Sensitization of Mechatosensitive Gastric Vagal Afferent Fibers in the Rat by Thermal and Chemical Stimuli and Gastric Ulcers

Yu-Ming Kang, Klaus Bielefeldt, and G. F. Gebhart

Departments of Pharmacology and Internal Medicine, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, Iowa 52242

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Kang, Yu-Ming, Klaus Bielefeldt, and G. F. Gebhart. Sensitization of mechatosensitive gastric vagal afferent fibers in the rat by thermal and chemical stimuli and gastric ulcers. J Neurophysiol. 91: 1981–1989, 2004; 10.1152/jn.01097.2003. In the present study we examined the effects of acute thermal and chemical stimuli and gastric ulceration on mechatosensitive gastric vagal afferent fibers. Single-fiber recordings were made from the cervical vagus nerve. Mechanosensitive afferent fibers were identified by response to gastric distension (GD). Intragastric pressure was maintained below 3 mmHg during intragastric instillation of saline, heated saline, HCl, or glycocholic acid. Responses to graded GD (5–60 mmHg, 20 s, 4-min interval) were determined before and after 30-min exposure to thermal or chemical stimuli. All mechanosensitive fibers studied were C-fibers (mean CV: 1.07 ± 0.07 m/s). Saline (37°C) did not affect resting activity or alter responses to GD, but exposure to heated saline (46°C) significantly increased resting activity and sensitized responses to GD. The decrease in resting activity was hydrochloric acid concentration dependent (0.025–0.2 N), but responses to GD were sensitized after 30-min exposure to 0.1 N HCl (n = 7). The bile acid glycocholic acid significantly increased resting activity and desensitized responses to GD at an intragastric pH of 7, and similarly increased resting activity but sensitized responses to GD (n = 6) at an intragastric pH of 1.2. Vagal afferents recorded in rats with gastric ulcers had significantly greater resting activity and responses to GD than sham ulcer rats; intragastric instillation of glycocholic acid (pH 1.2) further increased afferent fiber excitability. These findings indicate that acute gastric thermal and chemical stimuli alter the response characteristics of mechanosensitive vagal afferents in the absence of inflammation or structural damage. Accordingly, acute sensitization of gastric afferents through different stimulus modalities may contribute to the development of dyspeptic symptoms. In the presence of gastric inflammation, mechanosensitive vagal afferents exhibit a further increase in excitability.

INTRODUCTION

Functional gastrointestinal disorders, such as nonulcer dyspepsia and irritable bowel syndrome, are characterized by altered sensations, principally discomfort and pain (Mc Namara et al. 2000; Mujica et al. 2001; Naliboff et al. 1997). It is generally held that spinal visceral afferents convey information to the CNS that is interpreted as uncomfortable or painful, whereas vagal afferents play little or no role in painful sensations that arise from the gastrointestinal tract. We previously reported that gastric ulceration produces hypersensitivity to gastric distension (Ozaki et al. 2002) and that mechanosensitive gastric afferent fibers sensitize (Ozaki et al. 2001). Although most studies suggest that pain associated with gastrointestinal disorders is conveyed to the CNS by the splanchnic innervation of the stomach, there is growing evidence that gastric vagal afferent input may also contribute to the altered sensations associated with functional gastrointestinal disorders.

Gastric vagal afferents terminate principally in the nucleus of the solitary tract in the dorsal medulla and from there information is distributed to higher centers involved in stress and emotional state (Berthoud and Neuhuber 2000). In addition to mechanical stimuli, other modalities of stimulation, principally chemical stimuli, may trigger symptoms in patients with functional gastrointestinal disorders. Limited information is available about chemo- and thermosensation from the gastrointestinal tract as related to discomfort and pain. Interestingly, gastric injury attributed to acid exposure activates vagal rather than spinal afferents (Schuligoi et al. 1998), suggesting a role for vagal input in chemonociception. Consistent with these findings, we have recently shown that vagotomy, but not splanchnic nerve resection, abolishes the aversive response to instillation of acid into the stomach (Lamb et al. 2003).

Considering the polymodal character of visceral afferent fibers and the potential role of vagal afferent input in chemonociception, we hypothesized that acute thermal or chemical stimuli would sensitize vagal afferent mechanosensitive fiber responses to gastric distension. Portions of these data were previously reported in abstract form (Kang et al. 2003).

METHODS

General procedures

Male Sprague–Dawley rats (440–460 g, Harlan, Indianapolis, IN), housed under a 12-h light and dark cycle with free access to water and food, were used for all experiments. Animal care and use followed the guidelines of the American Physiological Society. The experimental protocol was approved by the Animal Care and Use Committee of The University of Iowa. Food, but not water, was withheld for 24 h before surgery. Rats were anesthetized initially with an intraperitoneal injection of pentobarbital sodium (Nembutal, Abbott Laboratories, Abbott Park, IL; 45–50 mg/kg) and subsequently maintained with a constant intravenous infusion of pentobarbital (5–10 mg kg⁻¹ h⁻¹). The right femoral vein and artery were cannulated for infusion of fluid and pentobarbital anesthetic and monitoring blood pressure and heart rate, respectively. Mean arterial pressure was maintained at >80 mmHg with supplemental intravenous injection of 5% dextrose in saline administered in a bolus of 1–1.5 ml as required. Core body temperature was monitored with a rectal thermoprobe (Yellow Springs...
Instruments, Yellow Springs, OH) and maintained at 36°C by a hot water–circulating heating pad placed under the rat and an overhead feedback-controlled heat lamp. At the end of experiments, rats were killed with an overdose of pentobarbital.

The abdomen was opened by a transverse epigastric incision 4–5 cm in length to expose the stomach. To measure afferent fiber conduction velocity, the right vagus nerve was isolated from the distal esophagus and a pair of Teflon-coated, 40-gauge stainless steel wires stripped at the tips were placed around the nerve and sealed with nonconductive Wacker gel (Wacker Silicone, Adrian, MI). For fluid gastric distension (GD), an orogastric tube (Tygon, 2.3 mm OD, 1.3 mm ID) was inserted and secured by a ligature around the esophageal–gastric junction. Another Tygon tube (3.9 mm OD, 2.4 mm ID) was introduced distally into the stomach through the pylorus and secured by a ligature placed caudal to the pyloric sphincter; the duodenum was ligated close to the pyloric ring. The distal catheter was connected to a reservoir containing saline at 37°C. Gastric fluid distension was triggered by a distension-control device with the outflow catheter closed. A pressure transducer connected to the outflow catheter continuously monitored intragastric pressure. The abdomen was closed with silk sutures.

**Vagal afferent activity**

The right vagus nerve below the nodose ganglion was exposed by a ventral midline incision in the neck. The sternocephalomandibular, sternohyoideus, and omohyoid muscles were removed. The neck skin was laterally tied to the frame to make a pool for warm mineral oil (37°C). The nerve was dissected away from the carotid tissue sheath, decentralized close to its entry to the nodose ganglion, and placed over a black microscope base. The perineural sheath was removed in the pool of warm mineral oil (37°C), the nerve was split into thin bundles, and fine filaments were teased from the bundle to obtain a single unit. Electrical activity of the single unit was recorded by placing the fiber over one arm of a bipolar silver–silver chloride electrode. Action potentials were displayed on a storage oscilloscope after low-noise differential amplification. Action potentials were processed through a window discriminator and counted (1-s bin width) on-line using the Spike2/CED 1401 data-acquisition program. Peristimulus time histograms, intragastric pressure, and blood pressure were continuously displayed on-line.

**Experimental protocol**

To measure conduction velocity, the vagus nerve was stimulated with a single 0.5-ms square-wave pulse at 3–8 mA, and the conduction latency (time between stimulus artifact and evoked response) was recorded. The distance between stimulation and recording electrodes was measured postmortem. Fibers were classified on the basis of their conduction velocities as C-fibers (<2.5 m/s) or thinly myelinated Aδ fibers (>2.5 m/s).

Mechanosensitive gastric afferents in the vagus nerve were identified by response to GD (40 mmHg, <5 s). If a fiber responded to the test stimulus, a stimulus–response function (SRF) to distending pressures of 5, 10, 20, 40, and 60 mmHg, 30 s at 4-min intervals was determined. Resting (baseline) activity was recorded for 5 min, after which a thermal or chemical stimulus was applied by instilling into the stomach saline (37°C), heated saline (46°C, pH 7), HCl (0.025, 0.05, 0.1, 0.2 N), or glycocholic acid (2.5 mM, 5 mM, 10 mM, at a pH value of either 7 or 1.2). Intragastric pressure was maintained <3 mmHg during the 30 min of intragastric instillation of saline, heated saline, HCl, or glycocholic acid. After 30 min, the fluid was removed and another SRF to distending pressures of 5–60 mmHg was obtained. The osmolality of all solutions was adjusted to 300 mOsm using D-mannitol. For 0.2 N HCl, NaCl with identical osmolality served as control. Intragastric pressure was maintained <3 mmHg during application of thermal or chemical stimuli to avoid mechanical stimulation. To monitor the temperature of the perfusate, a thermoprobe (Physitemp) was introduced into the stomach through the proximal catheter. At the end of the protocol for each fiber, the abdomen was opened, the stomach was exposed, and the mechanosensitive receptive field was located by probing the serosal surface of the stomach with a fine, blunt rod.

**Gastric kissing ulcers**

In other rats, we produced gastric ulceration by injection of acetic acid into a clamped area of the stomach (Lamb et al. 2003). Briefly, the stomach was exposed by a midline incision and the dorsal and ventral stomach was placed between a pair of circular clamps (10 mm ID). In the kissing ulcer (KU) group, 100 μl of 60% acetic acid (HAc) was injected into the lumen between the clamps and withdrawn after 45 s. Controls received an identical injection of sterile saline. The abdominal cavity was subsequently rinsed with sterile saline, the incision closed, and rats allowed to recover until day 5, a time at which gastric balloon distension or instillation of HCl into the stomach produces significantly increased visceromotor responses (Lamb et al. 2003).

**Assessment of gastric injury**

The stomach was examined after completion of the experimental paradigm (i.e., after 30-min exposure to the luminal stimulus and repeating the SRF). The stomach was removed, opened along the greater curvature, pinned flat (mucosal side up), and examined under a dissecting microscope to qualitatively assess morphological damage. The stomach was then fixed in 10% buffered formalin, sectioned, and stained with hematoxylin and eosin. Mucosal structure, hemorrhage, and inflammatory infiltrate were assessed by an individual unaware of treatment.

**Drugs and chemicals**

Glycocholic acid was obtained from Sigma Chemical (St. Louis, MO), HCl from Fisher Scientific (Fair Lawn, NJ), and D-mannitol from Amresco (Solon, OH).

**Data analysis**

The resting activity of a fiber was counted for 60 s before GD, and the response to GD was determined as the increase in discharge during GD above its resting activity (imp/s). SRFs to graded GD were plotted for each individual fiber, and a least-squares regression line was obtained from the linear part of the SRF. The regression line was extrapolated to the ordinate (representing distension pressure) to estimate the response threshold. The response to intragastric instillation of 37 or 46°C saline, HCl, or glycocholic acid was determined as the maximum (peak) change in resting activity during the period of instillation of the fluid (imp/s). The time to the peak change was also determined. The mean change in resting activity was determined by counting fiber activity during the 5 s before and after the peak change in resting activity (imp/s). Changes were not considered significant if they did not differ from control by more than ±2 SDs of mean resting activity. Quantitative data are expressed as means ± SE. Results were analyzed using one-way ANOVA for repeated measures or Student’s t-test. A value of P < 0.05 was considered statistically significant.

**RESULTS**

**Fiber sample**

Thirty-six mechanosensitive gastric afferent fibers were studied before and after acute intragastric exposure to thermal or chemical stimuli. The conduction velocity of this
sample of fibers was \(1.09 \pm 0.07 \text{ m/s (range: 0.57–1.83 m/s)}\) and their resting activity was \(2.04 \pm 0.21 \text{ Hz (range: 0.03–5.4 Hz)}\). Evaluation of the individual SRFs (Fig. 1) revealed a homogeneous sample of fibers with an extrapolated response threshold of \(2.88 \pm 1.03 \text{ mmHg}\), consistent with previous reports from this laboratory (Ozaki et al. 1999). The receptive fields of the 36 fibers were located in the corpus (17), corpus and antrum (7), corpus and cardia (5), and corpus and fundus (7) and are presented in Table 1 along with other characteristics of the sample. An additional 12 mechanosensitive gastric vagal afferent fibers were studied in rats 5 days after gastric ulceration (or sham treatment). The conduction velocity of this second sample of fibers was \(0.95 \pm 0.09 \text{ m/s (range: 0.8–1.56 m/s)}\). Their receptive fields were located in the corpus (7), corpus and antrum (2), and corpus and fundus (3) and are presented in Table 1. In rats with KUs, the receptive fields of 5 fibers involved the inflamed area of the experimental ulcer, whereas the receptive field for one fiber was in the corpus, 0.9 cm proximal of the ulcer.

**Effects of intragastric instillation of 37°C saline**

The effect of gastric instillation of 37°C saline (a control group) was tested in 7 fibers. Saline did not significantly alter either resting activity or responses to gastric distension when tested after 30-min exposure to 37°C saline (Fig. 2). The extrapolated response threshold before and after 37°C saline infusion did not change nor did the slopes of the SRFs. These and other data are summarized in Table 1.

**Effect of intragastric instillation of 46°C saline**

Thermal sensitivity was tested in 8 gastric vagal mechanosensitive fibers. The representative fiber illustrated in Fig. 3B shows that intragastric instillation of 46°C saline significantly increases resting activity (peak \(5.7 \pm 0.6 \text{ Hz}\); mean \(4.02 \pm 0.6 \text{ Hz, n = 8; P < 0.01}\;\text{see Table 1}\) at a latency of \(93.7 \pm 18.1 \text{ s (range: 35–182 s)}\). After exposure to 46°C for 30 min, the SRF to GD (5–60 mmHg) was shifted significantly to the left [Fig. 3A; \(F_{(9,63)} = 26.4, P < 0.001\)].

![FIG. 1. A: individual stimulus-response functions (SRFs) and mean SRF (±SE; inset) of 36 vagal gastric mechanosensitive fibers. B: photomicrographs (×200) demonstrating intact gastric mucosa after 30-min exposure to 46°C saline (a), 0.1 N HCl (b), or 5 mM glycocholic acid (c). d: photomicrograph (×50) from an ulcerated stomach [kissing ulcer (KU)]. Granulation tissue covers the ulcerated area with extension of the inflammatory infiltrate into the muscularis propria and adjacent mucosa (light).](image-url)
Effects of intragastric instillation of HCl

As illustrated in Fig. 4, exposure to 0.1 N HCl (pH 1.0) decreased spontaneous activity and sensitized responses of mechanosensitive gastric vagal fibers to GD. Hydrochloric acid (0.1 N) exposure significantly reduced resting activity to 0.6 ± 0.3 Hz (n = 7) at a latency of 224 ± 22 s (range: 137–284 s). The reduction in resting activity was HCl concentration dependent (0.025–0.2 N) (Fig. 4A; see also example in Fig. 4C). After exposure of the luminal surface of the stomach to 0.1 N HCl for 30 min, the SRF to GD was shifted significantly to the left [F(9,54) = 21.2, P < 0.001], consistent with sensitization to mechanical stimulation. The data are summarized in Table 1.

Effects of intragastric instillation of glycocholic acid, pH 7

The bile acid glycocholic acid was tested in 8 mechanosensitive fibers and, as illustrated in Fig. 5, exposure of the stomach to 5 mM glycocholic acid increased resting activity, but desensitized responses to GD. The effect of glycocholic acid on resting activity, studied in a different group of 7 fibers, was concentration dependent (2.5–10 mM, pH was adjusted to 7 by adding NaOH) (Fig. 5A). At a concentration of 5 mM, glycocholic acid significantly increased resting activity of mechanosensitive vagal nerve fibers to a peak of 6.2 ± 1.0 Hz (P < 0.01) at a latency of 254 ± 44 s (range: 89–370 s). Resting activity increased to a mean 4.24 ± 0.8 Hz (P < 0.01, n = 8; see Table 1). After 30-min exposure to 5 mM glycocholic acid, the slope of the SRF to gastric distension was significantly reduced [F(9,63) = 22.8, P < 0.001] compared with control conditions (Fig. 5B and Table 1).

Effects of intragastric instillation of glycocholic acid, pH 1.2

We chose a combination of the chemical stimuli HCl and the conjugated primary bile acid glycolcholic acid, which can be found in duodenal reflux in concentrations exceeding 1 mM (Bechi et al. 2000). The proton concentration was adjusted to a physiologically relevant pH, given that duodenogastric bile reflux typically occurs in the presence of gastric acid. In 6 gastric vagal mechanosensitive fibers, instillation of 2.5 mM glycolcholic acid and 0.05 N HCl (pH = 1.2) significantly increased resting activity to a peak of 6.1 ± 1.6 Hz (Fig. 6) at a latency of 190 ± 26 s (range: 86–236 s). Resting activity increased to a mean 3.86 ± 1.04 Hz (P < 0.05, n = 6; see Table 1). After 30-min exposure to 2.5 mM glycolcholic acid/0.05 N HCl (pH = 1.2), the SRF to GD was significantly shifted to the left [F(9,45) = 9.9, P < 0.001], consistent with sensitization to mechanical stimulation (Fig. 6, see also Table 1).

Effects of thermal and chemical stimulation on gastric mucosal damage

Macroscopically, no lesions were seen after any of the protocols described above (i.e., distension, infusion of a chemical, or thermal stimulation and repeated distension). This was consistent with microscopic examination of the stomach, which demonstrated an intact epithelium without evidence of mucosal injury (Fig. 1). Occasional dilated blood vessels were seen within the luminal third of the mucosa under control conditions and after exposure to heated saline, acid, and bile acid as well. Some exfoliated cells were seen in extruded mucus in control animals (n = 1), after heated saline (n = 1), and after acid (n = 2) or bile infusion (n = 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conduction Velocity (m/s)</th>
<th>Mean Spontaneous Activity (imp/s)</th>
<th>Response at 5 mmHg GD</th>
<th>Receptive Field Location</th>
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<td>Saline (37°C)</td>
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<td>Before</td>
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<td>1.84 ± 0.31</td>
<td>2.94 ± 0.62</td>
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<td>(n = 61)</td>
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<tr>
<td>Before</td>
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<td>3.72 ± 1.16</td>
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<tr>
<td>After</td>
<td>(n = 8)</td>
<td>4.02 ± 0.58*</td>
<td>9.42 ± 1.75*</td>
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<td>0.1 N HCl</td>
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<tr>
<td>Before</td>
<td>1.33 ± 0.16</td>
<td>2.98 ± 0.65</td>
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<td>After</td>
<td>(n = 7)</td>
<td>0.56 ± 0.28*</td>
<td>10.21 ± 2.52*</td>
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<td>5 mM Glycocholic acid (pH 7)</td>
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<td>Before</td>
<td>1.20 ± 0.18</td>
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<td>5.79 ± 1.37</td>
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<td>After</td>
<td>(n = 8)</td>
<td>4.24 ± 0.78*</td>
<td>3.67 ± 2.17</td>
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<td>2.5 mM Glycocholic acid (pH 1.2)</td>
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<tr>
<td>Before</td>
<td>1.02 ± 0.15</td>
<td>1.75 ± 0.64</td>
<td>2.96 ± 1.44</td>
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<td>After</td>
<td>(n = 6)</td>
<td>3.86 ± 1.04*</td>
<td>11.85 ± 4.18*</td>
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<td>Sham ulcer</td>
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<td>0.85 ± 0.06</td>
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<td>3.33 ± 0.75</td>
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<td>After</td>
<td>(n = 6)</td>
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<tr>
<td>Kissing ulcer (KU) 2.5 mM glycocholic acid (pH 1.2)</td>
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<td>Before</td>
<td>1.04 ± 0.12</td>
<td>3.23 ± 1.05</td>
<td>11.74 ± 3.25</td>
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<tr>
<td>After</td>
<td>(n = 6)</td>
<td>6.35 ± 1.37*</td>
<td>36.19 ± 8.06*</td>
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Values are means ± SE before and after 30-min intragastric exposure to indicated treatment. * Significantly different (P < 0.05) from corresponding control (before) value.
Effects of intragastric instillation of glycocholic acid, pH 1.2, in rats with gastric ulcers

In sham-treated rats, the resting activity and responses to gastric distension of 6 gastric vagal mechanosensitive fibers did not differ from data collected in naïve rats (Table 1 and Fig. 7). Thus clamping of the stomach and exposing its luminal surface to sterile saline did not alter response characteristics of the fibers studied, and this is consistent with the absence of gastric inflammation (see Lamb et al. 2003). In 6 gastric vagal mechanosensitive fibers recorded in rats 5 days after production of ulcers, the mean resting activity was significantly greater than that in fibers recorded in sham ulcer rats and the SRF was significantly shifted to the left (Fig. 7). Interestingly, the magnitude of change in excitability of the fibers studied was similar to that produced by acute instillation of either 46°C saline, 0.1 N HCl, or 2.5 mM glycocholic acid at pH 1.2 (Table 1). Intragastric instillation of 2.5 mM glycocholic acid and 0.05 N HCl (pH = 1.2) in rats with gastric ulcers further and significantly increased both resting activity (from 3.23 ± 1.05 to 6.35 ± 1.37 Hz) and responses of these mechanosensitive fibers to gastric distension \[F_{(9,45)} = 20.9, P < 0.001; \text{Fig. 7}].

DISCUSSION

The present study documents the polymodal character of mechanosensitive gastric vagal afferent fibers, consistent with prior reports indicating that the majority of vagal afferents in the rat are sensitive to more than one stimulus modality (e.g., Berthoud and Neuhuber 2000; Wei and Wang 2000). Gastric vagal afferents were identified by their response to distension of the stomach and all fibers studied also responded to thermal or chemical stimuli. In this study we did not test fibers for responses to all 3 stimulus modalities, but preliminary results suggest that many mechanosensitive gastric vagal afferent fibers respond to both thermal (46°C) and acid stimuli (either HCl or glycocholic acid). Significantly, 30-min exposure to chemical or thermal stimuli sensitized responses of these fibers to gastric distension. Although not studied here, preliminary investigation (Kang, Bielefeldt, and Gebhart, unpublished observations) suggests that sensitization after such exposure can last ≤60 min.

All fibers tested responded to thermal stimulation with an increase in resting activity, consistent with evidence that the TRPV1 (VR1) receptor, a heat sensor, is expressed by more than 80% of nodose ganglion neurons (Michael and Priestley 1999). Because we did not want to damage the stomach, we did not test the effects of intragastric temperatures >46°C and so cannot comment on whether some of the mechanosensitive fibers studied also may have expressed other members of the family of temperature-gated ion channels identified in rat vagal sensory neurons (e.g., TRPV2, TRPm8; Ichikawa and Sugi-moto 2003; Zhang et al. 2003).

FIG. 2. Effect of intragastric instillation of 37°C saline on responses to gastric distension. A: mean SRFs (± SE) of 7 fibers before and 30 min after instillation of 37°C saline into the stomach. B: responses of a representative vagal afferent fiber to gastric distension before and 30 min after instillation of 37°C saline into the stomach.

FIG. 3. Effect of intragastric instillation of 46°C saline on responses to gastric distension. A: mean SRFs (± SE) of 8 fibers before and 30 min after instillation of 46°C saline into the stomach. B: responses of a representative vagal afferent fiber to gastric distension before and 30 min after instillation of 46°C saline into the stomach.
The effects of exposure of the stomach to 46°C saline contrasts with results obtained during the intragastric instillation of HCl, where luminal exposure to 0.1 N HCl significantly decreased resting activity. Because the gastric vagal afferent fibers studied here were identified based on their responses to mechanical stimulation, it is unlikely that the decrease in resting activity after intragastric HCl contributes to chemosensation or chemonociception. Like heated saline, however, acute acid exposure also sensitized responses to subsequent mechanical distension of the stomach. These data clearly show that acid (and other chemical stimuli, e.g., bile) can alter the properties of afferent neurons that do not play a major role in chemosensation, primarily by changing their response to other stimulus modalities. We encountered no fibers that were excited by HCl, consistent with reports that only a small number of vagal afferents are activated by luminal exposure to acid (Page and Blackshaw 1998; Page et al. 2000, 2002; Sekizawa et al. 1999). Additional studies are needed to determine whether chemosensitive gastric vagal afferents can be similarly sensitized by different stimulus modalities.

Vagal afferents are also important in the regulation of gastric acid secretion (Ramos et al. 1992), but the localization of vagal afferents involved in chemosensation or chemonociception is not known. Several mechanosensitive endings, on the other hand, have been localized. Many vagal afferent fibers within the gastric wall terminate as intraganglionic laminar endings in myenteric ganglia (see Phillips and Powley 2000 and Powley and Phillips 2002 for reviews), where they may receive input from or interact with submucosal and myenteric neurons. Intraganglionic laminar endings (IGLEs) are densely distributed in the corpus and antrum of the stomach (the location of 33 of receptive fields of the total 48 fibers studied here) and are thought to respond to tension in smooth muscle. In addition to IGLEs, gastric vagal afferents terminate in intramuscular arrays (IMAs) that are thought to respond to stretch of smooth muscle. Finally, mucosal terminations of the vagus have been described that express the TRPV1 receptor, suggesting that they may be involved in thermo- and chemosensation (Berthoud and Neuhuber 2000; Patterson et al. 2003).

It is not known which, if any, of these structures is chemosensitive. In recent studies using organ-nerve in vitro preparations, the majority of acid-sensitive fibers also responded to gentle mucosal stroking, suggesting that their endings terminate within the mucosal layer (Page and Blackshaw 1998; Page et al. 2002). Of 5 tension sensitive gastric vagal afferents studied in an in vitro gastroesophageal preparation, none responded to acid, whereas 2 responded to chemical stimulation with α,β-methylene ATP or bile (Page et al. 2001). Whether the tension receptors were IGLEs was not known, but was likely. In contrast, of 18 gastric mucosal vagal afferents studied...
in the same in vitro gastroesophageal preparation, 11 responded to chemical stimuli. There is also a smaller number of nerve endings that extend into the submucosa and mucosa, where they may be excited by mechanical and perhaps chemical stimulation of the mucosal layer.

The bile acid glycocholic acid produced effects dependent on the pH of the solution instilled into the stomach. Instillation of 5 mM glycocholic acid (pH 7) significantly increased resting activity and desensitized responses to GD, whereas instillation of 2.5 mM glycocholic acid and 0.05 N HCl into the stomach. A: responses of a representative gastric vagal afferent fiber before and 30 min after instillation of 2.5 mM glycocholic acid and 0.05 N HCl into the stomach.

Blackshaw 1998), we did not observe activation of silent afferents by bile acids. Additional studies are needed to determine the effects of pH and bile acid concentration and relative potency of different bile acids on vagal afferents. These findings do, however, reveal that bile acid across a range of concentrations increases the resting activity of mechanosensitive vagal afferents and thus could contribute to the development of dyspeptic symptoms.

The most interesting result of the current study is that acute thermal and chemical stimuli significantly alter mechanosensitivity of gastric vagal receptors. Most studies have examined the effect of tissue injury or inflammation on the function of afferent nerves. Such interventions typically trigger the production of multiple signaling molecules, such as growth factors and cytokines, leading to changes in neuron properties attributed to altered gene transcription (Bielefeldt et al. 2003; Toma et al. 2000; Winston et al. 2001). We hypothesized that application of thermal and chemical stimuli may rapidly alter nerve function and potentially contribute to peripheral sensitization. Prior investigations in healthy humans demonstrate that gastric infusion of acid sensitizes the stomach to concomitant mechanical stimulation (Coffin et al. 2001). However, the mechanisms of this acute modulation are unclear. Experiments with different mechanical stimulation paradigms suggested that summa-

![Figure 6](http://jn.physiology.org/)

**FIG. 6.** Effect of intragastric instillation of the bile acid glycocholic acid (2.5 mM) and 0.05 N HCl (intragastric pH = 1.2) on responses to gastric distension. A: mean SRFs (±SE) of 6 fibers before and 30 min after instillation of 2.5 mM glycocholic acid and 0.05 N HCl into the stomach. B: responses of a representative gastric vagal afferent fiber before and 30 min after instillation of 2.5 mM glycocholic acid and 0.05 N HCl into the stomach.

![Figure 7](http://jn.physiology.org/)

**FIG. 7.** Effects of gastric ulceration on responses to gastric distension. A: mean SRFs (±SE) of 6 fibers from rats 5 days after sham ulcer surgery (unfilled circles) and of 6 fibers from rats 5 days after production of KUs before (filled circles) and 30 min after (filled squares) instillation of 2.5 mM glycocholic acid and 0.05 N HCl into the stomach (pH 1.2). B: responses of a representative gastric vagal afferent fiber from a rat with a KU before and 30 min after instillation of 2.5 mM glycocholic acid and 0.05 N HCl into the stomach.
tion at spinal or more central synapses in the form of visceral-visceral rather than viscerosomatic convergence contributed to the change in visceral sensation (Serra et al. 1995, 1998). The present experiments addressed the role of peripheral nerves and provide direct evidence that thermal and chemical stimulation change the mechanosensitive properties of primary vagal afferents. We did not observe macro- or microscopic changes in the mucosal architecture. Although macroscopic and light microscopic examination cannot rule out subtle structural changes that might result in increased diffusion of luminal contents into the mucosa, the present data allow us to conclude that the results were not simply the result of gastric injury. Increasing resting activity alone, as was produced by thermal and bile acid stimulation, can likely contribute to sensations from the stomach. There is growing evidence that acid stimulation of the resting activity alone, as was produced by thermal and bile acid stimulation, can likely contribute to sensations from the stomach. The present data suggest that normal gastric contents, such as acid and bile or heated solutions, can directly affect the tissue. Additional studies are needed to characterize the underlying mechanisms.

The present data suggest that normal gastric contents, such as acid and bile or heated solutions, can directly affect the sensitivity of mechanosensitive vagal afferents and likely other sensory pathways in the gut. Although it is generally held that the vagus nerve does not contribute a significant role to the pain associated with functional gut disorders, it plays a central role in emotional reactions to visceral stimuli and may mediate other subjectively perceived information, thus contributing to the broad spectrum of dyspeptic symptoms.

Clearly, acute exposure of the stomach to noninjurious stimuli led to increased mechanosensitivity of vagal afferent fibers. We did not examine the duration of increased excitability and assume it to be fully reversible and time-limited. Interestingly, the magnitude of changes produced by acute exposure of the stomach was not different from the magnitude of changes in resting activity or response to gastric distension produced by gastric ulceration. The excitability of gastric vagal mechanosensitive endings, however, was shown to increase further when chemical stimulation was applied to the inflamed, ulcerated stomach.

In the aggregate, these data suggest a role for gastric vagal afferent fibers in nociception. Intragastric instillation of noninjurious chemical or thermal stimuli, applied either in the normal, uninflamed stomach or chemical stimuli acting on already sensitized mechanoreceptive endings in an ulcerated stomach, significantly affected the response properties of gastric mechanoreceptors. Thermal and chemical stimuli typically increased resting activity and responses of vagal mechanoreceptors across the dynamic range of distending pressures tested.

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Present address of K. Bielefeldt: Division of Gastroenterology, Department of Internal Medicine, University of Pittsburgh, Pittsburgh, PA 15261.

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