Mechanosensitive Properties of Gastric Vagal Afferent Fibers in the Rat

NORIYUKI OZAKI, J. N. SENGUPTA, AND G. F. GEBHART
Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, Iowa 52242

Ozaki, Noriyuki, J. N. Sengupta, and G. F. Gebhart. Mechano-sensitive properties of gastric vagal afferent fibers in the rat. J. Neurophysiol. 82: 2210–2220, 1999. Single, teased fiber recordings were made from the decentralized right cervical vagus nerve (hyponodal) of the rat. A total of 67 afferent fibers that responded to gastric distension (GD) were studied: 9 fibers were stimulated by phasic balloon GD, 58 by more natural fluid GD. All balloon GD–responsive fibers had resting activity (3.1 imp/s), and 57/58 fluid GD responsive fibers had resting activity (1.3 imp/s). All balloon GD–responsive fibers exhibited a dynamic response to phasic distension followed by slow adaptation, whereas fluid GD–responsive fibers exhibited increasing responses as intragastric pressure increased, followed typically by slow adaptation. Responses to graded GD were studied in all fibers, and all gave increasing responses to increasing pressures (5–60 mmHg). Thresholds for response varied between 0 and 18 mmHg. Mean response thresholds for two durations of fluid GD (30 and 60 s) were 5.6 and 3.9 mmHg; the mean response threshold to phasic balloon GD (30 s duration) was 5.3 mmHg. The potential sensitizing effect of platelet activating factor (PAF, 50 or 100 ng $\cdot$ kg$^{-1}$ $\cdot$ min$^{-1}$ for 20 min) infused into the gastric artery was studied in 20 fibers. Fifteen fibers exhibited an increase in spontaneous activity; intragastric pressure also slightly increased during PAF infusion. The increase in activity produced by PAF was attenuated in the presence of the PAF receptor antagonist WEB 2086. After PAF-induced acute inflammation of the stomach, three of five fibers studied did not exhibit any change in response to graded GD. The present study characterized distension-sensitive afferent fibers in the right cervical vagus innervating the stomach of the rat by balloon GD and fluid GD. The results document that all distension-sensitive gastric vagal afferent fibers encoded the intensity of GD, but none had response thresholds in what might be considered the noxious range. PAF infusion activated mechanosensitive gastric vagal afferent fibers, but acute inflammation produced by PAF did not sensitize responses to GD.

INTRODUCTION

The upper abdominal gastrointestinal (GI) tract receives dual innervation by the vagus and spinal visceral nerves (primarily the splanchnic nerve). These afferent nerve fibers play an integral part in conveying information from the gut to the CNS relative to regulatory functions (e.g., absorption, secretion, storage, propulsion, and emptying) and conscious sensations (e.g., satiety, nausea, discomfort, and pain). The most compelling sensations that arise from the GI tract are discomfort and pain. Although considerable information has accumulated about the morphology and neuropeptide contents of afferent fibers innervating the GI tract, our understanding of the functional role of these fibers remains limited.

Most nonhuman animal studies have focused on the roles of visceral afferent fibers in GI motility, secretion, and other regulatory functions (for reviews see Cervero 1994; Sengupta and Gebhart 1994; 1995). It is generally held that vagal afferent fibers do not convey nociceptive information, but vagal afferent fibers innervating the inferior-posterior surface of the heart (Meller and Gebhart 1992) and the upper respiratory tract (Barnes 1991; Coleridge and Coleridge 1984) may contribute to visceral pain sensation. Previous studies of vagal afferent fibers have addressed their involvement in regulatory mechanisms (e.g., motility) and chemosensitivity under normal physiological conditions. Their mechanosensitivity, particularly associated with discomfort and pain, has not been studied.

Functional bowel disorders are characterized by altered sensory perceptions (e.g., bloating, discomfort, and pain), which can arise from changes in peripheral or/and central neuron excitability (see Mayer and Gebhart 1994). The aim of this study was to gain information about the response characteristics of vagal afferent fibers innervating the stomach. Accordingly, we quantitatively characterized the mechanosensitive properties of vagal afferent fibers innervating the stomach, investigating the reproducibility and intensity-coding properties of these afferent fibers to graded fluid and balloon gastric distension (GD). To determine whether acute inflammation of the stomach would provide insight into afferent mechanisms that could contribute to altered sensations, we also examined the effect of platelet activating factor (PAF) on gastric afferent fibers and their response to GD. We chose to use PAF because it is an endogenous mediator of inflammation (Zimmerman et al. 1992), and its content in both gastric juice and mucosa is increased in patients with ulcers (Ackerman et al. 1990; Sobhan et al. 1992). Some of these data have been presented previously in abstract form (Ozaki et al. 1998; Sengupta et al. 1997).

METHODS

General procedures

Experiments were performed on 52 male Sprague-Dawley rats (Harlan, Indianapolis, IN; 400–500 g). Food, but not water, was withheld for 24 h before surgery. The 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  $\cdot$ kg$^{-1}$  $\cdot$ h$^{-1}$). The right femoral vein was cannulated for infusion of fluid and anesthetic. The right femoral artery was cannulated and connected to a pressure transducer for monitoring blood pressure and heart rate. The 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. The trachea...
was intubated to permit artificial ventilation with room air. The rat was paralyzed with pancuronium bromide (0.2–0.3 mg/kg iv) and mechanically ventilated with room air (~70 strokes/min, 2–2.5 ml stroke volume). Supplemental doses of pancuronium bromide (0.2–0.3 mg · kg⁻¹ · h⁻¹) were given to maintain paralysis during the experiment. Core body temperature was maintained at 36°C by a hot water circulating heating pad placed under the rat and an overhead feedback-controlled heat lamp (thermoprobe inserted into the rectum; Yellow Springs Instrument, Yellow Springs, OH). At the end of experiments, rats were killed with an overdose of pentobarbital. The experimental protocol was approved by the Institutional Animal Care and Use Committee, The University of Iowa.

The abdomen was opened by a transverse epigastric incision 4–5 cm in length. To measureafferent fiber conduction velocity, a bipolar wire electrode was placed on the vagal trunk at the esophageal–gastric junction in some experiments. The right vagus nerve was isolated from the esophagus, and a pair of Teflon-coated, 40-gauge stainless steel wires stripped at the tips were placed around the nerve and sealed with nonreactive Wacker gel (Wacker Silicone, Adrian, MI).

For phasic balloon GD (n = 8 rats), a 2.0–2.5 cm long, 2–3 cm diam flaccid, flexible latex balloon was placed surgically in the stomach through the fundus. The balloon occupied approximately two-thirds of the proximal stomach. The pylorus was not obstructed, and there was no blockage of gastric emptying. The outside diameter of the balloon when inflated was greater than the intraluminal diameter of the stomach of the rat. Therefore the pressure measured during GD reflected actual intragastric pressure. The balloon catheter was connected to a distension control device via a low-volume pressure transducer (see Gebhart and Sengupta 1996 for details).

For fluid GD (n = 44 rats), the stomach was intubated with flexible plastic tubing (Tygon, Fisher Scientific Co., Pittsburgh, PA; 2.3 mm OD, 1.3 mm ID) via the mouth, esophagus, and cardia. The catheter was secured by a ligature around the esophageal–gastric junction. Another Tygon tube (3.9 mm OD, 2.4 mm ID) was introduced distally through the pylorus and was secured by a ligature placed caudal to the pyloric sphincter; the duodenum was ligated close to the pyloric ring. Another Tygon tube (3.9 mm OD, 2.4 mm ID) was introduced distally through the pylorus and was secured by a ligature placed caudal to the pyloric sphincter; the duodenum was ligated close to the pyloric ring. For GD, the oral catheter was connected to a reservoir containing saline at room temperature. Constant pressure distension was controlled by the distension control device with the distal catheter clamped. Intragastric pressure was monitored by connecting the distal catheter via a three-way stopcock to a low-volume pressure transducer. The left gastric artery was freed from connective tissue under a stereomicroscope and cannulated for infusion of PAF in 22 rats. The abdomen was closed with silk sutures.

Recording of afferent nerve activity

The right vagus nerve was exposed by a ventral midline incision in the neck. The sternocecidomastoid, sternohyoid, and omohyoid muscles were removed. The skin was reflected laterally and 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 micro-base plate. 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. A fine strand of connective tissue was placed over the other pole of the electrode for differential recording.

Action potentials were monitored continuously by analogue delay and displayed on a storage oscilloscope after low noise DC differential amplification. Action potentials were processed through a window discriminator and counted (1-s binwidth) on-line using the SPIKE2/CED 1401 data acquisition program. Peristimulus time histograms, intragastric pressure, and blood pressure were displayed on-line continuously. Data were also recorded on tape for later analysis.

Experimental protocol

Mechanosensitive gastric muscle afferents in the vagus nerve were identified by response to a test stimulus of GD (40 mmHg, <5 s). This intensity of GD was chosen because it is in excess of the mean response threshold for high-threshold mechanosensitive fibers innervating other viscera (see Sengupta and Gebhart 1995). If a fiber responded to 40 mmHg GD, a stimulus-response function (SRF) to distending pressures of 5, 10, 20, 30, 40, and 60 mmHg, 30 or 60 s at 4-min intervals was determined.

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 delay (time between stimulus artifact and evoked response) was recorded. The conduction distance was measured postmortem. Fibers were classified on the basis of their conduction velocities; those with conduction velocities <2.5 m/s were considered unmyelinated C-fibers, and those with conduction velocities >2.5 m/s were considered thinly myelinated Aδ-fibers.

The effect of PAF on mechanosensitive gastric afferent fibers was tested as were responses to GD following the acute inflammation produced by PAF. PAF in 0.25% bovine serum albumin (BSA in saline) was infused via the left gastric artery (50 or 100 ng · kg⁻¹ · min⁻¹ for 20 min). The volume of infusion was <0.1 ml/min. Because PAF produces smooth muscle contraction (Stimers and O’Flaherty 1983), changes in activity of these mechanosensitive fibers to PAF may reflect an indirect effect due to contraction. To test whether responses to PAF were direct or secondary to muscle contraction, 12 rats were pretreated with loperamide (50 mg/kg iv) 5 or 25 min before the experiment in an attempt to eliminate gastric smooth muscle contractions; one rat was treated with loperamide during PAF infusion. The effect of intragastric artery infusion of vehicle (0.25% BSA in saline) was tested in nine rats. To determine whether drug effects were produced at the PAF receptor, the PAF receptor antagonist WEB 2086 (Hines and Fisher 1992) was administered intravenously 5 min before starting the intragastric artery infusion of PAF. WEB 2086 was given in a dose of 250 mg/kg, and PAF was infused at 50 ng · kg⁻¹ · min⁻¹ for 20 min. In addition, WEB 2086 was tested in other experiments where it was injected 5 min after starting the infusion of PAF. The effect of acute inflammation produced by PAF was tested on seven mechanosensitive afferent fibers. Responses of the fibers to graded GD were determined 30 and 90 min after the 20-min infusion of PAF.

At the end of the protocol for each fiber, the abdomen was opened, and the mechanosensitive receptive field was located by probing the stomach with a fine, blunt glass rod. At the end of an experiment, stomachs from treated and untreated rats were removed, opened along the greater curvature, pinned flat (mucosal side up), and examined under a dissecting microscope to qualitatively assess inflammation.

Drugs

Platelet activating factor (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) was obtained from Sigma Chemical (St. Louis, MO). An aliquot of a stock 2 mg/ml solution of PAF in chloroform was evaporated and reconstituted in 0.25% (wt/vol) BSA in 0.9% (wt/vol) saline when required. WEB 2086 was kindly provided by Dr. Rory A. Fisher (University of Iowa) and was dissolved in 0.9% saline immediately before use. Loperamide hydrochloride was obtained from Sigma and was dissolved in 0.9% saline.

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). Stimulus-response functions (SRF) 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 then was extrapolated to the ordinate (rep-
resenting distension pressure) to estimate response threshold. The response to PAF infusion was determined as the maximum discharge during infusion (imp/s).

All data are expressed as means ± SE. Results were analyzed using Student’s t-test or ANOVA. A value of P < 0.05 was considered statistically significant.

RESULTS

Fiber sample

A total of 67 vagal afferent fibers (in 52 rats) were studied, all of which responded to GD. In the presence of the balloon in the stomach (n = 9 fibers in 8 rats), fibers exhibited ongoing activity (3.1 ± 1.5 imp/s, mean ± SE; range: 0.02–14.2 imp/s). Of the 58 fibers (in 44 rats) that responded to fluid GD, 1 had no spontaneous activity, and 57 were spontaneously active (1.3 ± 0.2 imp/s; range: 0.01–5.5 imp/s; P < 0.05 vs. balloon GD). This group of 57 spontaneously active fibers consisted of 29 in which the gastric artery had been cannulated (1.4 ± 0.3 imp/s) and 28 in which the artery was not cannulated (1.3 ± 0.3 imp/s).

Conduction velocities of 27 fibers that responded to fluid GD were measured by electrical stimulation of the vagus nerve. All tested fibers were unmyelinated C-fibers (mean conduction velocity: 0.70 ± 0.06 m/s, range 0.4–2.2 m/s).

Responses to GD

The nine fibers tested with balloon GD typically gave an initial dynamic response followed by a slowly adapting response during maintained GD (60 mmHg; e.g., Fig. 1A). Slow adaptation to a tonic discharge was generally observed at all intensities of GD.

Fibers tested with fluid GD (60 mmHg) typically gave slowly incrementing responses as intragastric pressure increased, and either began to slowly adapt during maintained pressure or sustained responses until termination of distension. Examples of responses to fluid GD are shown in Figs. 1 and 2. Because distension pressure typically became maximal at the end of 30-s fluid GD, there was no opportunity to observe adaptation during distension (e.g., see Fig. 1B). Fibers tested with longer duration, 60-s fluid GD (n = 46) did permit observation of adaptation during stimulation: 38 fibers were slowly adapting (e.g., Fig. 2C). The remaining eight fibers were either nonadapting (e.g., Fig. 1C) or appeared to quickly adapt near or at peak distending pressure as if the stomach had relaxed (although intragastric pressure did not decrease).

Following termination of either phasic balloon or fluid GD, many fibers gave evidence of a period of poststimulus inhibition (Fig. 1). The frequency of discharge fell below the resting level of activity after termination of GD in 5/9 fibers tested with 30-s balloon GD, 3/12 fibers tested with 30-s fluid GD, and 17/46 fibers tested with 60-s fluid GD. Most fibers tested also exhibited prolonged afterdischarge at rates greater than resting. Although we did not follow the duration of all afterdischarges, afterdischarges in six of nine fibers tested with 30-s balloon GD continued for 23 s to >145 s after termination of 60 mmHg GD. Eight of 12 fibers tested with 30-s fluid GD gave afterdischarges that ranged from 43 to >220 s (Fig. 2B). Fourteen of 16 fibers tested with 60-s fluid GD and 26/30 fibers tested with 60-s fluid GD in stomachs with the gastric artery catheterized gave afterdischarges that ranged from 12 to >190

FIG. 1. Response patterns of vagal afferent fibers to gastric distension (GD, 60 mmHg). In each record, the response is illustrated as a peristimulus time histogram (1-s binwidth), and the intragastric pressure is illustrated below. A: example of the response to phasic balloon GD, 30 s. B: example of the response to fluid GD, 30 s. C: example of the response to fluid GD, 60 s. Note in each example poststimulus inhibition.
There was no relationship between afterdischarge and response thresholds of the fibers (see Table 1). The characteristics of gastric vagal afferent fibers to 60 mmHg GD are summarized in Table 1.

Eighteen of 67 fibers tested with GD exhibited intermittent phasic changes in resting activity that were maintained during and after termination of distension (Fig. 3). These changes were not noted to be associated with spontaneous changes in intragastric pressure.

**Stimulus-response functions (SRFs)**

Responses to graded balloon or fluid GD were studied in all 67 fibers. SRFs of fibers in the different experimental groups are given in Fig. 4. Extrapolation of the linear portion of individual SRFs revealed that gastric vagal afferent fibers exhibited a range of thresholds for response to GD. The mean response threshold to 30-s balloon GD was 5.3 ± 1.7 mmHg (range 0–13.8 mmHg), to 30-s fluid GD 5.6 ± 1.8 mmHg (range 0–15.5 mmHg), to 60-s fluid GD 3.9 ± 1.1 mmHg (range 0–13.2 mmHg), and to 60-s fluid GD in gastric artery-catheterized stomach 5.7 ± 1.0 mmHg (range 0–18.3 mmHg; Table 1).

**FIG. 2.** Responses of vagal afferent fibers to graded intensities (5–60 mmHg) of gastric distension (GD). Responses are illustrated topmost in each record as peristimulus time histograms (1-s binwidth); intragastric pressure is presented below. A: responses of a fiber to 30-s balloon GD. B: responses of a fiber to 30-s fluid GD. C: responses of a fiber to 60-s fluid GD.

**FIG. 3.** Responses of a vagal afferent fiber to graded intensities of fluid distension (60 s). Responses are illustrated as peristimulus time histograms (1-s binwidth); intragastric pressures for the 3 intensities of distension are illustrated at the bottom. This fiber exhibited regular, periodic bursts of activity that were maintained during distension. These bursts of activity, associated in magnitude with distending pressure, were not associated with changes in intragastric pressure reflected in the pressure record nor with the respiratory cycle.

<table>
<thead>
<tr>
<th>Method/Duration of GD</th>
<th>Balloon 30 s</th>
<th>Fluid 30 s</th>
<th>Fluid 60 s, Gastric Artery Catheterized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (sample)</td>
<td>9</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Spontaneous activity, imp/s</td>
<td>3.1 ± 1.5</td>
<td>1.9 ± 0.5</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Adaptation (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slow</td>
<td>9</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Non</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Post-GD inhibition: % of sample</td>
<td>55.6 (5)</td>
<td>25.0 (3)</td>
<td>43.8 (7)</td>
</tr>
<tr>
<td>Duration (s)</td>
<td>4.0 ± 1.0</td>
<td>12.0 ± 6.0</td>
<td>11.6 ± 3.4</td>
</tr>
<tr>
<td>Post-GD afterdischarge: % of sample</td>
<td>66.7 (6)</td>
<td>66.7 (8)</td>
<td>87.5 (14)</td>
</tr>
<tr>
<td>Duration (s)</td>
<td>23–145</td>
<td>43–220</td>
<td>59–190</td>
</tr>
<tr>
<td>Response threshold (mmHg)</td>
<td>5.3 ± 1.7</td>
<td>5.6 ± 1.8</td>
<td>3.9 ± 1.1</td>
</tr>
<tr>
<td>Maximum response to 60 mmHg (imp/s)</td>
<td>40.9 ± 4.1</td>
<td>53.4 ± 6.5</td>
<td>43.6 ± 3.0</td>
</tr>
<tr>
<td>Latency to maximum response(s)</td>
<td>10.4 ± 3.2</td>
<td>18.1 ± 1.6</td>
<td>26.0 ± 2.3</td>
</tr>
</tbody>
</table>

Values are means ± SE with number of fibers in parentheses. GD, gastric distension.
Mean response thresholds did not differ between groups.

The mean SRFs of vagal afferent fibers of the SRF to GD are shown in Fig. 6. When responses to the more natural fluid filling of the stomach were compared with responses to phasic balloon GD, response magnitude generally was greater to fluid GD, particularly during the second half of the period of distension. Although GD was delivered at a constant pressure whether by balloon or fluid, the rate at which the final pressure was achieved and the duration of the target pressure differed for the two types of GD. We would have expected responses to phasic balloon GD to be of greater magnitude than fluid GD, but this was apparent for only the first 10 s of GD. The slope and mean response magnitudes ofafferent fibers to 60-s fluid GD in gastric artery catheterized stomach were significantly lower than responses to the same stimulus in naive stomach ($F = 32.8; P < 0.01$).

**Gastric receptive fields (RFs)**

Receptive fields in the stomach were found for 2/12 fibers tested with 30-s fluid GD, 2/16 fibers tested with 60-s fluid GD, and 9/30 fibers tested with 60-s fluid GD (gastric artery catheterized). No RFs were located in the cardia or fundus; all 13 RFs were associated with the corpus. Characteristics of these fibers are presented in Table 2.

**Effect of PAF on gastric mechanosensitive vagal afferent fibers**

The effect of gastric artery infusion of PAF (20 min) was tested in 20 fibers, in the absence or presence of loperamide, 13 during 50 ng \cdot kg^{-1} \cdot min^{-1} PAF and 7 during 100 ng \cdot kg^{-1} \cdot min^{-1} PAF. Spontaneous activity increased in 9/13 and 6/7 fibers during 50 and 100 ng \cdot kg^{-1} \cdot min^{-1} infusion of PAF. Mean spontaneous activity increased from 1.3 ± 0.3 imp/s to a mean maximum response 16.9 ± 2.9 imp/s ($n = 9$) during 50 ng \cdot kg^{-1} \cdot min^{-1} PAF infusion and from 0.5 ± 0.3 imp/s to 15.2 ± 2.7 imp/s ($n = 6$) during 100 ng \cdot kg^{-1} \cdot min^{-1} PAF.

**Fig. 5.** Frequency histograms of extrapolated response thresholds of gastric vagal afferent fiber responses to gastric distension (GD). For each of the 4 groups, the mean ± SE response threshold is illustrated (●).
Figure 7 shows examples of the effect of infusion of 100 ng·kg⁻¹·min⁻¹ PAF. One fiber was tested to a second infusion of PAF started 15 min after conclusion of the first infusion. The fiber responded to the first PAF infusion but did not respond to the second PAF infusion (Fig. 7B).

The effect of gastric artery infusion of 0.25% BSA was tested in nine fibers. No fibers exhibited increases or decreases in spontaneous activity during BSA infusion. In six fibers, BSA was tested before PAF infusion, five of which gave increases in spontaneous activity to PAF (Fig. 8). In two fibers, BSA was tested 15 min after PAF infusion. Both fibers responded to PAF; neither of them responded to BSA.

Intragastric pressure increased during PAF infusion (see Figs. 7 and 8). The mean maximum increase in intragastric pressure was 4.1 ± 0.4 mmHg (n = 7). To test whether the response of afferent fibers to PAF was due to direct activation of the nerve ending or secondary to changes in tension of the smooth muscle during PAF–induced contractions, the effect of PAF was tested in 13 fibers 5 min after loperamide (50 mg/kg iv) administration. Seven of 10 fibers tested with 50 ng · kg⁻¹ · min⁻¹ PAF and 2/3 fibers tested with 100 ng · kg⁻¹ · min⁻¹ PAF exhibited a response to PAF after loperamide treatment, suggesting a direct action of PAF. The response to PAF (doses combined) in the absence of loperamide was 15.9 ± 2.2 imp/s (n = 6) and in the presence of loperamide was 14.8 ± 3.0 imp/s (n = 9; P > 0.05). However, the intragastric pressure still increased slightly (mean maximum increase: 3.7 ± 0.4 mmHg, n = 13) during infusion of PAF.

To test whether the PAF receptor was involved in the increases in spontaneous activity during PAF infusion, the effect of the specific PAF receptor antagonist WEB 2086 (125 mg/kg iv) was tested in five fibers. The antagonist was injected into the femoral vein during PAF infusion into the gastric artery. The fiber exhibited an increase in spontaneous activity only at the beginning of PAF infusion, but intragastric pressure increased. B: responses of another fiber to 2 infusions of PAF, 15 min apart. This fiber responded to the 1st, but not 2nd infusion of PAF.

Intragastric pressure was maintained after WEB 2086 administration (102.3% of intragastric pressure before WEB 2086).

The mechanosensitive properties of five fibers were tested after infusion of 50 or 100 ng · kg⁻¹ · min⁻¹ PAF (20 min) into the gastric artery. The spontaneous activity of three fibers increased during PAF infusion. Responses to GD did not change at any of the pressures of GD tested (5–60 mmHg) either 30 or 90 min after PAF infusion. Figure 10 shows an example of responses of an afferent fiber to graded GD before, 30 and 90 min after infusion of PAF, and Fig. 11 illustrates the mean SRFs of fibers before and 30 and 90 min after PAF infusion.

Macroscopic examination of PAF-treated stomachs at the end of experiments revealed acute damage relative to stomachs taken from untreated rats. Damage consisted of both hemor-
rhagic and nonhemorrhagic lesions. A nonhemorrhagic lesion was defined as one that had a shallow erosion without any trace of blood; hemorrhagic lesions had deeper damage with blood at the erosion site.

**DISCUSSION**

The principal findings of the present study are that high-threshold mechanosensitive receptors were not present in the sample of vagal afferent C-fibers studied and that PAF did not sensitize these receptors to GD. These results suggest that gastric vagal afferent fibers likely play no role in acute gastric pain. The fibers studied, however, did encode increasing intensities of GD and thus likely contribute to altered gastric sensations. Phasic balloon GD-responsive fibers typically exhibited a dynamic response followed by slow adaptation during GD, whereas fluid GD-responsive fibers exhibited an increasing response as intragastric pressure increased, followed by either slow adaption or no adaptation during GD. SRFs to graded GD revealed that gastric vagal C-fibers have a range of thresholds for response, but no response thresholds exceeded what might be considered an intensity in the noxious range. Gastric distension-responsive fibers also responded to PAF, an effect mediated by the PAF receptor, but were not sensitized by PAF-produced gastric inflammation.

**Vagal innervation of the stomach**

Electron microscopic studies of the abdominal vagus nerve in the rat estimate that there are 10,000 unmyelinated fibers (Gabella and Pease 1973). Quantitative studies of subdiaphragmatic dorsal and ventral vagal branches innervating abdominal...
visceral organs revealed that dorsal and ventral gastric branches, celiac branches, and hepatic branches of the vagus consist primarily of unmyelinated afferent fibers; the total number of unmyelinated fibers in the rat has been estimated to exceed 99% of all vagal fibers (Precht and Powley 1990).

The conduction velocity of vagal afferent fibers in the stomach has been reported to range from 6.5 to 13 m/s (Paintal 1954b) or <1.3 m/s (Iggo 1957) in the cat, 2–12 m/s in the goat (Iggo 1955), 1.1 ± 0.1 m/s in the dog (Takeshima 1971, 1974), 1.0–1.7 m/s in the sheep (Falempin et al. 1978), 0.91 ± 0.21 m/s in the ferret (Andrews et al. 1980), and 0.4–1.6 m/s in the rat (Davison and Clarke 1978, 1988). The majority of gastric muscle afferent fibers in the rat are unmyelinated C-fibers. The present results are in accord with these morphological and electrophysiological studies. Conduction velocities of 27 fibers tested were unmyelinated C-fibers, 26 having a conduction velocity ≤1.0 m/s. Because it was not possible in all experiments to place stimulating electrodes at the gastroesophageal junction, some of the fibers studied may have been more rapidly conducting Aδ-fibers.

Gastrointestinal distension-responsive afferent fibers are generally considered to be muscle afferent fibers. The location of distension-sensitive afferent fibers in muscle layers has been demonstrated by removal of the underlying mucosa and submucosa (Cottrell 1984; Iggo 1957; Takeshima 1971) without impairing responses to distension. Recent morphological studies have also confirmed the locations and structures of the sensory endings in the stomach. Berthoud and Powley (1992) injected DiI in the nodose ganglion of the rat and reported that labeled vagal afferent fiber endings terminated in longitudinal and circular muscle layers of the stomach and duodenum, sending collaterals to the myenteric plexus, mucosa, and submucosa.

Vagal afferent fibers supplying the stomach have ongoing activity, and some exhibit periodic bursts with the respiratory cycle (Andrews et al. 1980; Blackshaw et al. 1987; Davison and Clarke 1988; Grundy et al. 1995; Iggo 1955; Paintal 1954b; Takeshima 1971). The spontaneous activity of gastric afferent fibers in these reports ranged from 1.5 to 38 imp/s, consistent with resting activity between <1 and 14 imp/s in the present study. We also observed phasic, regular changes in activity that were maintained during GD (e.g., Fig. 3). These bursts, however, did not cycle with respiration (~70/min).

**Response to GD**

Responses of GD-sensitive vagal afferent fibers to phasic gastric distension (constant volume) were reported to consist of three major components during and immediately after distension: 1) a dynamic response (initial burst), 2) a static or sustained response, with or without random fluctuations during maintained GD, and 3) a pause or silent period after the termination of GD (Blackshaw et al. 1987; Davison and Clarke 1988; Falempin et al. 1978; Iggo 1955, 1957; Leek 1969; Paintal 1954b; Takeshima 1971, 1974). The dynamic response consists of a burst of discharges at the onset of phasic distension. This high-frequency of discharge is likely due to an initial, high tension that develops during active resistance offered by smooth muscle and local excitatory reflexes produced by intrinsic nerves. The afferent discharge then declines during the period of distension, believed to be due to reflexive relaxation of the stomach. With termination of the distending stimulus, the discharge of fibers typically falls below predistension spontaneous activity; occasionally there is transient silence. The duration of the silent period has been suggested to be related to the magnitude of distension and to the rate of deflation (Davison and Clarke 1988). It has also been reported that during phasic, step inflation of the stomach, the initial dynamic response was greatest at low volumes of distension; as the distension volume was increased, the dynamic response decreased. However, the static response of the fibers remained unaltered and followed linearly the step increases in gastric volume (Davison and Clarke 1978). In the present study, constant pressure, phasic balloon distension (30 s duration) led typically to 1) a dynamic, initial burst, 2) a slowly adapting response, and 3) a pause or silent period at the termination of GD. However, the initial dynamic response was not greatest at low pressures of GD as was reported for low volumes of distension. This likely reflects differences in the methods of GD as well as the 4-min intertrial interval in the present study (rather than step increases) between distensions. Davison and Clarke (1978) incremented intragastric volume stepwise with a 10-s interval or no interval between steps. Gastric vagal afferent fiber responses to fluid distension exhibited slowly increasing responses clearly associated with increasing intragastric pressure. When gastric pressure plateaued (during 60-s fluid distension), fibers typically exhibited slow adaptation. In contrast with balloon GD, and consistent with the rate of pressure increase, fibers did not give dynamic responses at the onset of fluid GD because fluid distension slowly increases initial tension that may not develop active resistance in smooth muscle. Some investigators have reported that a small number of afferent fibers in the vagus nerve exhibit rapid adaptation during phasic distension (Falempin et al. 1978; Niijima 1962; Takeshima 1971, 1974), which was observed in a small number of fibers studied here.

Some fibers exhibited spontaneous fluctuations in discharge at rest as well as during and after distension. Such fluctuations suggest gastric motor activity (peristalsis), although we did not observe intragastric pressure waves when studying these fibers. The fluctuations may alternatively reflect muscle contraction of localized peristalsis in the stomach; localized peristalsis may not affect or be reflected in intragastric pressure of the whole stomach. Two types of distension-sensitive afferent fibers were identified in the vagus nerve of dog (Takeshima 1971, 1974) and ferret (Andrews et al. 1980). Stretch receptors (sensitive to distension) primarily innervate the fundus and corpus, and tension receptors (sensitive to distension and contraction) primarily innervate the antrum and pylorus in these animals. In the present study, those fibers exhibiting spontaneous fluctuations may have been associated with tension receptors, although we did not discern a pattern of response properties associated with location of receptive field.

Response patterns of vagal afferent fibers to balloon and fluid GD differ (Andrews et al. 1980; Blackshaw et al. 1987; Davison and Clarke 1978, 1988; Falempin et al. 1978; Grundy et al. 1995; Iggo 1955, 1957; Leek 1969; Niijima 1962; Paintal 1953, 1954a,b; Yoshida-Yoneda et al. 1996). Certainly, fluid GD more closely approximates a natural stimulus, although the stomach is not normally filled as quickly as in these (and other) experiments. Fluid GD also permits measurement of changes in intragastric pressure more accurately, likely because the stomach is more uniformly filled by fluid than by the balloon,
which is surgically inserted into the fundus and accordingly distends the fundus more than corpus, antrum, and pyloric regions.

The right gastric artery was catheterized in some experiments. We noted that the slope and magnitude of the SRF to 60-s fluid GD in catheterized stomach were significantly lower than responses to the same stimulus in naive stomach. One might have expected responses to GD to be greater (i.e., sensitized) if not the same when the additional insult of catheterization was added to the experimental protocol. Interestingly, further insult by inflammation with PAF also did not sensitize fiber responses to GD (see Effect of PAF infusion).

Thresholds for response to distending stimuli have been reported for many hollow viscera (for review see Sengupta and Gebhart 1995). Afferent fibers having low and high thresholds for response have been described, and function has been assigned on the basis of response threshold. Low thresholds for response are interpreted as indicating a role for the fibers in regulatory functions in addition to conscious sensations associated with nonpainful distention (e.g., fullness, bloating, nausea, etc.) (Cervero and Jäning 1992). High thresholds for response have been taken as evidence for the presence of specific nociceptors that give rise to discomfort and pain (Cervero 1994). In the present study, gastric vagal afferent fibers that responded, whether to balloon or fluid GD, exhibited a range of thresholds, none of which exceeded 20 mmHg. These findings are consistent with previous reports (Andrews et al. 1980; Davison and Clarke 1978, 1988; Iggo 1957) and suggest the absence of high-threshold, potentially nociceptive, gastric vagal afferent fibers. The absence of high-threshold, mechanosensitive afferent fibers may be characteristic of vagal afferents in general. No high-threshold esophageal afferent fibers were found in an earlier study of the opossum esophagus (Sengupta et al. 1989).

Effect of PAF infusion

PAF is an endogenous phospholipid that is an important mediator of inflammation (Zimmerman et al. 1992). PAF is also a potent vasodilator (Blank et al. 1979); PAF increases vascular permeability (McManus et al. 1981) and stimulates polymorphonuclear leukocytes to aggregate (O’Flaherty et al. 1981). An elevated content of PAF is present in the gastric juice of patients with erosive gastritis and oesophagitis (Šobhani et al. 1992). PAF content has also been reported to be increased in fundic, antral, and duodenal mucosa from patients with duodenal ulceration (Ackerman et al. 1990). We thus examined the effect of PAF on gastric vagal afferent fibers.

Local intra-arterial infusion of PAF causes an initial, dose-related contraction of the rat stomach followed by a second contraction after termination of infusion of PAF (Esplugues and Whittle 1989). Peripheral infusion of tetrodotoxin reduces the initial contraction and abolishes the following contraction produced by PAF. It has been suggested that stimulation of neuronal activity by PAF underlies the gastric motility response. In the present study, GD-sensitive fibers responded to PAF infusion, an effect mediated by the PAF receptor. Intragastric pressure slightly increased during PAF infusion, perhaps reflecting smooth muscle contraction of the stomach. We attempted to address this issue through the use of loperamide to prevent smooth muscle contraction. Loperamide did not affect fiber responses to PAF infusion or eliminate the increase in intragastric pressure produced by PAF. Because WEB 2086 attenuated the effects of PAF on fiber activity, but not intragastric pressure, the PAF-induced increase in fiber activity is unrelated to smooth muscle contraction.

Acute inflammation of the stomach was also produced by PAF. Administration of exogenous PAF to laboratory animals causes gastric ulcerations in the rat (Esplugues and Whittle 1988; Rosam et al. 1986) and small bowel inflammation (Gonzalez-Crussi and Hsueh 1983; Hsueh et al. 1986; Wallace and Whittle 1986). Chronic idiopathic dyspepsia (CID), a functional bowel disorder, is a clinical syndrome of the upper abdominal GI tract without any identifiable cause by diagnostic evaluation (Talley and Phillips 1988). It has been speculated that the etiology of CID involves either hypersensitivity of primary afferent fibers and/or CNS neurons (for reviews see Mayer and Gebhart 1994; Mayer and Raybould 1990). Although not a model of CID, we used PAF to determine whether inflammation induces sensitization of fiber responses to GD. None of the vagal afferent fibers studied exhibited altered responses to the range of GD pressures tested (5–60 mmHg), unlike the sensitization of visceral primary afferent fibers documented for other nerves (Häbler et al. 1993; Sengupta et al. 1996; Su et al. 1997a,b). Decreasing blood drainage from the mucosa by aggregation of neutrophils (Valone and Goetzl 1983), neutrophil-derived free radicals (Droy-Lefaix et al. 1991), or increased vascular permeability (Hatakeyama et al. 1991) could contribute to PAF-induced gastrointestinal damage. Mustard oil, which has been documented to sensitize afferent fibers innervating the urinary bladder (Häbler et al. 1993; Su et al. 1997a) and colon (Su et al. 1997b), is considered to be a neurogenic inflammatory agent that directly activates unmyelinated afferents and releases various peptides (Koltzenburg and McMahon 1986; Patacchini et al. 1990). Different mechanisms for producing inflammation may affect the induction of sensitization of visceral primary afferent fibers. Alternatively, GD-responsive vagal afferent fibers in the rat may sensitize to stimuli not tested here.

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Address for reprint requests: G. F. Gebhart, Dept. of Pharmacology, The University of Iowa College of Medicine, 2-471 Bowen Science Bldg., Iowa City, IA 52242.

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REFERENCES


