Experimentally induced ulcers and gastric sensory-motor function in rats

Y. M. Kang,1 K. Lamb,1 G. F. Gebhart,1 and K. Bielefeldt2
1Department of Pharmacology, University of Iowa, Iowa City, Iowa; and
2Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania

Submitted 4 June 2004; accepted in final form 22 September 2004


First published September 23, 2004; doi:10.1152/ajpgi.00250.2004.—Prior studies have demonstrated that inflammation can sensitize visceral afferent neurons, contributing to the development of hyperalgesia. We hypothesized that both afferent and efferent pathways are affected, resulting in changes in motor and sensory function. Kissing ulcers (KU) were induced in the distal stomach by injecting 60% acetic acid for 45 s into a clamped area of the stomach. In controls, saline was injected into the stomach. A balloon catheter was surgically placed into the stomach, and electromyographic responses to gastric distension were recorded from the acromiotrapezius muscle at various times after ulcer induction. The accommodation reflex was assessed by slowly infusing saline into the distally occluded stomach. Gastric pressure changes in response to vagal stimulation were measured in anesthetized rats. Contractile function of circular muscle strips was examined in vitro using force-displacement transducers. KU caused gastric hypersensitivity that persisted for at least 14 days. Fluid distension of the stomach led to a rapid pressure increase in KU but not in control animals, consistent with an impaired accommodation reflex. Gastric ulcers enhanced the contractile response to vagal stimulation, whereas the effect of cholinergic stimulation on smooth muscle in vitro was not changed. These data suggest that inflammation directly alters gastric sensory and motor function. Increased activation of afferents will trigger vagovagal reflexes, thereby further changing motility and indirectly activating sensory neurons. Thus afferent and efferent pathways both contribute to the development of dyspeptic symptoms.

visceral hyperalgesia; sensitization; gastric motility

ABDOMINAL PAIN OR DISCOMFORT is common and leads to many physician visits and diagnostic tests in the United States. Yet in more than half of the patients, no structural or biochemical abnormalities are identified, leading to the diagnosis of so-called functional diseases, such as noncardiac chest pain, nonulcer dyspepsia, or irritable bowel syndrome. Visceral hypersensitivity has been identified in patients with a variety of such functional disorders, documenting a state of nervous system sensitization (9, 26, 31, 32, 43). Changes in the properties of primary afferent neurons (peripheral sensitization) and abnormal processing of sensory information within the central nervous system (central sensitization) likely both contribute to the development of this visceral hypersensitivity (10, 14, 15, 21).

A subgroup of patients reports the onset of symptoms after an episode of acute gastrointestinal infection, pointing at a role of intestinal inflammation in the pathogenesis of these functional disorders (33, 39). This is consistent with several recent studies demonstrating subtle inflammatory changes within the mucosa or deeper layers of the intestinal wall (40, 44). Cells within the inflammatory infiltrate produce and release a variety of mediators that can alter nerve function. We have recently shown that experimentally induced gastritis and gastric ulcers sensitize gastric afferents, resulting in visceral hypersensitivity (28). This was associated with significant changes in the properties of gastric sensory neurons, indicating that peripheral mechanisms contribute to the development of gastric hypersensitivity in these models (4, 6). Afferent and efferent pathways project to the same area and could thus be similarly affected by peripheral inflammation, such as gastric ulcers. Although the modulation of sensory pathways has been studied extensively in models of visceral hyperalgesia, very little is known about the concomitant changes in efferent nerve function. We hypothesized that gastric inflammation induced by intraluminal acetic acid causes hypersensitivity and alters efferent nerve function, which in turn may change motility and secretion and thereby indirectly affect visceral afferents. We tested sensory function by testing aversive responses to gastric distension and indirectly examined the function of efferent pathways by testing the accommodation reflex and gastric motility changes triggered by electrical stimulation of the vagus.

MATERIALS AND METHODS

Animals

All experiments were performed using male Sprague-Dawley rats (Harlan, Indianapolis, IN; 400–500 g). Food, but not water, was withheld for 24 h before surgery. The experimental protocol was approved by the Institutional Animal Care and Use Committee of The University of Iowa.

Induction of Gastric Ulcers

Kissing ulcer model. We adapted a previously described model by inducing gastric ulcerations [called kissing ulcers (KUs)] through intraluminal exposure to acetic acid (41, 42). Briefly, rats were deeply anesthetized with 45–50 mg/kg ip pentobarbital sodium (Nembutal; Abbott Laboratories, Abbott Park, IL). The stomach was exposed through a midline incision and placed in a circular clamp (inner diameter, 10 mm) with the midbody located in the clamp. In the KU group, 100 µl of 60% acetic acid were injected into the restricted space created by the clamp and was completely withdrawn after 45 s. Controls received an identical injection of sterile saline.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Assessment of Gastric Insult

Stomachs from randomly selected rats of both groups were removed, opened along the greater curvature, pinned flat, and fixed in 10% formalin. The mucosal surfaces of the stomachs were macroscopically examined for the presence and extent of ulcers, hemorrhagic lesions, and/or nonhemorrhagic lesions. For histological examination, tissue was taken from an area of the glandular stomach affected by the ulcer or corresponding regions in control animals and placed in 10% formalin overnight. After 48–72 h, the tissue was embedded in paraffin, cut on a microtome (6-μm sections), and stained conventionally with hematoxylin and eosin.

Surgical Preparations

Electromyogram electrode implantation. Animals were deeply anesthetized with pentobarbital (see Kissing ulcer model), and sterilized, multistranded, Teflon-insulated, 40-ga stainless steel wires (Cooner Wire, Chatsworth, CA) were implanted in the acromiotorapezius muscle by using aseptic techniques. The electrode leads were tunneled subcutaneously and externalized at the back of the head for easy access during experiments.

Balloon implantation. Balloons for gastric distension were placed surgically at the time of electromyogram (EMG) electrode implantation and induction of gastric injury. A 3- to 4-cm-long left lateral epigastric incision was made to expose the stomach. As described previously (28), latex balloons (diameter: 2.0–2.5 cm) were inserted through the fundus into the stomach. The polyethylene tubing balloon inflation was exteriorized at the back of the neck along with the EMG electrodes. Because the diameter of the inflated balloon exceeded that of the rat stomach, the pressure measured during distension reflected intragastric pressure.

Experimental protocol. Starting on day 3 after balloon implantation, visceromotor responses were recorded by quantifying EMG activity recorded with electrodes implanted in the acromiotorapezius muscle. The EMG before (baseline) and during rapid constant-pressure distension (rate of 100 ml/s) of the stomach (10–80 mmHg, 20 s) was amplified and filtered (×10,000, 300–5,000 Hz; A-M Systems, Everett, WA), digitized, and integrated using the SPIKE2/CED1401 data-acquisition interface. The raw EMG was rectified and quantified by calculating the area under the curve. The both raw and integrated EMG were continuously displayed on an oscilloscope and recorded. Each distension trial consisted of three segments: a 10-s predistension period, a 20-s distension period (10–80 mmHg), and 10 s after termination of gastric distension. Distension steps were separated by 60 s. Response to gastric distension was defined as the percent increase in EMG activity above baseline during the distension period. To evaluate gastric hyperalgesia after induction of KU, the magnitude of the visceromotor response to graded gastric distension was characterized in rats 3, 7, 10, and 14 days and 6 wk after either acetic acid or saline injection into the gastric lumen.

Vagal stimulation. After an overnight fast, rats were anesthetized initially with an intraperitoneal injection of pentobarbital sodium and subsequently maintained with a constant intravenous infusion of pentobarbital (5–10 mg·kg⁻¹·h⁻¹). A tracheotomy was performed to ensure airway patentity. The right femoral vein and artery were cannulated as described in Vagal stimulation. After obtaining baseline values, 10 ml of normal saline was instilled into the stomach at a rate of 5 ml/min. Pressure changes were monitored for an additional 5 min before the stomach was drained.

Gastric accommodation. To examine the accommodation reflex, animals were fasted overnight and the stomach was exposed and cannulated as described in Vagal stimulation. After obtaining baseline values, 10 ml of normal saline was instilled into the stomach at a rate of 5 ml/min. Pressure changes were monitored for an additional 5 min before the stomach was drained.

Data Analysis

All data are expressed as means ± SE. Results were analyzed by using Student’s paired or nonpaired t-test, two-way ANOVA, or two-way ANOVA for repeated measures, followed by Tukey’s test for multiple comparisons if warranted. A value of P < 0.05 was considered statistically significant.

RESULTS

Morphology

Consistent with prior observations (41, 42), the intraluminal instillation of acetic acid created ulcers at the ventral and dorsal surface of the stomach. Although the mucosa remained completely intact in saline controls (Fig. 1A), lesions extended beyond the muscularis mucosae, with loss of the epithelium and significant inflammatory infiltrate in the submucosa and muscularis propria in animals with KU (Fig. 1B). The inflammation extended into the immediately adjacent mucosa. However, samples obtained at a distance of ~5 mm from the ulcer did not show obvious inflammation. Ulcerations were seen at day 3, with a peak in the ulcer size on day 5. On day 10, the majority of the injured area was covered by epithelium, and only a small concentric scar could sometimes be recognized macroscopically on day 14 (Fig. 1C).

Visceromotor Response

Stepwise gastric distension (pressure control) reproducibly triggered an increase in EMG activity measured from the acromiotorapezius muscle. Compared with control, saline-treated rats, the stimulus-response curve to gastric distension differed significantly between KU rats and controls, consistent
with the development of hypersensitivity. This was seen at day 3 and persisted for 14 days (Fig. 2A). Although results obtained in KU animals differed significantly from controls at all time points \(F = 11.3; P < 0.01\), there were no significant differences between stimulus-response functions on the different days of testing after gastric ulceration. To determine whether the changes in visceromotor response persisted, we tested animals 6 wk after induction of gastric ulcers. At that time, no lesion was seen macroscopically and responses to gastric distension did not differ from controls (Fig. 2B).

**Gastric Accommodation**

In saline-treated animals, instillation of 10 ml into the stomach (volume control) caused a slow pressure increase of 10.6 ± 1.0 mmHg above baseline \((n = 4)\). Within 5 min, the pressure decreased by about one third to 6.8 ± 0.5 mmHg. Bilateral vagotomy significantly altered the changes in intra-
gastric pressure in response to saline instillation. As shown in Fig. 3A, there was a rapid initial rise in gastric pressure to a peak pressure of 17.4 ± 1.3 mmHg (F = 20.3, P < 0.01 compared with control). Considering the early rise in intragastric pressure, we determined the slope during the initial filling phase (1–20 s) and compared it to the late filling phase (101–120 s).

In control animals, the initial pressure rise was slow (0.78 ± 0.07 mmHg/ml) and became more pronounced at the end of saline infusion (2.09 ± 0.72 mmHg/ml). Vagotomy caused a significant increase in the initial rate of rise (3.34 ± 0.07 mmHg/ml, F < 0.01 compared with control condition), whereas the late phase remained unaltered (1.66 ± 0.16 mmHg/ml; not significant). Compared with saline treatment, KU induction significantly increased the peak intragastric pressure measured after 10-ml saline instillation (20.9 ± 1.7 mmHg; n = 4; F = 45.2, P < 0.01 compared with control animals; Fig. 3B). Within 5 min, the pressure fell by about one third to 12.5 ± 0.7 mmHg. In contrast to saline-treated animals, KU led to a pressure increase early during gastric instillation of saline (1.71 ± 0.26 mmHg; F = 9.1, P < 0.01 compared with control animals). Bilateral vagotomy further enhanced this pressure rise to 2.76 ± 0.08 mmHg/ml (P < 0.05), although it did not significantly alter the late changes in intragastric pressure (3.31 ± 0.58 vs. 5.07 ± 0.86 mmHg/ml before and after vagotomy; not significant). Because the early pressure rise most clearly reflected functional changes due to vagotomy or inflammation, we compared the relative increase in slopes between the groups. Although vagotomy increased the initial rate of pressure increase in saline-treated animals by a factor of 4.6 ± 0.6, the effect was significantly lower in KU animals with a 1.8 ± 0.4-fold increase (T = 3.7, P < 0.01).

**Efferent Vagal Stimulation**

Electrical stimulation of the right vagus nerve triggered an increase in gastric pressure. Immediately after cessation of electrical stimulation, a prominent contraction was noted, followed by a slow relaxation (Fig. 4). The increase in baseline pressure and the phasic activity during stimulation as well as the subsequent contraction and relaxation depended on stimulation frequency.

Compared with saline controls, stimulation of the right vagus nerve triggered a significant increase in on contractions (Fig. 5A; n = 4; F = 50.12, P < 0.01), off contractions (Fig. 5B; F = 10.74, P < 0.05), and off relaxation (Fig. 5C; F = 13.36, P < 0.05) in KU animals.

To eliminate potentially confounding influences due to sensitization of the intact left vagal afferents, we performed another series of experiments with bilateral cervical vagotomy 30 min before stimulating the right vagus nerve. Qualitatively, the responses to vagal stimulation after bilateral vagotomy were similar to responses obtained after unilateral vagotomy. There was a frequency-dependent increase during electrical stimulation followed by a brief off contraction and subsequent off relaxation. However, the amplitude of these responses was significantly lower compared with experiments performed with an intact left vagus. Despite the reduction in magnitude of response, contraction during stimulation (Fig. 6A; n = 6; F = 21.49, P < 0.01) and relaxation after stimulus cessation (Fig. 6B; F = 10.07, P < 0.05) were enhanced after gastric injury in bilateral vagotomized rats compared with saline controls, whereas the off contraction was not significantly affected (Fig. 6C; F = 1.51, not significant).

**Fig. 4.** Gastric pressure changes in response to vagal stimulation. Representative trace showing the pressure changes during and after electrical stimulation of the right vagus at 5 Hz (bar). The dotted line indicates gastric baseline pressure.
Muscle Contractility In Vitro

Considering the significant increase in gastric pressure during vagal stimulation, we examined the properties of circular muscle strips obtained from the ulcerated site and adjacent and distant sites from the ulcer. Although strips from the ulcer generated lower tension (0.68 \pm 0.2 g; n = 4) compared with those from unaffected areas of the stomach (1.22 \pm 0.08 g; n = 4), there was no shift in the concentration dependence of carbachol effects (F = 1.1, not significant; Fig. 7).

DISCUSSION

We have recently demonstrated that severe gastritis induced by acetic acid injection into the gastric wall causes behavioral changes consistent with hypersensitivity (28). Because this model leads to transmural inflammation involving most of the stomach, we investigated the effects of a more localized injury. Consistent with prior reports, a 45-s luminal exposure of the stomach to 60% acetic acid resulted in deep ulcerations that persisted for \(\sim 14\) days (41, 42). However, in contrast to the intramural injection of acetic acid (27, 28, 34), the injury in this KU model was spatially restricted. Consistent with a recent study showing enhanced responses to chemical stimulation (20), the results described above demonstrate hypersensitivity to mechanical stimuli with enhanced responses to gastric distension at higher luminal pressures, similar to effects seen with mild gastritis induced by iodoacetamide (28).

Inflammation triggers the production of mediators, such as cytokines and growth factors, many of which affect nerves and contribute to the development of peripheral sensitization. Prior studies have focused on the role of such target-derived factors altering the function of afferent neurons (22, 23). We have recently shown (5) that nerve growth factor (NGF) increases after gastric injury and alters the properties of gastric sensory neurons. We did not investigate the role of NGF or other target-derived factors in this study. However, our results are consistent with an effect of inflammatory mediators or growth factors on afferents innervating the stomach. Although we noted a gradual healing of the ulcers within 14 days, the response to gastric distension remained enhanced throughout this time period. Six weeks after injury, the appearance of the gastric mucosa and the behavioral response to gastric distension did not differ from that in sham-treated animals. In addition to the responses to gastric distension, we also noted...
changes in the accommodation reflex and contractile response to efferent vagal stimulation, pointing at concomitant effects on efferent pathways. Receptors for NGF and effects of interleukin-1β have been identified in neurons of the dorsal motor nucleus of the vagus (16, 24). In the context of our findings, it raises the question of whether target-derived factors similarly interact with receptors on the terminals of efferent neurons.

Distension of the stomach with 10 ml of normal saline caused an increase in gastric pressure, which became more prominent after bilateral vagotomy, because the nerve lesion abolishes the vagovagal mediated accommodation reflex (36). Interestingly, localized gastric injury in the distal stomach altered the pressure-volume relationship, similar to results obtained after bilateral vagotomy. Whereas the inflammatory infiltrate alters the biomechanical properties of the affected area, thereby decreasing the compliance of the stomach, our data point to an impaired accommodation reflex in addition to changes in compliance such as localized injury (KU) having the most striking effect during the early phase of the distension, which was primarily affected by vagotomy in control animals.

The gastric responses to vagal stimulation support the interpretation of an altered neural regulation of gastric function. Electrical stimulation of the right vagus nerve after bilateral vagotomy triggered a typical response pattern with a sustained contraction during stimulation, followed by a brief contraction and prolonged relaxation after stimulus cessation (35, 37). This pattern is a composite of the differential effects of vagal stimulation on the proximal and distal stomach with fundic relaxation and antral contractions (2). Considering the location of the experimentally induced injury within the body of the stomach, inflammatory changes should primarily affect the antral response. However, contraction and relaxation were both significantly affected by gastric injury. Our approach did not enable us to determine whether the observed decrease in gastric pressure was due to altered fundic relaxation, as also suggested by the impaired accommodation reflex, or changes in antral motility.

The increased response to vagal stimulation was further enhanced when the left vagus remained intact because contractile activity could activate gastric tension receptors, leading to increased vagal efferent discharge in the intact left vagus nerve and augmented contractility, consistent with a role of vagovagal reflexes (7, 30). However, sensory fibers may also release mediators in the periphery that may affect motility (8, 38). We have previously shown that inflammation significantly alters the properties of gastric afferents (4, 11, 18). Because vagal stimulation may lead to antidromic excitation of afferent fibers, sensitized afferents may contribute to the motility changes described above.

Although we were primarily interested in the extrinsic innervation of the stomach, inflammation within the gastric wall certainly affects both intrinsic and extrinsic nerves. Several investigators have described the effects of acute or chronic injury of myenteric neurons (17, 19, 29). Vagal efferent neurons innervate the majority of myenteric neurons in the stomach (46). It is therefore possible that changes in properties of intrinsic nerves and/or changes in the signaling between these intrinsic neurons and interstitial cells or smooth muscle cells contributed to sensitization of efferent pathways. Experiments after prior selective ablation of afferent or efferent vagal fibers and direct recordings of vagal efferent fibers or neurons in the dorsal motor nucleus of the vagus are needed to more definitively determine whether gastric inflammation affects the excitability of vagal efferent neurons.

Finally, the functional changes observed could at least in part be due to effects on the tunica muscularis, because prior studies have demonstrated changes in smooth muscle function due to inflammation (25). However, the similar dose-response function after cholinergic stimulation with the muscarinic agonist carbachol along with the enhanced contraction during and the increased relaxation after vagal stimulation suggest that inflammation altered neuromuscular signaling, rather than simply changing muscle function. Again, this involves intrinsic and extrinsic nerves as well as interstitial cells of Cajal (ICC), which may play a role in the signaling between nerves and muscle cells (45). Considering previously published reports on structural and functional changes in ICC during inflammation (1, 12, 13), it is possible that ICC contributed to the altered motility in response to vagal stimulation.

In summary, localized ulceration of the stomach enhanced responses to gastric distension consistent with the development of impaired accommodation reflex, increased contractility, and heightened response to electrical stimulation.
of visceral hypersensitivity. These effects lasted for at least 14 days and were still present when the initial injury was healed. Changes in gastric accommodation were consistent with impaired vagovagal reflexes. This was at least in part due to effects on the efferent signaling to the stomach, as shown by altered responses to efferent vagal stimulation. Although the discussion about potential causes of functional diseases often focuses on changes in motility or visceral sensation, our results demonstrate that sensory and motor function are both affected in an animal model of visceral hypersensitivity, suggesting that both may contribute to the development of symptoms.

Sensitization of afferents will enhance activation of sensory neurons, leading to increased input to the dorsal vagal complex and activation or inactivation of vagal motor neurons, which will directly affect gastric motility. The concomitant sensitization of efferent vagal pathways and/or enteric neurons will further alter motor function, potentially further activating sensory neurons and thereby worsening symptoms.

GRANTS
The study was supported by grants from the National Institutes of Health (NS 35790 & NS 19912).

REFERENCES