H. pylori stimulates gastrin release from canine antral cells in primary culture

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Lehmann, Frank S., Neal Schiller, Timothy Cover, Ritchard Hatch, Rein Seensalai, Kimitoshi Kato, John H. Walsh, and Andrew H. Soll H. pylori stimulates gastrin release from canine antral cells in primary culture. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G992–G996, 1998.—Patients chronically infected with Helicobacter pylori are known to have hypergastrinemia. Previous studies have demonstrated the stimulation of gastrin from isolated G cells by monocytes and cytokines. The aim of this study was to determine if H. pylori can directly stimulate gastrin secretion. The secretion of gastrin from canine G cells in 48-h primary cultures was investigated using either live H. pylori bacteria or various bacterial extracts from three well-characterized strains. Whole bacterial sonic extracts and water-extracted surface proteins, but not PBS extracts, from strains 43579 (CagA+/VacA–), 60190 (CagA+/VacA–), and 60190:v1 (CagA+/VacA–) significantly stimulated gastrin release. Controls demonstrated that gastrin stimulation by the sonic extracts was not due to a direct toxic effect on G cells. We conclude that H. pylori produces a soluble factor(s), which can directly stimulate gastrin release in enriched canine G cell cultures. This stimulatory effect may play an important role in the H. pylori-associated hypergastrinemia and subsequent development of peptic ulcer disease.

Helicobacter; extracts; G cells

Helicobacter pylori (HP) is the causative agent of chronic superficial gastritis in humans, and infection with this organism is a major factor contributing to the pathogenesis of peptic ulcer disease (3). The mechanism by which HP predisposes the duodenum to ulceration is currently unknown. One attractive hypothesis is that the infection stimulates increased release of gastrin, which in turn induces acid secretion, and that this is the excessive duodenal acid load that causes ulceration (19). Numerous studies have confirmed that duodenal ulcer patients as well as asymptomatic subjects infected with HP have increased plasma gastrin concentrations in the basal state (18) as well as after stimulation by a meal (11), bombesin (12), or gastrin-releasing peptide (2). Eradication of the infection results in a marked fall in basal and meal-stimulated gastrin release (11).

The mechanism(s) by which HP affects gastric endocrine cells is unclear. It has been suggested that products from the bacterium itself, inflammatory cells or cell products, or a combination of these might influence endocrine cell function (10). Previously we reported the stimulation of gastrin release from cultured canine G cells by monocytes and tumor necrosis factor-α (TNF-α) (15, 16). The stimulatory effect of TNF-α has now been confirmed in different G cell preparations (1, 26). However, a direct effect of HP on gastrin release has not been described. Using enriched canine G cell primary cultures, we examined the effect of three well-characterized strains of HP on gastrin release. G cells were incubated with either actively motile exponential-phase cultures or bacterial extracts.

METHODS

Cell separation, enrichment, and culture. Canine G cells were prepared and cultured from adult mongrel dogs as previously described in detail (25). Briefly, the antral mucosa tissue was separated from the submucosa and minced, and cells were dispersed by sequential incubation with collagenase and EDTA. Dispersed cells were washed, collected by centrifugation, and filtered through nylon mesh. The cells were separated by velocity sedimentation using a Beckman elutriator. Cell fractions that have been shown to contain maximal gastrin immunoreactivity (25) were collected, and elutriated cells were centrifuged and resuspended in DMEM–F12, supplemented with 2% newborn calf serum, 100 µg/ml amikacin, 8 µg/ml insulin, 2 mM glutamine, and 0.1 µg/ml hydrocortisone. Cells were plated on Matrigel (Collaborative Research, Waltham, MA)-coated 24-well plates at a concentration of 1 × 10^6 cells/well and incubated for 2 days in a humidified atmosphere of 5% CO₂–95% air at 37°C.

Gastrin release. All release studies were performed using mucosal antral cells maintained in short-term culture, which resulted in an enrichment of G cells to 8–12% of the viable cell population (9). Somatostatin cells accounted for ~1.5% and mucous cells for the remainder. Before release studies, each multiwell plate was washed twice with release medium (9). Monoclonal somatostatin antibody CURE S6 (10^–7 M) was included in all experiments to rule out any indirect effect on gastrin stimulation from somatostatin cells. S6 antagonizes the effects of exogenously administered and endogenously released somatostatin in the stomach (27). Somatostatin content in the G cell preparations was <5 pmol/10^6 cells (9), which is blocked by S6 at a concentration of 10^–7 M (27). After the plates were incubated for 2 h at 37°C in 5% CO₂–95% air with either control DMEM culture medium, live HP bacteria, or various bacterial extracts, the G cell supernatants were collected and the cells were detached from the base of unstimulated wells to determine the cell content of gastrin (16). All release studies were performed in triplicate. Three wells on each plate incubated without stimulant indicated basal release, and three wells received 10^–11 M bombesin as a positive control for gastrin release. The percentage of gastrin release was then calculated by dividing the amount of gastrin in the test supernatant by the amount remaining in the adherent cells (9).

Gastrin assay. Gastrinlike immunoreactivity was measured by radioimmunoassay as described previously, using antiserum 1611 in a final titer of 1:80,000 and tracer prepared by iodination of gastrin-17 by the chloramine-T method. This assay recognizes all COOH-terminal fragments of gastrin.
longer than four residues but does not detect glycine-
extended gastrin variants (25).

HP strains. Three well-characterized strains were used for
these studies: 1) American Type Culture Collection (ATCC)
strain 43579 (14), 2) strain 60190 (17), which is also known as
ATCC 49503, and 3) strain 60190:v1, a cytotoxin-deficient
derivative of 60190 (7). Strains 43579 and 60190 were initially
isolated from human gastric biopsies and are positive for
CagA and vacuolating toxin, whereas 60190:v1 is an isogenic
mutant in which the VacA gene has been disrupted by
insertion of a kanamycin resistance gene (7).

Expression of CagA strain 43579 was assessed by immu
noblots of whole cells, using anti-CagA antiserum as de
scribed previously (6). The vacuolating cytotoxic activity of
strain 43579 was evaluated by incubating HP culture super
natants with HeLa cells for 18 h and monitoring cell vacula
tion with the neutral red uptake assay, as described previ
ously (4). Exponential-phase broth cultures of each strain
were prepared by growth in Brucella broth (Difco, Detroit,
MI) containing 5% heat-inactivated FCS and 1% IsoVitaleX
(Becton-Dickinson Microbiology, Cockeysville, MD) at 37°C in
a humidified 12% CO2 incubator. Previous growth curves,
assessed by phase-contrast microscopy, optical density, and
bacterial colony determination, have demonstrated that expo
nential phase growth occurs at about 36 h when cells are
incubated in the culture medium described above. The optical
density of the cultures before use was determined to be 620
nm, and the organisms were centrifuged and resuspended in
DMEM at a concentration of 107 bacteria/well. The highest
0.05, statistically significant. When tested at 106
and 105 bacteria/ml, no stimulatory effect was induced
by any of the strains.

HP sonic extracts. Incubation of canine G cells with
whole cell sonic extracts obtained from 48-h plate-
grown cultures of each strain significantly enhanced
gastrin release (P < 0.01, n = 5). There was a modest
but still significant increase induced by 72-h broth
cultures from strain 43579 but not the other two strains
(Fig. 2). Strain 43579 stimulated gastrin release by
53% (48 h) and 37% (72 h) above basal values, strain
60190 stimulated gastrin release by 32% (48 h) and
18% (72 h) above basal values, and strain 60190:v1
stimulated gastrin release by 50% (48 h) and 3% (72 h)
above basal values. The protein concentration of all
sonic extracts was 0.3 ± 0.1 mg/ml (48 h) and 1 ± 0.2
mg/ml (72 h).

Water-extracted surface components. Extracts from
each strain prepared by vigorous vortexing in double-
distilled water were able to cause significant (P < 0.01,
n = 5) stimulation of gastrin release, regardless of
whether the bacteria were cultured for 48 or 72 h (Fig.
1).
This was all the more impressive since the preparations contained less than 10 µg of protein/ml. Strain 43579 stimulated gastrin release by 41% (48 h) and 25% (72 h), strain 60190 by 41% (48 h) and 53% (72 h), and strain 60190:v1 by 33% (48 h) and 49% (72 h) above basal values. The bacteria remained viable after water extraction as determined by phase-contrast microscopy and colony-forming unit determination. PBS extracts. There was no effect on gastrin release when PBS extracts prepared from 48- or 72-h cultures from any of the three strains were added to the monolayers (0.7 ± 0.1% basal vs. 0.7 ± 0.1 with PBS extracts, n = 5). The protein concentration of the PBS extracts was <10 µg/ml (48 h and 72 h).

Responsiveness of G cells after release experiments. As a control for confirming cell viability after treatment with sonic extracts, the responsiveness of the treated G cells to bombesin stimulation was determined. Previously unstimulated G cells showed a 1.5-fold increase of gastrin release after bombesin stimulation, similar to cells that had been previously exposed to HP sonic extracts (n = 3; Fig. 4).

DISCUSSION

We report here for the first time that live HP bacteria or bacterial extracts can directly stimulate the release of gastrin from canine antral G cells. These experiments demonstrate that HP produces a soluble factor(s), probably surface exposed, which can cause G cell cultures to release enhanced levels of gastrin. This observation is consistent with the hypothesis that HP can directly contribute to the damage observed in gastroduodenal disease.

It is now well established that duodenal ulcer patients chronically infected with HP have increased basal and stimulated gastrin release. Circulating gastrin may be responsible for driving basal acid secretion, which is increased in duodenal ulcer patients (19). Recently, it was demonstrated that the fall in gastrin after HP eradication was accompanied by a proportional fall in basal acid secretion (22). Reversion of hypergastrinemia and increased basal secretion may contribute to the duodenal ulcer remission after HP eradication.

The presence of HP in the gastric antrum is associated with mucosal inflammatory cells such as neutrophils, lymphocytes, monocytes/macrophages, and plasma cells (3, 20). HP secretes a potent chemotactic factor, possibly urease, for mononuclear and polymorphonuclear inflammatory cells and stimulates monocytes to release several cytokines, including TNF-α (8, 13). In a previ-
ous study we demonstrated gastrin secretion in isolated G cells by stimulated monocytes and TNF-α (16). The inflammatory response induced by HP may in turn stimulate monocytes and TNF-α secretion, which may have an additional impact on G cell function.

Water-extracted surface proteins stimulated gastrin release in our experiments. Mai et al. (20) found that this extraction procedure had essentially no contamination by lipopolysaccharide. Because these proteins are shed from the bacterial cell, this material may be similar to that released from HP in vivo (20). HP usually does not appear to invade the mucosa, and therefore the release of soluble proteins could be an important factor in stimulating gastrin release. Our observation that actively motile intact HP stimulate gastrin release is consistent with this hypothesis.

We examined bacterial extracts at two different time points to determine whether the bacterial gastrin stimulatory factor(s) was produced primarily in the midexponential (48 h) or early stationary (72 h) phase. Although the water extracts were equally potent in gastrin stimulation using either 48- or 72-h cultures, the sonic extracts prepared at 48 h were more effective than those prepared at 72 h. Although the explanation for this remains unknown, one possibility is that cytoplasmic or periplasmic proteases produced during later growth stages may be released by sonication and may alter the gastrin stimulatory activity. Additional studies are planned that will characterize the gastrin-stimulating factor(s) present in water-extracted surface proteins from these HP strains.

Two major phenotypic characteristics known to differ among HP strains are production of a vacuolating cytotoxin (5) and the presence of a cytotoxin-associated protein encoded by CagA (6). These two related phenotypes are known to be potentially important virulence factors that may affect the clinical outcome of HP infection (7). The mechanisms whereby CagA or vacuolating toxin could be related to the pathogenesis of ulcer disease are unknown. Restimulation of G cells with bombesin after treatment with sonic extracts demonstrated that these monolayers were still viable and intact, indicating that stimulation of gastrin release occurred without cell damage by enzymes or cytotoxic activity. In addition, disruption of the VacA gene did not influence the stimulatory effects of sonicates and water extracts on gastrin release in our experiments, suggesting that the importance of this cytotoxin as a virulence factor is unrelated to its potential to stimulate gastrin release.

Our whole organism studies provided only modest increases of gastrin stimulation over baseline at the highest level of bacteria we used (10⁷/ml) for the two VacA⁺ strains, whereas at lower concentrations no stimulatory effect was induced by any of the strains. HP live cells of strain 60190:v1 also induced gastrin release, although the results were not statistically significant. It is probable that at higher concentrations of the VacA-deficient strain, we would have seen significant levels of gastrin secretion. A dose-response curve with higher concentrations was not performed, because increasing bacterial numbers to 10⁹/ml or more might be much greater than physiologically reasonable.

Eradication of HP in infected patients results in increased synthesis and release of somatostatin (23). It has been suggested that suppression of somatostatin might explain the increased gastrin release in HP-infected patients (23). However, the stimulatory effect on gastrin release by HP observed in this study occurred in the presence of the somatostatin antibody S6, suggesting that HP causes a direct effect on G cell-mediated gastrin release. The stimulatory effect on gastrin release by mononuclear cells and cytokines has also been observed with S6 (1, 16). However, these observations do not exclude an additional, independent effect on somatostatin release.

We have provided evidence that three HP strains, incubated with canine G cell primary cultures as either actively motile bacteria, soluble sonic cell extracts, or water-extracted surface proteins, stimulate gastrin release. HP and cytokines may both play an important role in HP-induced hypergastrinemia.

REFERENCES


