Cephalic phase of acid secretion involves activation of medullary TRH receptor subtype 1 in rats

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Martínez, Vicente, Maria Dolores Barrachina, Gordon Ohning, and Yvette Tachén. Cephalic phase of acid secretion involves activation of medullary TRH receptor subtype 1 in rats. Am J Physiol Gastrointest Liver Physiol 283: G1310–G1319, 2002. First published August 28, 2002; 10.1152/ajpgi.00222.2002.—Mechanisms involved in the cephalic phase of gastric acid secretion were studied in awake fasted rats with chronic gastric fistula and exposed to the sight and smell of chow for 30 min. Acid secretion was monitored using constant intragastric perfusion and automatic titration. Sham feeding induced a peak acid response reaching 82 ± 7 μmol/10 min within 20 min compared with the average 22 ± 2 μmol/10 min in controls. The sham-feeding response was abolished by intracisternal pretreatment with the TRH1-receptor antisense oligodeoxynucleotides or subcutaneous injection of atropine, whereas TRH1 mismatch oligodeoxynucleotides had no effect. Serum gastrin was not altered by the sham feeding and increased by refeeding. Gastrin antibody did not block the rise in acid during sham feeding, although the net acid response was reduced by 47% compared with the control group. Glycine-gastrin antibody, indomethacin and nitro-1-arginine methyl ester had no effect. Atropine and gastrin antibody decreased basal acid secretion by 98 and 75%, respectively, whereas all other pretreatments did not. These results indicate that the cholinergic-dependent acid response to sham feeding is mediated by brain medullary TRH1 receptors in rats.

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The cephalic phase of digestive events is associated with a small increase in basal acid secretion (30%) (9, 50). Furthermore, the increase in acid secretion is elicited by sham feeding and has been amply demonstrated in dogs and humans (9, 22). Pavlov’s pioneering work in dogs (41) and later reports in dogs and humans (1, 21) showed that the cephalic phase of gastric acid secretion was abolished by vagotomy and muscarinic blockade and may contribute >50% of the overall postprandial response (18). Although the primary role of vagal cholinergic pathways in the gastric acid response to sham feeding has been well established, the central signaling mechanisms involved in vagal activation have received little attention. The cephalic phase response has been largely studied in dogs and humans, thereby limiting the ability to perform more comprehensive investigations of central mechanisms (1, 21, 41).

Vagal efferent fibers innervating the stomach originate mainly from neurons located in the dorsal motor nucleus (DMN) of the vagus (4). Several neuropeptides and transmitters have been reported to act on DMN neurons to influence gastric function through vagal efferent pathways in rats (24, 48). Convergent neuroanatomical and electrophysiological evidence supports a physiological excitatory action of TRH on DMN neurons (46). TRH-containing projections from the caudal medullary raphe nuclei, namely the raphe pallidus, raphe obscurus, and the parapyramidal region innervate the dorsal vagal complex (28). TRH delivered to DMN neurons or injected intracisternally stimulates the activity of preganglionic vagal motor neurons, gastric vagal efferent fibers, and gastric myenteric neurons (32, 34, 51). This results in a vagal cholinergic-dependent stimulation of gastric acid secretion, motility, emptying, and mucosal blood flow in rats and cats (15, 46, 49). TRH actions in the brain are mediated by an interaction with two TRH receptors, subtypes 1 (TRH1) and 2 (TRH2) (5, 6). In support of TRH interacting with TRH1 receptors to stimulate vagal outflow, mapping studies showed that TRH1 is densely expressed in DMN neurons, whereas TRH2 mRNA is not detected in this region (12). In addition, targeting the TRH1 receptor using antisense oligonucleotides results in peptide specific blockade of intracisternal TRH-induced vagally mediated stimulation of gastric motor function and cytoprotection in rats (30, 31, 45, 54). Previous studies indicated that medullary TRH is involved in 2-deoxy-D-glucose and acute cold exposure-induced vagally mediated stimulation of gastric motor function (31, 39).

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In the present study, we investigated whether medullary TRH is involved in the acid response to sham feeding. First, we established an experimental model in rats to study the cephalic phase of gastric acid secretion induced by exteroceptive stimulation (vision and olfaction of food in fasted animals) under conditions of continuous gastric perfusion and automatic titration of acid in the perfusate. The acid response to sham feeding was compared with that of an established vagal stimulant (2-deoxy-D-glucose) and peripheral secretagogue (histamine) under similar experimental conditions of gastric acid collection. Second, we examined the physiological role of endogenous medullary TRH receptors in the gastric secretory response to sham feeding using intracisternal pretreatment with antisense oligodeoxynucleotides against the TRH1 mRNA. Such functional knockdown of the TRH1 receptor has been previously characterized (31, 45). Finally, to gain further insight into the peripheral mechanisms involved in the secretory response to sham feeding in rats, we assessed the role of muscarinic receptors and gastrin as well as the possible modulatory action of transmitters released into the stomach by central vagal stimulation, namely prostaglandins and nitric oxide (NO) (16, 55).

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (250–300 g, Harlan Laboratories, San Diego, CA) were housed under controlled conditions of temperature (22–24°C) and illumination (12:12-h light-dark cycle, starting at 6:00 AM) and maintained with Purina chow and tap water ad libitum. All experiments were performed between 8:00 AM and 1:00 PM in rats deprived of food for 20–22 h but allowed free access to tap water up to the beginning of the experiments. Protocols were approved by the Veterans Administration Animal Research Committee (animal component of research protocol number 96–080–08).

Drugs and treatments. Atropine (sulfate salt), histamine, 2-deoxy-D-glucose and nitro-L-arginine methyl ester (L-NAME) in powder form (all from Sigma, St. Louis, MO) were dissolved in 0.9% saline before use. Indomethacin (Sigma) was dissolved in 0.9% NaHCO3, and TRH (Peninsula Laboratories, Belmont, CA) was dissolved in sterile 0.9% saline immediately before use. TRH1 antisense oligodeoxynucleotides complementary to the first 18 bases downstream from the initiation codon of the rat TRH1 mRNA were synthesized with phosphorothionate derivatives of each nucleotide (5'-GAC GTT TTC ATT CTC CAT-3'; UCLA Molecular Biology Core, Los Angeles, CA). The alignment analysis for the first 18 bases downstream from the initiation codon showed significant differences between sequences of the rat TRH1 (MENETV) and rat TRH2 (MDGPSN) (5), indicating that the antisense selected should not react with the rat TRH2 mRNA. Mismatch antisense oligodeoxynucleotides (5'-GAT GTT CTC ACT CTC CAT-3') mutated at four different positions (underlined bases), but kept identical in composition to the antisense, were also synthesized and used as control treatment. The mismatched sequence displays noncomplementarity to any part of the TRH1 mRNA or to any other gene sequence in the Gene Bank database. The oligodeoxynucleotides were purified by polyacrylamide gel electrophoresis and diluted in sterile saline to a final concentration of 10 mg/ml. Aliquots (20 μl) were maintained at −70°C until use.

The gastric specific monoclonal antibody (MAb) (CURE no. 051091.1) and glycine (Gly)-extended gastric MAb (CURE no. 051092.21) (Digestive Diseases Research Center Antibody Core, Los Angeles, CA) were characterized previously and purified for in vivo immunoneutralization (2, 35). The gastric MAb has high-binding affinity and specificity for gastrin peptides containing 10 or more amino acids including the biologically active COOH terminus. CCK peptides or peptides containing an altered COOH terminus did not demonstrate significant bindings (35). The Gly-extended gastric MAb does not bind to gastrin or CCK-like peptides with an intact COOH terminus (2).

Intracisternal injections were performed by puncture of the occipital membrane with a Hamilton syringe in rats anesthetized with enflurane (4% vapor in O2; Etrand, Anaquest, Madison, WI) and placed in stereotaxic equipment as previously detailed (31). The total procedure (induction of anesthesia plus intracisternal injection) lasted between 5 and 7 min. The intracisternal, intraperitoneal, or subcutaneous injections were given in dosages of 10 μl/rat, 0.5 ml/rat, and 1–1.5 ml/rat, respectively.

Measurement of gastric acid secretion. Fasted rats were anesthetized with a mixture of ketamine hydrochloride (75 mg/kg ip; Ketaset, Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (5 mg/kg ip; Rompun, Mobay, Shawnee, KS). The stomach was exteriorized through a medial laparotomy, and its nonglandular portion was incised to insert a modified stainless steel Thomas fistula. The fistula was sutured to the stomach wall, exteriorized through the abdominal wall, and capped. Rats were housed after surgery in individual cages with direct bedding. Experiments started 1 wk after surgery, and during this period, animals were accustomed to being maintained in Bollman cages (2 h/day for 3 days) and to the experimental conditions of gastric perfusion.

Gastric acid secretion was measured in fasted awake rats placed in Bollman cages. The gastric fistula was uncapped, and the stomach was rinsed until cleaned with saline solution (0.9% NaCl, pH 7.0, 37°C) through the cannula. Thereafter, a double-lumen cannula was attached to the gastric fistula. Continuous intragastric perfusion was performed throughout the experiment with warm 0.9% saline solution (pH 7.0, 37°C) at a constant rate (3 ml/min), with a peristaltic pump (Signal Pump TMP-6L; Kagaku Sangyo, Toyo, Japan). Gastric perfuse was collected by drainage, and continuous measurement of pH and titration to pH 7.0 with 0.01 N NaOH were performed with an automatic titration system (TOA Autotitrator TSC-10A, TOA Electronics, Kobe, Japan). The titration system was connected to a computer for online data acquisition (sample frequency of 1 Hz) using Axotape computer software (version 1.2.01, Axon Instruments, Dallas, TX). At the end of the experiment, data collected were transferred to a spreadsheet (Microsoft Excel) for analysis.

Experimental protocols. In all the experiments, a 30- to 45-min period was allowed for stabilization, followed by a 30-min basal recording of acid secretion before any treatments or sham feeding, which consisted of exposing fasted rats to the sight and smell of standard Purina chow for a 30-min period.

Stimulation of gastric acid secretion by sham feeding, 2-deoxy-D-glucose and histamine. After basal gastric acid secretion was recorded, rats were exposed to sham feeding. Gastric secretion was monitored for consecutive 30-min periods before (basal), during, and after sham feeding. In separate experiments, rats were injected with either 2-deoxy-D-glucose (150 mg/kg ip), histamine (5 mg/kg sc), or respective vehicles

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(0.9% saline, intraperitoneal or subcutaneous) and gastric acid secretion was monitored for 30 min before (basal) and 60 min after the injection of acid stimulants.

**Effect of TRH: antisense or mismatch oligodeoxynucleotide pretreatment on the acid response to sham feeding.** Rats were injected intracisternally with either TRH antisense or mismatch oligodeoxynucleotides at a total dose of 200 μg/rat in two separate injections (100 μg/10 μl each) at 24-h intervals as in previous studies (30, 31). Twenty four hours after the second intracisternal injection, rats pretreated with TRH antisense or mismatch or antisense oligodeoxynucleotides were injected intracisternally with saline or TRH (1 μg/rat ic, 10 μl) or exposed to sham feeding for 30 min. Gastric acid secretion was monitored for 30 min before and 60 min after the start of treatment.

**Effect of gastrin and Gly-gastrin antibody pretreatment on the acid response to sham feeding.** Groups of animals were injected intraperitoneally with either gastrin MAb (16 mg/kg), Gly-extended gastrin MAb (16 mg/kg), or the appropriate dilution of PBS (1.5 ml) 14 h before the experiments. The regimen of antibody administration was based on previous immunoneutralization studies of endogenous and exogenous gastrin in rats (36). On the day of the experiment, gastric acid secretion was monitored for consecutive 30-min periods before, during, and after sham feeding in rats pretreated with gastrin MAb, Gly-gastrin MAb, or vehicle.

**Effect of sham feeding and feeding on serum gastrin levels.** Fasted rats were exposed to sham feeding or given ad libitum Purina chow for 15 or 30 min, then euthanized by CO₂ inhalation. The abdominal cavity was opened, and blood was collected directly from the portal vein, placed into ice-chilled tubes, centrifuged (2,000 g, 10 min, 4°C), and the serum was separated and stored at −70°C until assay. Gastrin levels were determined by a competitive RIA with a gastrin antibody (CURE #1160), as previously described (52). Gastrin values are expressed as picomoles per liter of serum.

**Effects of atropine, t-NNAME or indomethacin pretreatment on the acid response to sham feeding.** After a basal period, either atropine sulfate (2 mg/kg sc, t-NNAME (3 mg/kg ip), saline (1 ml/kg ip or sc), indomethacin (5 mg/kg ip), or vehicle (0.9% NaHCO₃, 1 ml/kg ip) was injected. Sham feeding started 15 min after t-NNAME, 30 min after atropine, and 45 min after indomethacin administration. Gastric acid secretion was monitored continuously before and after drug pretreatment and for 60 min after the beginning of sham feeding. The regimen of treatment was based on our previous studies (49, 55).

A maximum of 3–4 experiments was performed in each animal, and a recovery period of 4–5 days was allowed between studies in the same animal. The same treatment was given only once per animal. After in vivo immunoneutralization with gastrin MAb or Gly-gastrin MAb or pretreatment with TRHantisense or mismatch oligodeoxynucleotides, animals were not further used.

**Statistical analysis.** Results are expressed as means ± SE and represent gastric acid output (μmol) vs. time, at 10- or 30-min intervals (μmol/10 min or μmol/30 min). Basal gastric acid secretion per 10 min for the 30-min period before treatment was calculated as the average value of the three consecutive 10-min periods recorded as basal. Net gastric acid secretion was calculated by subtracting the mean basal gastric acid secretion from each posttreatment value. Comparisons between two groups were performed using paired or unpaired Student’s t-test, as appropriate. Comparisons between multiple groups were performed by one-way ANOVA with or without repeated measures, as appropriate, followed by a Student-Newman-Keuls multiple-comparisons test. P values <0.05 were considered statistically significant.

**RESULTS**

**Effect of sham feeding, 2-deoxy-d-glucose, and histamine on basal gastric acid secretion.** Conscious, fasted rats had a baseline secretion of 25.9 ± 1.8 μmol/10 min (n = 12), which did not significantly vary during the following 60-min period (22.0 ± 1.6 μmol/10 min, pooled data from 6 consecutive sampling periods after basal measurement), indicating that acid secretion was stable throughout the experimental period conditions.

Sham feeding induced by the sight and smell of Purina chow for 30 min increased gastric acid secretion, as monitored continuously by constant gastric perfusion and automatic titration of the perfusate in conscious fasted rats adapted to be maintained in Bollman cages (Fig. 1). Gastric acid secretion increased significantly within 10 min after the onset of the sham feeding procedure (47.4 ± 9.2 μmol/10 min, n = 8; P < 0.05 compared with basal values: 31.3 ± 2.5 μmol/10 min) and reached a peak stimulation at 20 min (81.6 ± 7.4 μmol/10 min; P < 0.05 vs. basal). Thereafter, gastric acid output declined during the last 10-min period of sham feeding and returned to basal levels 10 min after the end of sham feeding (40.7 ± 4.6 μmol/10 min; P > 0.05 vs. basal and control group) (Fig. 2A). Total gastric acid output during the 30-min sham feeding period was 194.6 ± 20.3 μmol/30 min, which represents a 136 ± 49% increase over basal values (Fig. 2B). In rats maintained in Bollman cages without sham feeding, the acid output during the same period was 77.6 ± 17 μmol/30 min and not significantly different from basal (Fig. 2B).

The intraperitoneal injection of 2-deoxy-D-glucose (150 mg/kg, n = 3) induced a peak acid response at 40 min after injection (60.5 ± 11.6 μmol/10 min; P < 0.05 vs. basal and saline control). Thereafter, the gastric acid secretion decreased toward basal levels (Fig. 3A). Accumulated acid output during the maximal response was 80.3 ± 16.3 μmol/30 min, which represents a 113.9 ± 5.1% increase over basal secretion.

Histamine (5 mg/kg sc, n = 4) significantly increased gastric acid secretion at 30 min after subcutaneous

![Fig. 1. Representative trace showing gastric acid output/10 s during a continuous recording of gastric acid secretion in a conscious rat with chronic gastric fistula and maintained in a Bollman cage. The recording displays the acid secretion during the basal period, sham feeding, and postsham feeding, showing stimulation of secretion linked with that period.](http://ajpgi.physiology.org/10.1210/ajpgi.283.6.g1312)
gastric acid output was 80.2 ± 16.8 μmol/30 min during the basal period and increased to 221.5 ± 25.8 μmol/30 min (n = 4; P < 0.05) during sham feeding (Fig. 4B). TRH₁ antisense oligodeoxynucleotides blocked sham feeding-induced stimulation of gastric acid secretion. The acid output was 109.8 ± 28.0 μmol/30 min and not significantly different from basal values (67.0 ± 13.3 μmol/30 min, n = 5) (Fig. 4B). In TRH₁ mismatch-treated rats, intracisternal TRH stimulated gastric acid secretion with a peak response observed at 20 min (46.3 ± 7.0 μmol/10 min; P < 0.05 vs. mean basal; 20.6 ± 2.7 μmol/10 min, n = 4; P < 0.05). Gastric acid secretion returned to basal values 30 min after peptide administration (18.9 ± 11.3 μmol/10 min; P > 0.05 vs. basal). TRH₁ antisense completely prevented the gastric acid response to intracisternal TRH (Fig. 5). The intracisternal injection of vehicle (saline) in the same experimental conditions did not significantly modify acid secretion throughout the experiment (data not shown).

Effect of TRH₁ antisense oligodeoxynucleotides on sham feeding and intracisternal TRH-induced stimulation of gastric acid secretion. Pretreatment with either TRH₁ mismatch or antisense oligodeoxynucleotides did not modify basal gastric acid secretion (antisense: 22.0 ± 1.8 μmol/10 min, n = 9; mismatch: 22.7 ± 2.0 μmol/10 min, n = 8; Fig. 4A) compared with non-treated animals (25.8 ± 8.2 μmol/10 min). In rats pretreated with TRH₁ mismatch oligodeoxynucleotides,
Fig. 4. TRH₁-receptor antisense oligodeoxynucleotides prevented sham feeding-induced stimulation of gastric acid secretion in conscious rats with chronic gastric fistula. Rats were pretreated intracisternally with TRH₁ antisense or mismatch oligodeoxynucleotides (100 μg twice, −48 and −24 h) before exposure to sham feeding. A: time course of gastric acid output/10 min; each point represents the mean ± SE of 4 rats/group; #P < 0.05 vs. basal secretion; *P < 0.05 vs. same time point in mismatch pretreated group. B: gastric acid output/30 min before and during a 30-min sham feeding. Each bar represents the mean ± SE of 4 rats/group; *P < 0.05 vs. all other groups (ANOVA).

Effect of atropine on sham feeding-induced stimulation of acid secretion. The average gastric acid secretion values during the 30-min periods before (33.7 ± 5.2 μmol/10 min) and after (23.7 ± 4.8 μmol/10 min, n = 4) the subcutaneous injections of saline were not significantly different (Fig. 6B). Sham feeding stimulated acid secretion to a peak response of 72.7 ± 19.9 μmol/10 min at 20 min in subcutaneous vehicle-pretreated rats. (Fig. 6A). Accumulated acid secretion during sham feeding in vehicle-treated animals (178.6 ± 11.0 μmol/30 min; P < 0.05 vs. basal secretion) was similar to that observed in rats without any pretreatment (Figs. 2B and 6B). Atropine (n = 3) inhibited basal acid secretion to 0.7 ± 0.4 μmol/10 min (P < 0.05 vs. saline) at 30 min postinjection (Fig. 6A). Under these conditions, sham feeding did not increase gastric acid secretion (0.2 ± 0.1 μmol/30 min; Fig. 6B).

Effect of gastrin and Gly-gastrin antibodies on sham feeding-stimulated gastric acid secretion. In rats injected intraperitoneally 14 h before the experiment with vehicle (n = 3) or Gly-gastrin MAB (n = 5), sham feeding significantly increased gastric acid output to 200.7 ± 23.6 μmol/30 min (P < 0.05 vs. basal secretion: 122.5 ± 21.2 μmol/30 min) and 196.8 ± 16.6 μmol/30 min (P < 0.05 vs. basal secretion: 110.8 ± 24.9 μmol/30 min), respectively (Fig. 7B). This represents a 101.7 ± 21.6 and 122.4 ± 55.4% increase over baseline secretion, respectively. Pretreatment with the gastrin MAB (n = 7) reduced basal gastric acid secretion to 9.6 ± 0.5 μmol/10 min, whereas basal acid output was 36.4 ± 2.7 and 34.1 ± 1.4 μmol/10 min in vehicle- or Gly-gastrin MAB-pretreated animals, respectively (P < 0.05; Fig. 7A). This represents a 76.5 ± 3.8 and 71.2 ± 4.6% decrease in basal acid secretion compared with animals pretreated with vehicle or Gly-gastrin MAB, respectively. Sham feeding induced a significant 144.0 ± 30.9% increase in gastric acid secretion over baseline in gastrin MAB-pretreated animals. However, the magnitude of the net gastric output induced by sham feeding was reduced by 47% compared with that of the control group (Fig. 7).

Sham feeding did not modify gastrin levels in portal blood at 15 or 30 min compared with fasted animals. Refeeding resulted in 201 ± 37 and 216 ± 59% elevation of serum gastrin over fasting values at 15 or 30 min of food exposure, respectively (Table 1).

Fig. 5. TRH₁-receptor antisense oligodeoxynucleotides prevents intracisternal TRH-induced stimulation of gastric acid secretion in conscious rats with chronic gastric fistula maintained in Bollman cages. Rats were pretreated as detailed in Fig. 4 legend and after a 30-min basal period, they were injected intracisternally with TRH. A: time course of gastric acid output/10 min. Each point represents the mean ± SE of 4 rats/group; #P < 0.05 vs. basal secretion; *P < 0.05 vs. same time point in mismatch pretreated group. B: gastric acid output/30 min before and after the intracisternal injection of TRH. Each bar represents the mean ± SE of 4 rats/group; *P < 0.05 vs. basal.
Effect of L-NAME and indomethacin on sham feeding-induced stimulation of gastric acid secretion. NO synthase blockade with L-NAME did not modify basal gastric acid secretion in conscious rats nor the secretory response induced by sham feeding. In L-NAME-treated animals, sham feeding increased acid secretion by 122.8 ± 36.0% over basal values, which was not significantly different from the sham feeding acid response in vehicle-treated animals (124.0 ± 51.9% over basal values; Table 2). Blockade of prostaglandin synthesis with indomethacin did not alter either basal or stimulated acid secretion induced by sham feeding. In vehicle- or indomethacin-treated animals, sham feeding increased acid secretion by 124.4 ± 39.3 and 166.5 ± 67.8%, respectively, over basal secretion (Table 2).

DISCUSSION

The present studies demonstrate that exteroceptive stimulation by the sight and smell of standard food chow for a 30-min period induced a consistent increase of gastric acid secretion over baseline in fasted rats fitted with chronic gastric fistula and accustomed to be maintained in Bollman cages. The acid response is specific to the sham feeding procedure, because there is no change in basal acid secretion in rats maintained

Table 1. Effect of sham feeding and refeeding on portal blood gastrin levels in awake fasted rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time, min</th>
<th>Serum gastrin, pmol/l</th>
<th>n</th>
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<tbody>
<tr>
<td>None</td>
<td></td>
<td>29.0 ± 6.1</td>
<td>4</td>
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<tr>
<td>Sham feeding</td>
<td>15</td>
<td>20.0 ± 1.7</td>
<td>4</td>
</tr>
<tr>
<td>Refeeding</td>
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<td>25.2 ± 2.7</td>
<td>5</td>
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<tr>
<td>Refeeding</td>
<td>15</td>
<td>87.4 ± 10.7*</td>
<td>5</td>
</tr>
<tr>
<td>Refeeding</td>
<td>30</td>
<td>91.8 ± 17.0*</td>
<td>4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Fasted rats received either no treatment or were exposed to sham-feeding or Purina chow for 15 or 30 min and immediately euthanized for collection of portal blood. *P < 0.001 vs. no treatment or sham feeding [ANOVA, F(4,17) = 14.903, P < 0.001].
under similar conditions without sham feeding. The stimulation of acid occurred with a short onset (within 10 min), reached a peak (~130% increase over baseline) at 20 min, and displayed a rapid return to basal 10 min after the cessation of sham feeding. The magnitude of the acid response was similar to that induced by the central vagal stimulant 2-deoxy-D-glucose (14). So far, the cephalic phase of acid secretion has been investigated almost exclusively in dogs and humans (9, 18, 20, 22). One earlier study (53) induced sham feeding in rats by training them to ingest a sucrose liquid diet, which drained out of the stomach through the open gastric fistula. This resulted in a 200% increase of acid secretion over basal. Under these conditions, the acid response should be attributed to first-order visceral afferent inputs, encoding taste information, chewing, and swallowing with a minimal gastric component, because food ingested was diverted through the open gastric fistula (17). In the present rodent model of sham feeding, exteroceptive olfactory and visual sensory inputs, without chemical orosensory-geal stimulation associated with the ingestion of food, were effective to stimulate acid secretion. These findings bear similarity with reports in humans showing that the combination of sight or smell of food results in a short-lasting 160% increase of gastric acid output (9, 19).

Convergent evidence indicates that TRH-signaling pathways in the brain medulla play a role in the acid response to sham feeding in rats. In the present study, intracisternal pretreatment with TRH$_1$ antisense oligodeoxynucleotides inhibited sham feeding-induced gastric acid secretion, as shown by the lack of a significant rise in acid secretion during the sham-feeding period. By contrast, TRH$_1$ mismatched oligodeoxynucleotide pretreatment did not impair the acid response to sham feeding. The efficacy of the TRH$_1$ antisense pretreatment is also demonstrated by the complete abolition of intracisternal TRH-induced stimulation of gastric acid secretion, whereas mismatched pretreatment had no effect. Although antisense-targeting displays varying efficiency in different systems (25), several reports showed that a similar regimen of TRH$_1$-receptor antisense administration was effective in blocking intracisternal TRH-induced vagal cholinergic-mediated gastroprotection against ethanol and stimulation of gastric emptying (30, 31, 54). This pretreatment also abolished TRH microinjected into the DMN-induced increase in intraluminal gastric pressure and pyloric motility (45). Specificity and lack of toxic effect of TRH$_1$ antisense pretreatment are supported by the unchanged vagal cholinergic-mediated gastric stimulatory response to intracisternal injection of peptide YY and the somatostatin subtype 5-prefering agonist BIM-23052 and microinjection of glutamate into the DMN (31, 45, 54).

Consistent with the activation of medullary TRH pathways in the cephalic phase, intracisternal injection of TRH mimicked the pattern of gastric acid response to sham feeding with a rapid onset and short duration. Previous studies showed that TRH induced a direct postsynaptic increase in the firing of DMN neurons (51). Likewise, the anticipation of feeding activates DMN neurons monitored by the induction of Fos expression (8). TRH or a stable TRH analog injected intracisternally or into the DMN-induced stimulation of gastric acid secretion is abolished by vagotomy and peripheral injection of muscarinic antagonists, including atropine and pirenzepine (47). In the present study, subcutaneous injection of atropine completely inhibited the rise in gastric acid secretion induced by sham feeding in rats. These results are in agreement with previous reports in dogs and humans showing that the acid response to sham feeding is abolished by low doses of atropine and primarily under peripheral cholinergic control (18–20). There is evidence in rats that atropine microinjected into the DMN decreases gastric acid secretion stimulated by hypothalamic input (37) while not influencing that stimulated by TRH injected into the DMN (38). The question of whether, in addition to the periphery, the atropine inhibitory effects of sham feeding also result from a central action on DMN neurons needs to be further investigated. The muscarinic-dependent acid secretion induced by sham feeding contrasts with the largely noncholinergic gastric acid phase induced by intragastric administration of a peppone meal (27).

Neuroanatomical data also support a role for medullary TRH and TRH$_1$ receptors in gastric vagal stimulation. There is a dense innervation of DMN neurons...
by TRH immunoreactive fibers in rats (28). In humans, TRH immunoreactive fibers represent the most prominent network innervating the DMN compared with 12 other neuropeptides studied (10). In addition, the highest TRH binding in the medulla is located in the medial DMN that contained preganglionic neurons projecting to the stomach (29). Collectively, the present results along with the expression of TRH₁ receptors on DMN neurons (12) provide evidence that TRH₁ receptor activation is involved in the cholinergic-dependent stimulation of gastric secretion observed during sham feeding in fasted rats. The possibility that other mediators acting in synergy with TRH could participate in the vagal-dependent stimulation of gastric secretion during sham feeding could not be excluded. In particular, the involvement of serotonin, colocalized with TRH in medullary neurons, is suggested by existing evidence that serotonin injected into the DMN with TRH potentiates the stimulation of gastric acid secretion while having no stimulatory effect of its own (48, 56). Afferent neuronal circuitries leading to TRH₁-receptor stimulation of gastric acid secretion by sham feeding may involve afferents activated by exteroceptive senses (olfaction and vision) from the orbital frontal cortex and central amygdala, which receive visual and olfactory inputs and project to the dorsal vagal complex (42).

Central injection of TRH, in addition to increasing gastric acid secretion, induces a vagally mediated stimulation of gastro-duodenal blood flow and motor function and pancreatic exocrine and endocrine secretion (40, 47, 49). Similar changes occurred during sham feeding in experimental animals and humans (11, 18, 19). Therefore, it may be speculated that activation of TRH₁ receptors in the DMN orchestrates other components of the cephalic phase that subserve optimizing both the digestive and metabolic processes under conditions of impending nutrient load (33). Glucopenia, induced by peripheral injection of 2-deoxy-D-glucose, and energetic demand, induced by cold exposure, also result in a central vagal-dependent stimulation of gastric function recruiting endogenous brain medullary TRH (39) and activation of TRH₁ receptor (31). By contrast, central TRH₁ receptors do not play a significant role in regulating basal cholinergic-dependent gastric acid secretion as previously reported for basal gastric emptying in awake rats (31). This can be inferred by the observations that basal acid secretion was inhibited by atropine and not modified by pretreatment with TRH₁ antisense oligodeoxynucleotides. However, the present study does not allow us to rule out the possible involvement of medullary TRH in the ongoing regulation of the sensitivity of vago-vagal reflexes that modulates gastric function (44) in addition to mediating vagal activation to specific stimuli.

Gastric acid response to central vagal stimulation may result from an interplay of peripheral mechanisms recruited by vagal cholinergic activation (46). The present data support the role of basal circulating gastrin in potentiating the cholinergic acid response to sham feeding. Gastrin immunoneutralization for 14 h reduced the basal acid secretion in awake rats, consistent with recent reports in anesthetized rats and in mice genetically gastrin deficient (13, 27). However, sham feeding still induced a 144% increase in acid secretion from the reduced baseline in gastrin antibody pretreated rats although the net acid response was dampened by 47%. In addition, sham feeding did not alter basal gastrin levels during the peak acid secretion, whereas refeeding for 15 or 30 min resulted in a threefold increase in gastrin levels in portal blood. In previous human studies, the sight and smell of food did not significantly increase gastrin plasma levels (9, 18, 19). These results indicate that direct cholinergic stimulation along with basal circulating gastrin are important to convey the full acid response to sham feeding in rats. The most likely target of endogenous basal gastrin is the enterochromaffin cells (26). Histamine is released by this cell type in response to gastrin and potentiates parietal cell response to acetylcholine (26). In the present study, we showed that histamine injected peripherally stimulates gastric acid secretion in conscious rats.

Various reports showed that vagal-dependent gastric responses to specific stimuli are modulated by the vagal-dependent local releases of prostaglandin E₂ and/or NO (3, 49, 55). Neither prostaglandin synthesis blockade with indomethacin nor NO synthase inhibition with L-NAME influenced the gastric acid secretion induced by sham feeding. Indomethacin and L-NAME were administered at doses that blocked gastric prostaglandin synthesis (55) and NO-dependent vagal stimulation of gastric mucosal blood flow (49). Consistent with these findings, we previously reported that neither basal nor TRH injected intracisternally at doses that stimulated acid secretion were modified by L-NAME and indomethacin in anesthetized rats (49, 55). In humans, the blockade of prostaglandin synthesis with indomethacin did not alter basal acid secretion and failed to affect the secretory response elicited by sham feeding (23). These data indicate that under conditions of sham feeding, the acid response is not modulated by gastric NO or prostaglandins.

In summary, the present findings demonstrate that an atropine-sensitive cephalic phase of gastric acid secretion can be elicited reproducibly by a sham-feeding procedure involving solely the sight and smell of standard food in fasted rats with a gastric fistula. Moreover, we showed that the acid response to sham feeding is blocked by TRH₁ antisense oligodeoxynucleotide pretreatment, whereas basal acid secretion is not influenced. This, along with existing evidence for a high density of TRH₁ receptors on DMN neurons (12) and the excitatory action of intracisternal TRH on DMN neurons, vagal efferent fibers, cholinergic myenteric neurons, and acid secretion (15, 32, 34, 51), strongly support a role for brain medullary TRH₁ receptors in the cholinergic gastric response to sham feeding. The cephalic phase of acid secretion is not associated with a rise in serum gastrin and is still observed in the presence of gastrin immunoneutraliza-
tion and prostaglandins or NO synthesis blockade, showing a primary mediation through cholinergic pathways. However, basal circulating levels of gastrin contribute to the magnitude of basal and sham feeding acid secretion. These results provide new insight into the underlying biochemical substrate in the brain involved in the acid response to sham feeding. It is suggested that medullary TRH is a major candidate for the cephalic phase of digestion.

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