H. pylori acutely inhibits gastric secretion by activating CGRP sensory neurons coupled to stimulation of somatostatin and inhibition of histamine secretion

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Zaki M, Coudron PE, McCuen RW, Harrington L, Chu S, Schubert ML. H. pylori acutely inhibits gastric secretion by activating CGRP sensory neurons coupled to stimulation of somatostatin and inhibition of histamine secretion. Am J Physiol Gastrointest Liver Physiol 304: G715–G722, 2013. First published February 7, 2013; doi:10.1152/ajpgi.00187.2012.—Acute Helicobacter pylori infection produces hypochlorhydria. The decrease in acid facilitates survival of the bacterium and its colonization of the stomach. The present study was designed to identify the pathways by which acute H. pylori infection inhibits acid secretion. In rat fundic sheets in an Ussing chamber, perfusion of the luminal surface with H. pylori in spent broth (10^3–10^5 cfu/ml) or spent broth alone (1:10^3 to 1:10^6 final dilution) caused a concentration-dependent increase in somatostatin (SST; maximal: 200 ± 20 and 194 ± 9% above basal; P < 0.001) and decrease in histamine secretion (maximal: 45 ± 5 and 48 ± 2% below basal; P < 0.001); the latter was abolished by SST antibody, implying that changes in histamine secretion reflected changes in SST secretion. Both responses were abolished by the axonal blocker tetrodotoxin (TTX), the sensory neurotoxin capsaicin, or the CGRP antagonist CGRP8-37, implying that the reciprocal changes in SST and histamine secretion were due to release of CGRP from sensory neurons. In isolated rabbit oxyntic glands, H. pylori inhibited basal and histamine-stimulated acid secretion in a concentration-dependent manner; the responses were not affected by TTX or SST antibody, implying that H. pylori can directly inhibit parietal cell function. In conclusion, acute administration of H. pylori is capable of inhibiting acid secretion directly as well as indirectly by activating intramural CGRP sensory neurons coupled to stimulation of SST and inhibition of histamine secretion. Activation of neural pathways provides one explanation as to how initial patchy colonization of the superficial gastric mucosa by H. pylori can acutely inhibit acid secretion.

achlorhydria; hypochlorhydria; gastric acid secretion; acid

HELCOBACTER PYLORI (H. pylori), an extracellular bacterium that specifically colonizes gastric epithelium and survives within the mucus layer (28, 38), infects the stomach of half the world’s population. Almost all patients infected with the bacterium develop chronic gastritis. The infection usually remains silent but can evolve into more serious disease such as peptic ulcer, adenocarcinoma, and lymphoma. The pathways by which H. pylori produces gastric disease are incompletely understood. H. pylori may decrease or increase acid secretion depending on the time course and the area of the stomach predominantly infected (45). Acute infection elicits hypochlorhydria in animals and humans (21, 25, 51, 67). The decrease in acid secretion is thought to facilitate survival of the bacterium and its colonization of the stomach (46, 53). Chronic infection may induce hypochlorhydria or hyperchlorhydria depending on the anatomic site of infection (62). Long-term infection of the gastric body and fundus (i.e., oxyntic mucosa) is associated with hypochlorhydria and increased risk for gastric cancer (17, 42, 45, 50). In contrast, chronic infection of the gastric antrum is associated with hyperacidity and duodenal ulcer disease (42, 45).

The main stimulants of acid secretion are 1) gastrin, released from antral G cells; 2) histamine, released from oxyntic enterochromaffin-like (ECL) cells; and 3) acetylcholine, released from antral and oxyntic intramural neurons (62). The main inhibitor of acid secretion is somatostatin (SST), released from antral and oxyntic D cells (62). Antral SST cells are anatomically and functionally coupled to G cells whereas oxyntic SST cells are anatomically and functionally coupled to ECL and parietal cells.

Only a minority of chronic H. pylori-infected individuals exhibit increased acid secretion. These patients are predisposed to develop duodenal ulcer disease, have antral predominant infection, demonstrate increased basal and stimulated gastrin and acid secretion, and manifest decreased antral SST concentration and increased antral gastrin concentration (18, 22, 26, 48). In the antrum, SST-containing D cells are closely coupled to gastrin-containing G cells and exert a tonic paracrine restraint on gastrin secretion (54, 62, 63). Removing this restraint, i.e., disinhibition, stimulates the secretion of gastrin, the main hormonal stimulant of acid secretion during meal ingestion. Gastrin stimulates the parietal cell directly and indirectly by releasing histamine from ECL cells (62). The precise mechanism by which H. pylori depletes SST concentration in the antrum of the stomach is not known, but some have speculated that release of proinflammatory cytokines may initiate the process (1, 2, 75). It should be noted, however, that most patients chronically infected with H. pylori manifest a pangastritis and produce less than normal amounts of acid (5, 16).

Successful initial colonization of the stomach is pivotal to the pathogenesis of H. pylori-associated disease. The bacterium must overcome innate defenses including gastric acidity. To facilitate this, acute H. pylori infection produces hypochlorhydria in humans (21, 25, 51). The precise pathway whereby acute infection inhibits acid secretion is not known, but studies have suggested that constituents and products of H. pylori (e.g., acid-inhibitory factor, lipopolysaccharide, vacuolating cytotoxin, ammonia/ammonium, and products of the cag pathogenicity island gene) as well as proinflammatory cytokines may act directly on parietal cells (20, 24, 29, 39, 40, 55, 57, 62). However, it is uncertain whether this explains how patchy acid secretion is impaired.
superficial infection of the oxyntic mucosa can induce acute hypochlorhydria in intact gastric tissues. In addition, water extracts of *H. pylori* applied to rat gastric mucosa inhibit gene expression of histamine decarboxylase, the enzyme responsible for generating histamine, but the mechanism has not been elucidated (40).

In oxyntic glands of the gastric body and fundus, SST cells are anatomically and functionally coupled to parietal and ECL cells. In this region, SST exerts a tonic paracrine restraint on acid secretion that is mediated directly as well as indirectly through inhibition of histamine secretion (59, 60, 62, 74). Since *H. pylori* colonizes predominantly the mucosal epithelial surface but parietal, ECL, and SST cells are located in the middle to lower portions of oxyntic glands (28, 38, 62), we postulate that activation of neural pathways by the bacterium may offer an explanation as to how initial superficial colonization can produce acute acid inhibition.

Animal models of *H. pylori*-induced disease have been established in various species including gerbils, monkeys, cats, guinea pigs, ferrets, pigs, mice, and rats (7). In the present study, we used rat oxyntic mucosal sheets mounted in an Ussing chamber and isolated rabbit oxyntic glands to identify pathways by which *H. pylori* may inhibit acid secretion. The rat closely mimics human physiology and has proven invaluable in elucidating the neural and paracrine pathways that regulate gastric endocrine secretion in health and disease (13, 40, 43, 63, 68, 71, 73). Likewise, isolated rabbit oxyntic glands have proven useful in examining the direct and paracrine regulation of acid secretion in response to secretagogues, inhibitors of secretion, and *H. pylori* (29, 36).

The present study demonstrates that products of *H. pylori* can acutely inhibit acid secretion directly and also indirectly by activating intramuscular calcitonin gene-related peptide (CGRP) sensory neurons in oxyntic mucosa that are coupled to stimulation of SST secretion. Activation of CGRP neurons offers an explanation as to how initial patchy superficial colonization of the stomach by *H. pylori* can induce acute hypochlorhydria in humans.

**MATERIALS AND METHODS**

*H. pylori* preparation. *H. pylori* strains 159, isolated from a patient with duodenal ulcer, and 49503, obtained from American Type Culture Collection (ATCC), were used. Both strains were analyzed by T. Cover (Vanderbilt University) as previously described (11, 12) and were determined to be positive for CagA by reverse-transcription polymerase chain reaction and vacuolating toxin by incubation of HeLa cells with supernatants from *H. pylori* broth filtrates. Bacteria were grown under microaerophilic conditions (10% CO2), as previously described (11, 12) and were harvested at the rate of 1 ml/min with Krebs bicarbonate solution at pH 7.4 containing 0.2% bovine serum albumin, 4% dextran, and 4.5 mM glucose. The luminal surface was perfused from the bottom of the chamber at the rate of 1 ml/min with sterile Mueller-Hinton broth (Difco Laboratories) at pH 7.4. The effluent from each hemichamber was collected separately at 5-min intervals via a catheter exiting the top of each chamber. The serosal perfusate was gassed with 95% O2:5% CO2 and the mucosal perfusate with 100% O2. Tetrodotoxin, capsaicin, CGRP8-37, and CGRP were delivered at the rate of 0.1 ml/min to the serosal surface and *H. pylori* in spent broth or spent broth alone was delivered to the luminal surface at the rate of 0.1 ml/min via a side arm close to each inlet. The entire preparation was contained within a Plexiglas chamber maintained at 37°C.

Isolated rabbit oxyntic glands. Oxyntic glands were isolated from rabbit fundus according to the methods of Chew and Berglindh (8). This preparation retains intact paracrine pathways but is devoid of neural pathways. The aorta was cannulated, heparin was injected, and the blood was drained. The celiac axis was perfused at 300 ml/min with oxygenated phosphate buffer. The stomach was excised and the fundic portion was removed by blunt dissection from the submucosa, minced with scissors, then treated with pronase (0.4 mg/ml; Roche Diagnostics, Indianapolis, IN) for 20 min followed by collagenase type I (0.8 mg/ml; Sigma Chemical) for 30–45 min. The suspension was gravity filtered through coarse nylon mesh. The glands were allowed to settle in 10-ml conical tubes, washed three times, and resuspended in medium containing (in mM) 132.4 NaCl, 5.4 KCl, 5.0 Na2HPO4, 1.0 NaH2PO4, 1.2 MgSO4, 1.0 CaCl2, 0.5 dithiothreitol, 10.0 glucose, 1.0 pyruvate, and 10.0 HEPES. The medium also included bovine serum albumin (2 mg/ml) and phenolsulphonphalein (10 mg/l).

Experimental design. For rat fundus in an Ussing chamber, a 30-min equilibration period was performed by an 80-min sampling period. The sampling period consisted of a 30-min control basal period, a 20-min period during which *H. pylori* in spent broth (103 to 106 cfu/ml) was perfused (1:1 to 1:100,000 dilution), or CGRP (1 nM) was perfused either alone or in combination with the axonal blocker tetrodotoxin (TTX; 5 mM; Sigma Chemical) or the selective CGRP antagonist CGRP8-37 (10 μM; Bachem, Torrance, CA) (15, 74), and a final 30-min control period. In other experiments, I) the sensory neurotoxin capsaicin (50 μM; Sigma Chemical) was perfused for 60 min before and during perfusion with *H. pylori* spent broth or 2) SST antibody (S775, 1:200 final dilution; a gift from Akira Arimura, Tulane University, Belle Chase, LA), previously shown to functionally block the effects of SST in this preparation (70, 71), was perfused for 30 min before and during perfusion with *H. pylori* spent broth. One-milliliter samples of the serosal superfusate were obtained at 5-min intervals and stored in 0.5-ml aliquots at −37°C for subsequent analysis.
For rabbit oxyntic glands, a 20-min equilibration period was followed by a 20-min period during which *H. pylori* spent broth at various dilutions (1:1,000,000 to 1:100 final dilution) was added either alone or in combination with histamine (50 μM; Sigma Chemical), TTX, and/or SST antibody (S775; 1:200 final dilution.) Acid secretion was calculated as the accumulation of the weak base aminopyrine in the glands.

Radioimmunoassay. SST concentration was measured in duplicate by radioimmunoassay as described in detail previously (58). SST antibody 1001 (final dilution 1:66,000) was a gift from Dr. Tadatada Yamada and Dr. John DelValle, University of Michigan. The limit of detection was 4 pg/ml of sample and the EC₅₀ was 58 ± 10 pg/ml of sample (mean ± SD; n = 8 assays). Interassay and intra-assay coefficients of variability were 11 and 8%, respectively.

Histamine concentration was measured in duplicate by use of a commercial radioimmunoassay kit (Aran, Westbrook, ME) as previously described (71). The kit includes tubes coated with monoclonal antibody against acylated histamine, acylating agent, and ¹²⁵I-histamine as tracer. The limit of detection was 0.1 nM histamine and the EC₅₀ was 8 ± 1 nM of sample (mean ± SD; n = 8 assays). Interassay and intra-assay coefficients of variability were 14 and 10%, respectively. The antibody reacts with CGRP but does not cross-react with amylase, adrenomedullin, calcitonin, histamine, or somatostatin.

Aminopyrine accumulation. Acid secretion was measured in triplicate by oxyntic gland accumulation of the ¹⁴C-labeled weak base aminopyrine. [¹⁴C]Aminopyrine (0.1 μCi; specific activity 80–90 μCi/mmol; New England Nuclear, Boston, MA) was added per 1 ml of rabbit oxyntic glands. After the 20-min experimental period, the glands were separated by centrifugation, radioactivity in the cell pellet was determined in a liquid scintillation counter, and aminopyrine accumulation was determined (3, 64). Aminopyrine ratio was measured by the radioactivity recovered in the pellet expressed as a percentage of the radioactivity present in the incubation medium (i.e., supernatant). The radioactivity accumulated in the pellet in the presence of 0.1 mM sodium thiocyanate was subtracted from all data to correct for trapped [¹⁴C]aminopyrine.

Data analysis. The SST and histamine responses to 1) CGRP and 2) *H. pylori* alone and in the presence of TTX, capsaicin, CGRP antagonist, and SST antibody were expressed as the mean increase or decrease from the preceding basal level during the 5 min immediately preceding the experimental period. Since similar results were obtained with use of *H. pylori* strains 159 and 49503, results were combined. Because concentrations of CGRP were at the lower limit of detection, CGRP concentration in each experiment was assayed by collecting the entire preceding basal effluent during the 20 min immediately preceding the experimental period and the entire 20-min experimental period in which *H. pylori* was perfused, lyophilizing the samples, and reconstituting them each in 1 ml of serumal superfusate. The CGRP response was expressed as the mean increase from the preceding basal level. The acid response to *H. pylori* in spent broth alone and in the presence of TTX and SST antibody was expressed as the mean increase or decrease in aminopyrine accumulation and as percent change from control basal or histamine-stimulated glands.

Changes in secretion were tested for significance by Student’s t-test for unpaired values and, as necessary, by ANOVA. All values are given as means ± SE of *n* experiments on different animals. Concentrations eliciting 50% of maximal response (EC₅₀) were calculated by sigmoid plot logistics (GraphPad Prism).

RESULTS

**Basal somatostatin, histamine, CGRP, and acid secretion.** Mean basal SST, histamine, and CGRP secretion in rat fundic sheets was reproducible between animals and reverted to initial control levels at the end of the experimental period (SST, 45.0 ± 5 and 41 ± 5 pg/min; histamine, 397 ± 35 and 434 ± 46 nmol/min; CGRP, 0.8 ± 0.4 and 1.1 ± 0.5 pg/min). Mean basal acid secretion in isolated rabbit oxyntic glands, measured as aminopyrine accumulation ratio, was 30 ± 2 and was reproducible between animals.

**Effect of *H. pylori* on somatostatin, histamine, and CGRP secretion in rat fundus.** Perfusion of the luminal aspect of rat fundic mucosal sheets mounted in an Ussing chamber for 20 min with *H. pylori* in spent broth (10⁵ to 10⁷ cfu/ml) elicited a concentration-dependent increase in SST and decrease in histamine secretion (Fig. 1). Maximal stimulation of SST secretion (199 ± 20% above basal level, *P* < 0.001, *n* = 10) and inhibition of histamine secretion (45 ± 5% below basal level,

![Fig. 1. Effect of *Helicobacter pylori* in spent broth (10⁵ to 10⁷ cfu/ml; left) and *H. pylori* spent broth alone (1:10⁵ to 1:10⁶ final dilution; right) on somatostatin (●) and histamine (○) secretion in rat fundic sheets mounted in an Ussing chamber. Data are mean responses ± SE during 20-min period of perfusion of 6–10 experiments each. Horizontal dashed line indicates level of basal secretion. *Significant difference from basal levels at *P* < 0.01.](http://ajpgi.physiology.org/doi/10.1152/ajpgi.00187.2012)
Perfusion of the luminal aspect of rat fundic mucosal sheets mounted in an Ussing chamber for 20 min with *H. pylori* spent broth (*H. pylori* bacteria removed; 1:10^5 to 1:10^6 final dilution) elicited a similar concentration-dependent increase in SST and decrease in histamine secretion (Fig. 1). Maximal stimulation of SST secretion (194 ± 9% above basal level, \( P < 0.001, \ n = 8 \)) and inhibition of histamine secretion (48 ± 2% below basal level, \( P < 0.01, \ n = 8 \)), expressed as the integrated 20-min response, was obtained with undiluted spent broth. Undiluted spent broth also elicited a reversible increase in CGRP secretion (156 ± 36% above basal level, \( P < 0.01, \ n = 6 \)). Because the spent broth alone elicited similar SST and histamine responses as the spent broth containing *H. pylori* bacteria, we used the spent broth alone in subsequent experiments.

Perfusion with *H. pylori* spent broth at 1:10 final dilution elicited a prompt, reversible increase in SST (153 ± 17% above basal level, \( P < 0.001, \ n = 8 \)) and decrease in histamine (40 ± 5% below basal level, \( P < 0.001, \ n = 8 \)) secretion (Fig. 2). Both responses were abolished by 1) the axonal blocker TTX (5 \( \mu M; \) Fig. 2), indicating that they were neurally mediated; 2) the sensory neurotoxin capsaicin (50 \( \mu M; \) Fig. 3), indicating that they were mediated by activation of sensory neurons; and 3) the CGRP antagonist, CGRP8-37 (0.1 \( \mu M; \) Fig. 3), indicating CGRP as the sensory transmitter. Consistent with this notion, CGRP (1 nM) elicited a prompt, reversible increase in SST (159 ± 9% above basal level, \( P < 0.001, \ n = 6 \)) and decrease in histamine (24 ± 3% below basal level, \( P < 0.01, \ n = 6 \)) secretion (Fig. 4) (69, 74).

To determine whether changes in histamine secretion were due to changes in SST secretion, experiments were performed in the presence of SST antibody (Fig. 5). SST antibody alone (1:200 dilution) for 30 min caused an increase in histamine secretion (24 ± 3% above basal level, \( P < 0.001, \ n = 8 \)), confirming previous studies showing that endogenous SST exerts an inhibitory paracrine influence on histamine secretion (70). SST antibody also abolished the decrease in histamine secretion, implying that the effect of *H. pylori* on histamine secretion was mediated by changes in SST secretion.

**Effect of *H. pylori* on basal and stimulated acid secretion in isolated rabbit oxyntic glands.** Addition of *H. pylori* spent broth (1:10^5 to 1:10^2 final dilution) inhibited basal acid secretion in a concentration-dependent manner (Fig. 6), implying that a product of *H. pylori* is capable of inhibiting acid secretion. At 1:100 dilution, basal acid secretion was inhibited by 40 ± 4% (\( P < 0.001, \ n = 10 \)). The response was not significantly affected by the axonal blocker TTX (5 \( \mu M; \ n = 8 \)) or SST antibody (1:200 final dilution, \( n = 8 \)), implying that the effect was not mediated by neural pathways or by stimulation of SST secretion (Fig. 6).

Histamine (50 nM to 0.5 mM) elicited a concentration-dependent increase in acid secretion. The \( EC_{50} \) value was 7 × 10^{-3} M, and maximal stimulation of acid secretion was obtained at a concentration of 50 \( \mu M \) (aminopyrine ratio = 128 ± 23 or 357 ± 14% above basal level; \( P < 0.001; \ n = 12 \)). Addition of *H. pylori* spent broth (1:10^6 to 1:10^2 final dilution) inhibited maximal histamine-stimulated acid secretion in a concentration-dependent manner (Fig. 6). At 1:100 dilution, histamine-stimulated acid secretion was inhibited by 30 ± 3% (\( P < 0.001, \ n = 10 \)).

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Fig. 2. Time course for the effect of *H. pylori* spent broth (1:10 dilution) alone and in combination with the axonal blocker tetrodotoxin (TTX; 5 \( \mu M \)) on basal somatostatin and histamine secretion in rat fundic sheets mounted in an Ussing chamber. Horizontal dashed line indicates level of basal secretion. Data are means ± SE of 8 experiments each.

Fig. 3. Time course for the effect of *H. pylori* spent broth (1:10 dilution) alone and in combination with the sensory neurotoxin capsaicin (50 \( \mu M; \) bottom) or the calcitonin gene-related peptide antagonist, CGRP8-37 (0.1 \( \mu M; \) top), on basal somatostatin and histamine secretion in rat fundic sheets mounted in an Ussing chamber. Horizontal dashed line indicates level of basal secretion. Data are means ± SE of 8 experiments each.
The present study examined the pathways by which acute infection with *H. pylori* inhibits acid secretion. We show, for the first time, that *H. pylori* activates neural pathways in oxyntic mucosa, specifically CGRP sensory neurons, that are coupled to stimulation of SST and thus inhibition of histamine secretion. We speculate that this pathway may be the dominant pathway by which acute infection with *H. pylori* produces the hypochlorhydria necessary for colonization and establishment of infection in humans.

*H. pylori*-induced hypochlorhydria is thought to be important in establishing initial colonization within the hostile environment of the stomach. Prior studies have focused on bacterial adherence to epithelial cells and direct interactions of bacterial virulence factors using isolated parietal cells and oxyntic glands, gastric epithelial cell lines, and oxyntic biopsies in short-term culture (6, 20, 29, 47, 55, 56). In isolated rabbit oxyntic glands, we show that *H. pylori* spent broth inhibits basal and histamine-stimulated acid secretion in a concentration-dependent manner. The responses were unaffected by the axonal blocker TTX or SST antibody, implying that a product of *H. pylori* contained in the broth is capable of inhibiting acid secretion directly, independent of neural pathways or changes in SST secretion (Fig. 6). The findings confirm and extend those previously reported in isolated rabbit, ferret, and human oxyntic glands as well as human biopsy specimens (6, 29, 34, 56). In rabbit oxyntic glands, a protein produced by *H. pylori*...
was reported to inhibit histamine-stimulated acid secretion (6). In isolated rabbit parietal cells and a human gastric epithelial cell line, H. pylori interfered with the transcription and translation of H^+/K^+-ATPase as well as its insertion into the parietal cell apical membrane (20, 55). Using the axonal blocker TTX and SST antibody, we provide additional evidence that the decrease in acid secretion induced by H. pylori in oxyntic glands occurs independent of neural pathways or release of SST.

Shortcomings of isolated cell and gland preparations as well as biopsies in culture are 1) lack of neural pathways, 2) loss of polarity and inability to distinguish whether H. pylori organisms and products gain access to their target cells from the luminal (or apical) or serosal (or basolateral) surface, and 3) inability to ascertain whether products of H. pylori can actually diffuse across the relatively large distance from the surface of oxyntic mucosa to reach the parietal cells, which are located in the middle and deeper regions of the glands. To overcome these obstacles and explore the existence of additional pathways, we performed experiments in rat gastric fundus (oxyntic glands) mounted in an Ussing chamber, a preparation that retains neural and paracrine pathways as well as polarity. Perfusion of the mucosal surface with H. pylori in spent broth or spent broth alone elicited a prompt concentration-dependent increase in SST and reciprocal decrease in histamine secretion (Figs. 1 and 2). Significant effects were observed at bacterial doses (10^3 to 10^6) that cause infection in rhesus monkeys and are estimated to be relevant in humans (35, 65). Changes in histamine secretion reflected changes in SST secretion. This was evident in experiments in which the influence of SST was eliminated by addition of SST antibody; under these conditions, H. pylori did not alter histamine secretion (Fig. 5). It should be noted that SST antibody by itself increased histamine secretion, consistent with previous studies demonstrating that endogenous SST exerts a tonic inhibitory influence on the secretion of histamine in this region of the stomach (9, 59, 62, 74). The SST and histamine responses to H. pylori were abolished by the axonal blocker TTX as well as the sensory neurotoxin capsaicin (Figs. 2, 3, and 7), implying that H. pylori activated sensory neurons (31, 33). Capsaicin, the pungent ingredient in red peppers, desensitizes afferent neurons through activation of specific receptors termed transient receptor potential ion channel of the vanilloid type 1 (TRPV1). In the stomach, only primary afferent neurons are sensitive to capsaicin, and pretreatment with a neurotoxic dose of capsaicin depletes afferent neuron-derived peptides from the stomach (32). The prime candidate for sensory transmitter is CGRP since 1) CGRP is present exclusively in capsaicin-sensitive sensory neurons innervating the gastric mucosa (23, 33, 52), 2) capsaicin pretreatment decreases gastric CGRP concentration (23, 33, 52), 3) CGRP is released from gastric sensory peripheral nerve endings upon activation of afferent neurons (19, 31), and 4) CGRP stimulates SST secretion in rat, mouse, dog, and human stomach (27, 37, 41, 74). Consistent with this notion, we show, in oxyntic mucosa mounted in an Ussing chamber, that 1) H. pylori increases CGRP secretion, 2) CGRP stimulates SST and inhibits histamine secretion (Fig. 4), and, most importantly, 3) both the SST and histamine responses induced by H. pylori are abolished by the selective CGRP antagonist, CGRP8-37 (Figs. 3 and 7) (69, 74).

The fact that similar changes in SST and histamine secretion were obtained when perfusing H. pylori in spent broth or spent broth alone implies that a product secreted by the bacterium activated CGRP neurons. Further work is needed to identify the precise component or product responsible for activation of sensory neurons. It should be noted, however, that we observed significant changes in SST (and histamine) secretion as early as 5 min (Fig. 2). H. pylori lipopolysaccharide inhibits acid secretion in gastric fistula-equipped animals and pylorus-ligated conscious rats, but only after 4 h (4, 49). Although proinflammatory cytokines can influence gastric SST secretion, the time course may be too fast for cytokine secretion and synthesis to take place. For example, in a mouse model infected with H. pylori, the first signs of gastric inflammation occurred 3 wk after inoculation (44). In AGS cells, VacA induced the production of proinflammatory cytokines (TNF-α, IL-8, IL-6, and IL-1β), but not until 4 h (66). H. pylori urease and deamidases hydrolyze urea to produce NH₃ (ammonia), which is rapidly converted to NH₄⁺ (ammonium) in the acidic lumen of the stomach. NH₃ has been proposed to facilitate initial colonization by buffering luminal acid and creating a thin neutral layer around the bacterium. It has been estimated, however, that the amount of ammonia produced by the bacterium accounts for the neutralization of only 5–7% of stimulated acid secretion (14, 24, 72).

During acute infection, H. pylori resides mainly within the mucus layer on the surface of gastric mucosa and perhaps also in the upper portion of oxyntic glands. The relatively high intraglandular pressures (~18 mmHg) generated during secretion and the antibacterial activity of mucins in the deeper regions of the glands are thought to impede passage of H. pylori and its products downward (30, 38). Since H. pylori is present in the upper regions of gastric mucosa whereas parietal, SST, and ECL cells are located in the middle and lower regions, we speculate that activation of CGRP sensory neurons may offer an explanation as to how initial patchy superficial colonization of the stomach can induce acute hypochlorhydria. It also offers an explanation as to why water extracts of H.
**H. PYLORI AND GASTRIC SECRETION**

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**pylori** applied luminally suppress the expression of histidine decarboxylase and reduce histamine content in rat gastric mucosa (40).

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


