peristalsis, relaxation of the proximal stomach, gastric emptying, and sensations associated with, for example, satiety, nausea, or discomfort. Any means of controlling this input may therefore have broad implications in several disease states. Our data suggest that modulation of ghrelin receptors on vagal afferents may provide such a means. A clear role for ghrelin has already been established in control of food intake. Exogenous infusion of ghrelin increases food intake of a buffet meal in humans (62) and increases meal frequency (18, 28). This is consistent with our finding that ghrelin reduces the response of tension receptors to circular stretch. Hence the signaling of presence and amount of food in the stomach would be reduced.

A variety of signals generated within the gastrointestinal tract are well known to influence food intake (13). Distention of the stomach inhibits feeding via stimulation of vagal afferent mechanoreceptors (25). In addition, the gastrointestinal tract releases various hormones that can influence food intake. Many gastrointestinal peptides regulate short-term food intake via vagal afferent neurons (7, 64). For example, leptin and CCK receptors are expressed in the nodose ganglion (14, 15, 50) and selectively activate vagal afferent neurons innervating the stomach and duodenum (51). Therefore, vagal afferents play a key role in the inhibition of food intake (42). Interactions between mechanical stimuli, e.g., gastric distention, and hormones such as CCK have been reported on vagal afferents but are controversial as to their effects on single subtypes of vagal afferents (10, 23, 33, 56). Our findings indicate that ghrelin should be added to the range of hormones involved in interaction with mechanical activation of gastric afferents and clearly demonstrate that this interaction is negative.

It has been suggested that ghrelin increases food intake by suppressing the discharge of vagal afferents (6, 22), but whether this is a direct action or via modulation of other vagal responses has remained unresolved. Using our in vitro flat-sheet preparation, we distinguish specific subtypes of vagal afferents, whereas in other studies whole nerve afferent discharge was recorded (6, 22) so the origin of the endings could not be determined. In our study, ghrelin selectively reduced mechanosensitivity but also inhibited the basal firing of mouse tension receptors, yet it had no effect on basal firing of other types of mouse and ferret afferents. The basal firing rate of tension receptors was higher in mouse than in ferret. The baseline mechanical forces within mouse tissue are likely to be different to those in ferret because of its smaller dimensions so that the afferents are signaling different resting levels of tension. In this case ghrelin would be expected to inhibit

Fig. 3. Effect of [D-Lys-3]-GHRP-6 on mouse gastroesophageal vagal afferents. Stimulus-response functions of gastric (A, n = 6) and esophageal (B, n = 4) tension receptors to circumferential tension. The responses are before (●) and after (○) exposure to [D-Lys-3]-GHRP-6 (0.1 μM). *P < 0.05 compared with control (2-way ANOVA). Bonferroni post hoc tests revealed that the significant differences occurred at the larger tension stimuli (3–5 g; not shown), which may be due to decreasing variance in responses at higher loads.

Fig. 4. Transcript expression and relative expression of ghrelin and ghrelin receptor in whole mouse nodose ganglia. A: PCR products of quantitative RT-PCR experiments separated on a 3% agarose gel to confirm the presence of ghrelin receptor and ghrelin transcripts. The sizes of the amplified products were confirmed, showing intense single bands, corresponding to the predicted sizes of ghrelin, ghrelin receptor and β-actin transcripts. In addition this validated the products obtained during quantitative analysis. B: quantitative RT-PCR data revealed that the ghrelin receptor had a significantly greater transcript expression than ghrelin (P < 0.05; Mann-Whitney test). Experiments were performed in at least triplicate. Fold differences are calculated relative to β-actin mRNA levels.
spontaneous activity in the mouse if it were decreasing mechanical sensitivity. The decrease in basal firing rate observed in multiunit recordings in mice and rats in vivo (6, 22) may therefore be due to a decrease in basal firing rate of tension receptors or to inhibition of ongoing mechanical activation. We propose that a component of ghrelin’s influence on food intake in mice is by inhibiting signaling of distention or contractions from the upper gastrointestinal tract that occur continually in both fasted and fed states. Another consequence of inhibition of signaling distention in the intact animal is the inhibition of reflex effects of distention on gastric motility via the central nervous system. One of the major vagovagal gastric reflexes via the caudal medulla is inhibitory (1). By inhibiting the input to this reflex, we would predict that ghrelin would reduce inhibition of gastric motor function. This is in keeping with studies showing that ghrelin increases gastric motility and emptying (6, 40) and that this is mediated via an action on vagal afferents. It must be noted, however, that there are direct central actions of ghrelin besides these (29) that may also serve to promote gastrointestinal motility in vivo, either by reducing nitricergic vagal inhibition and/or by promoting cholinergic vagal excitation of gastric motility. Whatever its mechanism of action, ghrelin is a potent gastric prokinetic that may have important therapeutic implications for conditions in which gastric emptying is delayed.

The ghrelin receptor is a G-protein coupled receptor that can regulate ionic currents (16). We have previously shown that the inhibitory effect of another G-protein coupled receptor agonist baclofen exerts its inhibitory effect on vagal afferent mechanosensation via calcium and potassium channels linked to the G-protein coupled GABAB receptor (48). It is possible that ghrelin is modulating mechanosensitivity of vagal afferents via similar mechanisms.

The half-life of physiologically active ghrelin in the blood is relatively short (~10 min) because n-octanoylated ghrelin is easily degraded by plasma esterase and becomes des-n-octanoyl ghrelin, an inactive form (4). The concentration of active ghrelin is therefore much higher closer to its site of release in the stomach, so vagal afferent endings in the stomach would be an obvious site of action for ghrelin. Ghrelin receptors have been found in nodose ganglion (22) and specifically in nodose neurons that project to the stomach of the rat (54). In the present study we confirm that ghrelin receptors are also present in mouse nodose ganglia. It has been shown that these receptors are transported to the periphery (22) where they are associated with nerve fibers presumably of vagal origin, in addition to intrinsic cells of the gut (20). In addition we also found expression of ghrelin in mouse nodose ganglia. We found that expression of ghrelin mRNA in nodose neurons was low compared with the ghrelin receptor, but nevertheless it raises the possibility of an autoregulatory role of ghrelin released from vagal afferent endings, in addition to the role of ghrelin released from the gastric endocrine cells. The autoregulatory role of ghrelin may be of more importance in esophageal afferents where there is less ghrelin in the tissue surrounding the afferents than in the stomach (31). We investigated a role for endogenous ghrelin on the mechanosensitivity of mouse tension receptors using the ghrelin receptor antagonist [D-Lys-3]-GHRP-6 and only observed an effect on gastric tension receptors, the antagonist having no effect on esophageal tension receptors. This implies an endogenous source of ghrelin in the stomach but not the esophagus, arguing against an autoregulatory role for ghrelin in the esophagus. Together with evidence from other studies, these findings would suggest the gastric mucosa is the primary source of endogenous ghrelin acting on vagal afferents.

In the present study we have shown that ghrelin primarily reduces afferent responses to tension in the mouse and mucosal stroking in the ferret. This is unlikely to be a technical issue, as we have observed inhibition of all types of afferents in both species previously with peptide agonists such as galanin (49). It is conceivable that this difference between mice and ferrets reflects the inability of rodents to vomit and to exhibit gastro-esophageal reflux, whereas ferrets like humans are able to vomit and do exhibit gastroesophageal reflux (11). Interestingly, it has been shown that ghrelin has antiemetic effects in the ferret (53) although its effect on ferret food intake has not been specifically evaluated. Both emesis and food intake inhibition are triggered by activation of mucosal receptors in the stomach and small intestine (2, 3, 12, 34, 35). Our observation of selective inhibition of mucosal receptors by ghrelin therefore fits in with its antiemetic effect. Mice, which do not vomit, may rely more on the ability of ghrelin to act on vagal tension receptors in the periphery to promote food intake.

In conclusion, the present study provides the first direct evidence for the inhibitory modulation of primary afferent mechanotransduction by the gastric peptide ghrelin. These results confirm GHS-R as a potential target for the treatment of obesity and possibly other disorders of upper gastrointestinal function. They also provide greater understanding of the mechanisms of action of ghrelin.

GRANTS

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REFERENCES


