Models of gastric hyperalgesia in the rat

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Ozaki, Noriyuki, Klaus Bielefeldt, J. N. Sengupta, and G. F. Gebhart. Models of gastric hyperalgesia in the rat. Am J Physiol Gastrointest Liver Physiol 283: G666–G676, 2002.—Despite the prevalence of dyspepsia, nonhuman models for study of gastric hyperalgesia are limited. We thus characterized responses to gastric distension (GD) in the absence of and after two different gastric insults. A balloon was surgically placed into the stomach, and electromyographic responses to GD were recorded from the acromiotrapezius muscle at various times after balloon placement. Rats received either 20% acetic acid (HAc) or saline injections into the stomach wall or 0.1% iodoacetamide (IA) in drinking water. Responses to GD were monotonic with increasing distending pressure (10–80 mmHg) and were reproducible from days 3–14 after balloon implantation. Both HAc injection and IA ingestion led to increased responses to GD (i.e., gastric hyperalgesia), which, in the case of HAc, persisted for 60 days after HAc treatment. HAc injection produced ulcers in all treated animals; IA ingestion produced no lesions. Myeloperoxidase activity significantly increased after HAc but not saline injection or IA ingestion. In the awake, unrestrained rat, visceromotor responses to GD are quantifiable, reliable, and reproducible. Significantly enhanced responses to GD were apparent in two models of gastric insult, both of which may be useful for the study of the mechanisms of gastric hyperalgesia.

acetic acid; gastric ulcer; gastric distension; iodoacetamide; visceromotor; stomach pain

Organic and functional dyspepsia are common clinical syndromes characterized by pain associated with the stomach. Organic dyspepsia can be caused by chronic peptic ulcer disease or by other identifiable pathophysiological or microbiological abnormality (e.g., H. pylori) (24, 33). The greater problem is functional dyspepsia, defined as persistent or recurrent pain or discomfort, centered in the upper abdomen, in the absence of structural or biochemical explanation for these symptoms (53). The US household survey of functional gastrointestinal disorders reported in 1993 (14) estimated that functional dyspepsia outnumbered peptic ulcer diseases 3:1 and was present in up to 25% of Americans. Because the symptoms of functional dyspepsia are indistinguishable from organic causes of upper abdominal pain, such as peptic ulcer disease, gastro-esophageal reflux, or a malignancy of the upper abdo-

menn, the health care costs of functional dyspepsia are considerable. Like irritable bowel syndrome, several lines of evidence suggest that the discomfort and pain of dyspepsia are associated with altered function of visceral afferent pathways (30).

Patients with functional dyspepsia have lower discomfort thresholds and altered viscerosomatic referral of sensations during balloon distension of the stomach (26, 27, 34). Mertz et al. (34) recently examined responses to and perception of gastric balloon distension in patients with functional dyspepsia. They found that 87% of functional dyspeptic patients had evidence of altered visceral afferent function, including discontiguous areas of referred sensations and lowered perceptual thresholds to fullness, discomfort, and pain. They concluded that altered sensations that characterize functional dyspepsia represent a visceral hyperalgesia.

Most nonhuman animal studies of visceral hyperalgesia have focused on the distal gastrointestinal tract and urinary bladder. Previous studies in the gastrointestinal tract have employed balloon distension of the rat colon to study visceral hyperalgesia. Intracolonic treatment with a variety of irritants, including acetic acid (HAc), glyceral, formalin, tur-
pentine, trinitrobenzenesulfonic acid, and zymosan (e.g., Refs. 7, 9, 15, 28, 35, 36, 39) lead to exaggerated responses to colonic distension (i.e., hyperalgesia). Comparable models of gastric hyperalgesia have not been reported. Using previously established experimental models of mild and severe gastritis (2, 49, 51), we investigated the effect of inflammation on the responses to gastric distension (GD) in awake animals. Some of these data have been presented previously in abstract form (41–43).

METHODS

Animals

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

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Surgical Preparation

Electromyogram electrode implantation. Animals were deeply anesthetized with pentobarbital (Nembutal, Abbott Laboratories, Abbott Park, IL; 45–50 mg/kg), and sterilized multistranded, Teflon-insulated, 40-gauge stainless steel wires (Cooner Wire, Chatsworth, CA) were implanted in striated muscles by using aseptic techniques. In preliminary experiments, electrodes were stitched into the rectus abdominis and external oblique peritoneal muscles (both in the pelvic and diaphragmatic areas) or into the acromioplatius, spinotrapezius, and sternomastoid muscles. The electrode leads were tunneled subcutaneously and externalized at the back of the head for easy access during experiments.

Balloon implantation. The balloon for GD was placed surgically at the time of electromyogram (EMG) electrode implantation (unless noted otherwise). Balloons for GD were 2.0–2.5 cm in diameter and made from latent glove fingers. The fabrication of balloons and means of GD are fully described elsewhere (17) and are only briefly outlined here. A 3- to 4-cm-long left lateral epigastric incision was made, and the balloon was placed in the stomach through the fundus, where it occupies ~60% of the proximal stomach. When placed properly and not inflated, the pylorus is not obstructed, and there is no blockage of gastric emptying. Although rats lost weight right after the surgery, they continued to eat and gain weight normally 1 wk after implantation of the gastric balloon. The polyethylene tubing for air inflation of the gastric balloon was exteriorized at the back of the neck along with the EMG electrode leads. Before surgical implantation of balloons, they were inflated to the approximate size of a baseball for several hours (typically overnight) to overcome resistance to inflation (see Ref. 17). The diameter of the balloon when inflated in situ was thus greater than the intraluminal diameter of the rat stomach, and the pressure measured during distension reflected intragastric pressure, which was continuously monitored.

Gastric Irritation

HAc ulcer model. To produce multiple small ulcers in the stomach, 10 μl of 20% HAc (vol/vol in sterile saline) were injected into 20 sites in the submucosal layer of the glandular portion of the dorsal and ventral stomach wall (which is at the end opposite the site of balloon placement), with care taken not to disturb the vascular supply of the stomach wall (51). In another group of rats, a larger volume, single 30-μl injection of 20% HAc was made into the stomach wall to produce a single ulcer. Controls received identical injections of sterile saline instead of HAc.

Iodoacetamide gastritis model. To produce a gastritis, drinking water was continuously supplemented with 0.1% iodoacetamide (IA) (2, 49). The control group received the same pH drinking water.

Experimental Protocol

In these experiments, visceromotor responses (VMRs) (17, 29) were recorded by quantifying muscle contractions detected from electrodes implanted in the acromioplatiopus muscle. In an attempt to reduce stress and movement, rats were accommodated to, and allowed to crawl into, a canvas garden glove after being brought to the laboratory. The EMG electrode leads and balloon catheter were accessed through a slit in the glove. The EMG before (baseline) and during constant-pressure GD (5–80 mmHg, 20 s) was amplified and filtered (×10,000, 300–5,000 Hz; A-M Systems, Everett, WA), digitized, and integrated by using the SPIKE2/CED1401 data-acquisition interface. The raw EMG was rectified and quantified by calculating the area under the curve (μV/s). Both the raw and integrated EMG were displayed continuously on an oscilloscope and recorded. Each GD trial consisted of three segments: a 10-s predistension period, 20 s of GD (5–80 mmHg), and 10 s after termination of GD. Response to GD is defined as the increase in EMG activity above baseline during GD. Data are reported either as EMG (i.e., area under the integrated EMG after baseline subtraction) or percentage control when comparing the same rats before and after treatment. The response to 80-mmHg GD is taken as 100%.

Stimulus-response functions (SRFs) to graded GD (5, 10, 20, 30, 40, 50, 60, and 80 mmHg; 20 s; 4-min interstimulus interval) were performed on all rats (n = 83). To estimate response threshold, SRFs to graded GD were plotted for each animal, and a least squares regression line was obtained from the linear part of the SRF. The regression line was then extrapolated to the ordinate (representing distension pressure) to estimate response threshold.

To determine the reproducibility of VMRs to GD, we characterized responses to repeated GD (60 mmHg, 20 s, 4-min interstimulus interval) and, in another group of rats, SRFs on days 1, 3, 5, 7, 10, and 14 after surgery. We did not measure responses at times beyond day 14 after balloon implantation because the latex balloon was weakened by prolonged exposure to low stomach pH. In experiments in which responses to GD were tested 60 days after HAc treatment, balloons were placed during a second surgery 3 days before testing.

To evaluate theafferent pathway conveying acute gastric nociception, a passive avoidance paradigm described by Jarvic and Essman (21) and adapted by Ness and coworkers (37, 39) was employed. Rats were assigned to one of three groups: vagotomy, splenectomy, or intact. All rats had gastric balloons surgically implanted and were handled for 10 min three times a day for 3 days before testing. In this behavioral test, rats were placed on a 15 × 15 × 10 cm platform in a 50 × 75 × 15 cm box without a top. Rats normally quickly step down from the platform to the floor of the box. The latency to step down was defined as the time from placement of the rat on the platform to placement of both front paws on the floor, measured to the nearest second with a stopwatch. When rats in a control group stepped down from the platform, the previously placed gastric balloons were not inflated. When rats in one of the distension groups stepped down from the platform, the previously placed gastric balloon was inflated to 100 mmHg for 20 s. Rats exposed to this high-intensity stimulus, which was chosen to maximize acquisition of the learned behavior, ate and drank normally after the experiment and gave no evidence of irreversible damage to the stomach.

Gastric Hyperalgesia

To evaluate gastric hyperalgesia after HAc injection, the magnitude of VMR to graded intensities of GD (5–80 mmHg) was characterized in rats before and 5 days after either HAc or saline injection. Based on published reports of gastric ulceration, we anticipated that the magnitude of visceral hyperalgesia would be greatest 4–8 days after HAc injection. To determine the time course of gastric hyperalgesia after HAc or saline injection, VMRs to graded intensities of GD (5–80 mmHg) were characterized over time by testing rats on days 3, 5, 7, 10, 14, and 60 after HAc injection.

To evaluate gastric hyperalgesia after oral ingestion of IA, the VMR to graded intensities of GD was characterized on
day 7 after IA ingestion for 7 days and, in another group of rats, on day 60 after IA ingestion for 7 days. The control groups in these experiments were given the same pH normal drinking water.

Assessment of Gastric Insult

Macroscopic examination. Stomachs from randomly selected, untreated or treated rats were removed, opened along the greater curvature, pinned flat, and fixed with cold 4% paraformaldehyde in 0.1 M phosphate buffer. The mucosal surfaces of the stomachs were macroscopically examined for the presence of ulcers and/or hemorrhagic and/or nonhemorrhagic lesions.

Histology. For histological examination, tissue was taken from an area of the corpus where an ulcer or healed ulcer was apparent and randomly from areas of the corpus in other treatment groups and placed in cold 4% paraformaldehyde in 0.1 M phosphate buffer overnight. After 48–72 h, the tissue was embedded in paraffin, cut on a microtome, and stained conventionally with hematoxylin and eosin.

Myeloperoxidase assay. The magnitude of gastric inflammation was quantified by measuring activity of myeloperoxidase (MPO), an enzyme contained in the primary granules of polymorphonuclear cells, which infiltrate inflamed tissue. The assay was performed by following methods described previously (25). Rats were randomly selected from groups described above, at different times after surgery and deeply anesthetized, and the stomach was removed. A small piece of fresh stomach (~450 mg) was placed in a beaker containing 1 ml of ice-cold 0.5% hexadecyltrimethylammonium bromide (Sigma, St. Louis, MO) in 50 mM potassium phosphate buffer (pH 6.0). The tissue was minced, homogenized for 10 min, and subjected to a freeze-thaw cycle (acetone-dry ice bath and warm running water). After the freeze-thaw, the suspension was centrifuged at 40,000 g for 15 min. The supernatant (0.1 ml) was immediately assayed by mixing it with 2.9 ml of potassium phosphate buffer containing 167 μl/ml o-dianisidine dihydrochloride (Sigma) and 0.0005% H₂O₂. Changes in spectrometric absorbency were measured at 460 nm and compared against a standard curve.

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.

Fig. 1. Visceromotor response to graded (5–80 mmHg, 20 s) gastric distension (GD) from abdominal (rectus abdominis, external oblique-diaphragmatic, external oblique-pelvic), neck (acromio-trapezius, sternomastoideus), and back (spino-trapezius) muscles. Electromyographic (EMG) responses from the acromio-trapezius muscle gave monotonically increasing responses with increasing distending pressure. Values are means ± SE; n, no. of rats.

Fig. 2. Representative EMG records of visceromotor responses to graded intensities of GD recorded from acromio-trapezius muscles 3 days after balloon and electrode implantation.
where appropriate, followed by Tukey’s test for multiple comparisons, if warranted. A value of $P < 0.05$ was considered statistically significant.

**RESULTS**

**Effect of Balloon and Electrode Implantation**

Rats were closely observed for signs of distress or discomfort after surgery. Subjectively, rats exhibited normal exploratory behavior after the balloon and electrodes were implanted. Body weight was determined on days 1, 3, 5, 7, 10, 14, and 21 after balloon and electrode implantation. Rats lost weight to a mean maximum $93.7 \pm 0.7\%$ of the free-feeding, presurgery weight ($458.6 \pm 27.6$ g) over 7 days after surgery and balloon implantation, after which body weight increased.

**VMRs to GD**

In preliminary experiments, EMG electrodes were implanted into the rectus abdominus and external oblique peritoneal muscles (in the pelvic or diaphragmatic areas) in one group of three rats and into the acromiotorapezius, spinotorapezius, and sternomastoideus muscles in another group of seven rats. Summary data of VMRs to GD 4 days after balloon and electrode implantation are presented in Fig. 1. Among the muscles examined, the acromiotorapezius muscles in the back of the neck produced vigorous VMRs that were graded with increasing intensities of GD ($5–80$ mmHg, 20 s). Accordingly, EMG electrodes were implanted into acromiotorapezius muscles for quantification of the VMR in all subsequent experiments. Figure 2 presents representative EMG recordings from one experiment, and Fig. 3 illustrates individual and mean (inset) SRFs for GD. Extrapolation of the linear portion...
of individual SRFs revealed thresholds for responses to GD between 5 and 59 mmHg. The overall mean response threshold was 31.8 ± 1.6 mmHg (see Fig. 3, inset).

Reproducibility of Responses to GD

To examine whether responses to GD were reproducible and reliable, eight rats were tested for response to repeated GD (60 mmHg, 20 s, 4-min interstimulus interval). Figure 4A shows the mean responses of eight rats to 10 successive distensions. The mean response after the 10th distension was 99.6 ± 31.8% of the response to the first distension (P > 0.05). Although there was obvious variability, responses are typically stable and reproducible to repeated GD.

Responses to graded intensities of GD in a group of five rats 1, 3, 5, 7, 10, and 14 days after balloon and EMG electrode implantation were also determined. The repeated-measures ANOVA revealed a significant difference between days (F_{5,209} = 4.5, P = 0.006) (Fig.

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Fig. 6. A–D: macroscopic and microscopic photographs of stomach taken from naive, saline-, acetic acid (HAc)-, and iodoacetamide (IA)-treated rats, respectively. Saline- and HAc-treated rats received multiple injections; IA-treated rats ingested 0.1% IA for 7 days. All examples were taken 7 days after the start of treatment. Arrows in C, ulcers produced by HAc injection; arrowheads in D, presence of hemorrhage.
Post hoc analysis revealed that the SRF 1 day after surgery significantly differed from that on all other days except day 10. This is perhaps due to somatic hyperalgesia contributed to by the epigastric incision, which is in the area of convergent cutaneous input to the thoracic spinal cord. There were no differences among SRFs on days 3–14 after surgery. Resting EMG activity was similarly stable over this time frame. These results indicate that responses to GD in unanesthetized rats can be readily quantified and are both reliable and reproducible.

Passive Avoidance Behavior

Naive rats placed on a platform readily step down and explore the environment. In contrast, rats receiving GD (100 mmHg, 20 s), when stepping down from the raised platform, learn after three trials to passively avoid the stimulus by remaining on the platform (Fig. 5). The mean step-down latency in intact rats on trial 4 and all subsequent trials was greater than 60 s. Accordingly, GD produces behavior in intact rats consistent with an interpretation that the stimulus is noxious. To assess whether vagal or splanchnic afferent nerves convey acute noxious information from the stomach to the central nervous system, rats were tested for acquisition of passive avoidance behavior 5–7 days after vagotomy or splanchnecomy. Figure 5 reveals that splanchnectomy, but not vagotomy, prevented acquisition of the avoidance behavior. Splanchnecotomized rats were no different in step-down latency than naive rats that received no GD.

Gastric Irritation

HAc model. Multiple ulcers are present 7 days after multiple HAc injection into the stomach wall. Well-defined deep ulcerations, with distinct raised margins and whitish exudates covering the ulcer, and small shallow ulcerations without raised margins were observed (Fig. 6C). The corresponding histology shows thickening of the stomach wall with frank damage and infiltration of polymorphonuclear leukocytes in the submucosa, muscularis, submucosal, and mucosa, extending beyond the area of ulceration. In support of the histological presentation, MPO activity significantly increased 7 days after HAc, but not saline, injection into the stomach wall (Fig. 7). A single well-defined deep ulcer was present 5 days after a single injection of 30 µl HAc (20%) (not shown). No saline-injected rats showed evidence of gastric ulceration, although the stomach wall appeared to be thickened (Fig. 6B).

IA model. Oral ingestion by rats of 0.1% IA in drinking water clearly irritates the mucosal surface of the stomach, but no ulceration was apparent after 7 days of IA ingestion (Fig. 6D). Histologically, the gastric wall appears slightly thickened, and there is minimal inflammatory infiltration. The macroscopic and histological evaluations are supported by assay for MPO activity. In contrast to HAc-injected stomachs, MPO activity 7 days after ingestion of 0.1% IA was different from that in untreated, naive stomachs (Fig. 7). Twenty-one days of IA ingestion did produce a significant increase in MPO activity, but the increase was significantly less than that produced by HAc injection into the stomach wall.

Gastric Hyperalgesia

HAc model. In one set of experiments, responses to GD were quantified before and 5 days after either multiple HAc or saline injections into the stomach wall. The SRF was unaffected by saline injection into the stomach wall but was shifted significantly leftward in HAc-injected rats (F1,246 = 4.48, P = 0.049; data not shown), documenting the presence of gastric hyperalgesia.

We thus characterized the duration of gastric hyperalgesia by testing HAc- and saline-treated rats before and 3, 5, 7, 10, and 14 days after treatment. In a different group of rats, we examined responses to GD (10–80 mmHg) 60 days after either saline or HAc injections in the stomach wall. Gastric hyperalgesia was evident at the first time of testing 3 days after HAc injection into the stomach wall and was sustained...
reveals responses to GD in HAc-treated rats at the lowest distending pressures tested (10 and 20 mmHg), intensities that produced no or very low responses in saline-treated rats, consistent with a reduction in response threshold.

In rats tested 60 days after saline or HAc injection into the stomach wall, gastric hyperalgesia was also evident ($F_{1,139} = 6.17$, $P = 0.014$). Examination of gastric tissue 60 days after HAc injection revealed healed ulcers both macroscopically and histologically (Fig. 9). Tissue assay for MPO confirms the long-lasting effects of HAc; MPO activity was significantly greater than in saline-injected rats at 60 days, although less than MPO activity 7 days after HAc injection (Fig. 7).

We also examined responses in 14 rats to GD 5, 7, and 10 days after a single 30-µl injection of HAc, comparing responses to saline-treated rats ($n = 8$). Responses to GD in HAc-treated rats were modestly enhanced, but were not significantly different from responses of the same rats before HAc injection or responses of saline-treated animals ($P > 0.05$; data not shown).

**IA model.** The addition of 0.1% IA to drinking water also leads to exaggerated responses to GD 7 days after IA treatment is initiated (Fig. 10; $F_{1,111} = 6.87$, $P = 0.034$). In contrast to the HAc model, enhanced responses to GD occurred only at distension pressures ≥40 mmHg (e.g., see Fig. 8). The response threshold to GD, accordingly, was not changed. Ingestion of 0.1% IA for 7 days led to a significant decrease in body weight (to 79.3 ± 1.2% of the presurgery weight: 450.6 ± 9.1 g). It was our intention to test rats after 21 days of IA ingestion, but rats continued to lose weight, and responses to GD were not considered reliable. To address whether the hyperalgesia-producing effects of IA were long lasting (as were effects of HAc), another group of rats was tested for response to GD 60 days after 7 days of ingestion of 0.1% IA. No evidence of persistent gastric hyperalgesia was found (Fig. 10; $F_{1,139} = 1.12$, $P > 0.05$). Other times after IA ingestion have yet to be tested. Examination of stomach tissue 60 days after limited (7 days) exposure to IA revealed normal architecture (Fig. 9C).

**DISCUSSION**

This report describes two models of gastric hyperalgesia based on responses in the rat to balloon distension of the stomach. The principal findings are that GD in awake rats is a reliable model of acute stomach pain in the rat. Consistent with the clinical literature (5, 57) and recordings of vagal and splanchnic afferent fiber responses to GD (10, 20, 44, 45), acute gastric pain is conveyed to the central nervous system by the splanchnic nerve. Second, the VMR to GD recorded from the acromiotrapezius muscle in the back of the neck was reproducible, reliable, and graded with the intensity of GD. These characteristics of response to GD, in concert with the ability to perform such studies in unanesthe-

![Graph](https://via.placeholder.com/150)
tized rats, meet important criteria for development of animal models of visceral nociception (37). Third, responses to GD were shown to be significantly enhanced in two very different models of gastric insult, providing evidence for gastric hyperalgesia in circumstances of gastritis and multiple gastric ulcers.

**Stomach Pain**

The present study documents that splanchnectomy, but not vagotomy, blocked passive avoidance behavior in rats to noxious GD. Vagal afferent fibers are not considered to play a significant role in nociception (see Ref. 37 for review), and this is supported by the absence of mechanosensitive gastric vagal afferent fibers with high thresholds for response to GD (45). The mean response threshold of vagal afferents to GD is ~5 mmHg GD. In contrast, a study of splanchnic afferent fiber responses to GD documents the presence of low- and high-threshold mechanosensitive fibers in the splanchnic nerve innervation of the stomach of the rat (44). A proportion of the fibers that we studied responded only to distension pressures ≥30 mmHg, an intensity that we consider to be in the noxious range, supporting clinical reports that acute gastric pain is signaled by activation of splanchnic afferent fibers. The response threshold of high-threshold mechanosensitive fibers in the splanchnic nerve also correlates well with the present behavioral data. VMRs to GD in unanesthetized rats are apparent at distending pressures ≥30 mmHg. Consistent with this response threshold and response thresholds of mechanosensitive gastric splanchnic afferent fibers are studies in humans in which all control and functional dyspeptic subjects

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**Fig. 9.** A–C: macroscopic and microscopic photographs of stomach taken from saline-, HAc-, and IA-treated rats, respectively. Saline- and HAc-treated rats received multiple injections; IA-treated rats ingested 0.1% IA for 7 days. All examples were taken 60 days after the start of treatment. Arrows in B, locations of healed ulcers.
report discomfort at distending pressures between 20 and 25 mmHg (Ref. 50; see Ref. 37 for review).

**VMRs**

The term “visceromotor” was used by MacKenzie (29) in relation to lower abdominal visceral pain. The VMR is a contraction of the peritoneal (skeletal) musculature. Adaptation of this response, which is a pseudofective response in the Sherringtonian context and is organized supraspinally (48), was achieved by recording EMG activity in the acromiotrapezius muscle in the back of the neck. The acromiotrapezius muscle gave responses to GD that were reproducible, reliable, and graded with the intensity of GD. Employing procedures described previously (17) and used also in the present study, Rouzade et al. (46) described a similar model for evaluation of gastric sensitivity by recording EMG responses in neck muscles in the rat. Although they used a larger balloon and distended the stomach for a longer duration (10 min), their results are qualitatively similar to those reported here and similarly establish balloon distension of the stomach in the rat as a useful model of visceral pain. In a related study, Tougas and Wang (54) examined changes in heart rate to GD in anesthetized rats, documenting gastric volume-dependent bradycardia over a similar range of distending pressures as used here.

For VMRs to be meaningfully related to nociception, several criteria must be met. We showed that VMRs to GD were reproducible. Importantly, responses were graded with the intensity of GD, and SRFs were reproducible between 3 and 14 days after surgery for balloon and EMG electrode implantation. At later time points, GD became unreliable because the latex balloon was weakened after 14 days, likely due to the low gastric pH. Interestingly, VMRs across the range of distending pressures tested (10–80 mmHg) 1 day after surgery were greater on average than on essentially all subsequent days of testing. We believe that this is due to somatic hyperalgesia contributed to by the epigastric incision, which is in the area of convergent cutaneous input to the thoracic spinal cord.

**Gastric Insult**

Injection of 20% HAc into the stomach wall has previously been used to produce gastric ulcers (e.g., Refs. 51, 52). When the stomach is examined at various times after HAc injection, day 5 was determined to be the time at which well-defined ulcers are present before gradually healing over 2 wk. These macroscopic changes are consistent with biochemical alterations, such as an increase in PGE2 production that peaks by about threefold around day 12 before gradually returning to normal (51). In the present study, we evaluated gastric hyperalgesia over time and found that gastric hyperalgesia was present on days 3–60 after HAc injection into the stomach wall. When stomachs were examined 7 days after HAc injection, multiple ulcers were apparent macroscopically, and histological examination of stomach tissue revealed frank damage to all tissue layers. Saline-injected rats did not develop ulcers, but stomachs were affected by the injections. The muscle layer was thickened, and there appeared to be submucosal edema. Assay for MPO activity did not reveal significant inflammation in saline-injected stomachs. When stomachs were examined 60 days after HAc injection, the ulcerated areas were reepithelialized. However, there was clear histological evidence of persistent damage, and MPO activity was significantly greater than in rats that had received saline injections 60 days earlier, which appeared normal macroscopically and histologically.

Endogenous sulfhydryl compounds are important to the maintenance of mucosal integrity in the gastrointestinal tract. They are essential for protection of gastric epithelial cells against oxidative stress (33, 40), functioning as nucleophilic scavengers of reactive oxygen species. Moreover, calcium-induced gastric mucosal injury is associated with a reduction in sulfhydryl compounds (50). In rats, the sulfhydryl blocker IA was previously reported to induce diffuse gastritis (23, 49).
associated with superficial erosions, mild inflammatory cell infiltration, and an increase in MPO activity (12, 23). In the present experiments, 7 days of ingestion of 0.1% IA in drinking water produced mucosal erythema and edema and a neutrophil infiltrate in the submucosa, but no frank epithelial damage with erosion or ulceration or an increase in MPO activity. The more extensive damage by IA reported by others may reflect differences in strains or suppliers of animals or addition of sucrose to the IA-containing drinking water.

Visceral Hyperalgesia

Enhanced responses to GD (i.e., gastric hyperalgesia) were apparent in both the HAc and IA models of gastric insult. In addition, the significant leftward shift in SRFs in the HAc-treated rats resulted in a reduction in response thresholds, consistent with development of hyperalgesia. Visceral hyperalgesia is also evident after experimental inflammation of other organs: colon (e.g., Refs. 7, 9, 19, 22, 36, 39), bladder (e.g., Refs. 1, 32), and ureter (18, 47). In the gastrointestinal tract, inflammation triggers synthesis and/or release of endogenous mediators, including prostaglandins, cytokines, kinins, neurotrophins, histamine, and serotonin. Some of these substances (e.g., bradykinin, serotonin, and histamine) can directly stimulate visceral afferent fibers, whereas others (e.g., cytokines) indirectly produce sensitization of visceral afferent fibers (6). Enhanced sensitivity to mechanical, chemical, and/or thermal stimuli can come about because of changes in the expression or function of ion channels or de novo expression of receptors in peripheral tissues (e.g., B1 receptor expression after chronic inflammation; Ref. 13). In the present study, HAc- and IA-treated rats exhibited significantly greater magnitude VMRs to GD. The enhanced responses could reflect sensitization of visceral afferent fibers innervating the stomach.

Although peripheral mechanisms such as sensitization of visceral afferent fibers most certainly contribute to gastric hyperalgesia, changes in the excitability of central neurons are also involved. Development of central sensitization is suggested by several studies that report increased spontaneous activity, reduced response thresholds, increased response magnitudes, prolonged afterdischarges, or expansion of cutaneous receptive fields of spinal dorsal horn neurons after inflammation or repetitive noxious visceral stimulation (e.g., Refs. 8, 16, 31). Also, c-Fos and c-Jun protooncogene products have been shown to increase in the lumbar sacral spinal cord after repeated colorectal distension at a noxious intensity (55, 56). These observations, coupled with the enlarged fields of pain referral in humans after repeated colonic distension (38) or patients with irritable bowel syndrome (11) or dyspepsia (27, 34), implicate central changes in visceral pain.

Conclusion

In the awake, unrestrained rat, VMRs to GD are quantifiable, reliable, and reproducible signs of acute gastric nociception. The splanchnic nerve, as opposed to the vagus nerve, principally conveys acute pain or discomfort produced by GD. HAc-induced stomach ulcers or IA-induced gastritis can exaggerate VMRs to GD and, we believe, can involve both vagal and splanchnic innervations of the stomach. Enhanced responses to GD reflect an alteration in the sensitivity of the stomach to noxious stimuli, contributed to by changes in the excitability of primary sensory neurons that innervate the stomach (e.g., Refs. 3, 4) and by changes in the central nervous system (which have yet to be documented).

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