H. pylori and transient lower esophageal sphincter relaxations induced by gastric distension in healthy humans

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Zerbib, Frank, Valérie Bichéler, Véronique Leray, Madeleine Joubert, Stanislas Bruley des Varannes, and Jean-Paul Galmiche. H. pylori and transient lower esophageal sphincter relaxations induced by gastric distension in healthy humans. Am J Physiol Gastrointest Liver Physiol 281: G350–G356, 2001.—The role of Helicobacter pylori infection in the control of lower esophageal sphincter (LES) motility, especially the occurrence of transient LES relaxations (TLESRs), was studied in eight H. pylori-positive and eight H. pylori-negative healthy subjects. During endoscopy, biopsy specimens were taken from the cardia, fundus, and antrum for determinations of H. pylori status, gastritis, and proinflammatory cytokine mucosal concentrations. LES motility was monitored during three different 30-min periods: baseline, gastric distension (barostat), and gastric distension with CCK infusion. Gastric distension significantly increased the TLESR rate, whereas CCK increased the rate of distension-induced TLESRs further and reduced resting LES pressure without significant differences between infected and noninfected subjects. H. pylori status did not influence resting LES pressure or gastric compliance. Cytokine mucosal concentrations were increased in infected patients, but no correlation was found with the TLESR rate, which was also independent of inflammation at the cardia, fundus, and antrum. These results suggest that H. pylori-associated inflammation does not affect the motor events involved in the pathogenesis of gastroesophageal reflux.

Gastritis; barostat; cytokines; cholecystokinin

HELCOBACTER PYLORI infection is widely recognized as the major etiologic factor in peptic ulcer disease, although its precise role in the pathogenesis of gastroesophageal reflux disease (GERD) is still debatable (25). Epidemiological studies have failed to show any correlation between H. pylori infection and GERD, indicating that these bacteria are as prevalent (19, 29) or even at a lower level (34, 35) in patients as in healthy subjects. With respect to the pathophysiology of GERD, most studies have focused on the complex interactions between H. pylori and gastric acid secretion (12, 13, 18). Nonetheless, GERD is a primary motility disorder in which impaired lower esophageal sphincter (LES) function plays a crucial role (16). In both healthy subjects and patients with GERD, reflux episodes result from transient LES relaxations (TLESRs) rather than permanently low resting LES pressure (LESP) (9, 11, 31). TLESRs are thought to be neurally mediated through involvement of a vago-vagal reflex (27) because they are triggered by gastric distension through stimulation of gastric mechanoreceptors located mainly in the subcardiac area (15). No data are currently available on the interaction between H. pylori infection and LES motility, especially TLESRs.

From a theoretical standpoint, H. pylori-associated gastritis may affect LES motility through indirect mechanisms such as a lowering of the threshold for TLESR triggering resulting from a putative influence on vagal afferent pathways, especially in the proximal stomach. H. pylori infection may also affect LES motility directly through inflammation at the gastroesophageal junction (“carditis”), a condition very similar to H. pylori-associated antral gastritis (17). Many animal studies showed that mucosal inflammation alters gastrointestinal motor and sensory functions, and some pointed to the role of locally produced cytokines such as interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α (7). Because the gastric mucosal levels of these proinflammatory cytokines are increased during H. pylori infection (1, 8, 30), similar mechanisms may be involved, with potential effects on motility or sensory functions.

The present study was designed to determine the influence of H. pylori status, gastritis, and cytokines released by H. pylori-associated inflammation on LES motility, especially TLESRs, in healthy humans. Motility studies were performed using a model of gastric distension-induced TLESRs that has been widely demonstrated to be appropriate for physiological (21) as well as pharmacological (3, 5, 20) studies in humans. Two different stimuli were used to trigger TLESRs, gastric distension alone (21) and gastric distension in combination with sulfated CCK-8 (CCK-8S) infusion, which was recently reported to increase TLESR rate (5).

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MATERIALS AND METHODS

Subjects

Sixteen healthy subjects (5 women and 11 men; 20–29 yr old) who had given their informed written consent to the protocol were studied. No subjects were taking medications or had a history of gastrointestinal (GI) symptoms or prior abdominal surgery except appendectomy. The study protocol had been previously approved by the local Human Ethics Committee (Comité Consultatif pour la Protection des Personnes dans la Recherche Biomédicale No. 2, Région des Pays de Loire, France).

Study Design

All subjects underwent upper GI endoscopy during which nine biopsy specimens were obtained from the cardia (2 cm below the Z line; n = 3), the fundus (n = 3), and the antrum (n = 3). For each site, two specimens were used for histological assessment (gastritis score and H. pylori infection) and one for mucosal cytokine measurements. In addition, blood samples were obtained for H. pylori serology (Pyloriset EIA-G; Orion Diagnostica, Espoo, Finland). Eight subjects had negative serology and histology for H. pylori (H. pylori negative), seven had positive serology and histology, and one had positive serology and negative histology. Therefore, eight subjects were considered to be H. pylori positive.

Each subject fasted for at least 8 h before the study. A folded barostat bag was then introduced into the stomach through the mouth, and an esophageal motility catheter was subsequently inserted through the nose. Saline infusion was administered during the study. After 15 min for stabilization, LES motility (i.e., resting LESP and TLESRs) was monitored for a first 30-min baseline period. Graded isobaric gastric distension was then performed by means of the gastric barostat to determine a constant pain threshold pressure. Therefore, gastric distension was performed at a constant intragastric pressure defined as 75% of the pain threshold pressure. The effect of gastric distension alone on LES motility was studied first during a 30-min period before CCK-8S (sincalide, Kinevac; Bracco Diagnostics, Princeton, NJ) was infused during another 30-min distension period. Between the two distension periods, a 30-min resting period was observed during which the bag was entirely deflated. Infusion of CCK-8S at a dose of 30 ng·kg⁻¹·h⁻¹ was started 10 min before the distension period (5).

Data were analyzed during three periods: baseline, gastric distension alone, and gastric distension with CCK. Because our purpose was to apply an increasing stimulus for TLESR triggering (and not to compare gastric distension alone with distension plus CCK), the order of the two distension periods was not randomized. However, CCK and saline were administered in a blinded manner to subjects during the distension periods.

Esophageal Motility

Our technique of esophageal motility recording has been described completely elsewhere (36). A standard motility catheter fitted with a 6-cm sleeve (Dentsleeve, Parkside, Australia) was used to monitor LES and esophageal pressures. The assembly was introduced through a nostril, swallowed, and positioned so that pressures could be recorded from the LES (sleeve), fundus (2 cm below the sleeve), esophageal body (side holes 5, 10, and 15 cm proximal to the sleeve), and pharynx (side hole 28 cm proximal to the sleeve to detect swallowing). The catheter was infused at 0.5 ml/min using a low-compliance hydraulic capillary infusion system (Arndorfer Medical Specialties, Milwaukee, WI) driven by a pressure head of nitrogen. The infusion system was connected to pressure transducers. Signals were recorded on a polygraph (Synectics Medical, Stockholm, Sweden), digitized, computer processed, and stored using commercially available software (Polygram, Synectics Medical). All esophageal recordings were performed with the subjects in a sitting position.

Gastric Barostat

The electronic barostat (INRA, Toulouse, France) measures air volume or pressure within an intragastric bag maintained by a feedback mechanism at a constant preselected pressure or volume level. When the stomach contracts, the barostat aspirates air to maintain a constant pressure (or volume) within the bag; when the stomach relaxes, air is injected. Thus the barostat was used to produce isobaric distension, with measurement of intragastric volume at graded distending pressure levels. The barostat consisted of a strain gauge linked by an electronic relay to an air-injection system. The polyethylene bag used was highly compliant and had an approximately spherical shape, with a diameter of ~18 cm and a capacity of 1,000 ml. It was connected to a strain gauge and the injection system by a single-lumen polyvinyl tube (16 F).

The folded barostat bag was introduced through the mouth into the stomach. Three hundred milliliters of air were injected via a syringe to unfold the bag, which was then connected to the barostat and completely deflated. For determinations of pain threshold pressure, intragastric bag pressure was inflated in 2-mmHg increments until the constant pain threshold was reached (maximum pressure 20 mmHg). Distension steps, which lasted 2 min, were separated by 1-min resting periods (the bag being entirely deflated). Bag volume was recorded on a potentiometer running at 10 mm/min (Servotrace; IPE Sefram, Paris, France), which allowed assessment of the pressure-volume relationship. The effect of gastric distension on LES motility, either alone or with CCK infusion, was studied through gastric distension performed at a constant intragastric pressure defined as 75% of pain threshold pressure (5). During these periods, bag volume was continuously monitored.

Histology

For histopathological assessment, biopsy specimens were stained with hematoxylin and eosin and modified Giemsa. H. pylori infection was diagnosed when the bacterium was present at histopathological examination. The biopsy specimens were studied and graded for the following parameters according to the updated Sydney system (10): activity (polymorphonuclear cell infiltration), chronic inflammation (mononuclear cell infiltration), glandular atrophy, intestinal metaplasia, and presence of lymphoid follicles. Each parameter was graded semiquantitatively according to the following scale: 0 (absent), 1 (mild), 2 (moderate), and 3 (marked). Gastritis was defined as the presence of inflammation or activity (score ≥1).

Cytokine Assays

For cytokine measurements, biopsy specimens were placed in 2 ml of normal saline and frozen at ~80°C until use. Samples were homogenized using a tissue homogenizer, and aliquots of supernatants obtained by centrifugation (10,000 g for 10 min) were assayed for total protein by a modified Lowry method and frozen at ~80°C until assay. The mean concentration of protein was 0.244 mg/ml (range 0.151–0.747 mg/ml).
IL-1β, TNF-α, IL-6, and IL-8 levels in biopsy homogenate supernatants were measured by ELISA using commercially available assay kits (Pelikine compact human ELISA kit; CLB, Amsterdam, The Netherlands). Assays were performed in duplicate according to the manufacturer's instructions. The sensitivity of each test was 0.2 (IL-6), 1 (IL-8), 1 (TNF-α), and 0.8 (IL-1β) pg/ml.

Data Analysis

LES motility. One investigator (F. Zerbib) analyzed all recordings. As previously described (36), resting LESP was measured every 3 min at end expiration relative to gastric pressure, averaged over 15-min intervals, and expressed in millimeters of mercury. TLESRs were defined according to Holloway et al. (22) as 1) absence of a pharyngeal swallow signal for 4 s before and 2 s after the onset of LES relaxation, 2) decrease in resting LESP of >1 mmHg/s, 3) time from onset to complete relaxation of ≤10 s, 4) nadir pressure of ≤2 mmHg, and 5) decrease in resting LESP to ≤2 mmHg for >10 s (excluding multiple rapid swallows).

Barostat bag volume. Bag volume was monitored for each pressure step during progressive gastric distension and during the two periods with continuous gastric distension. For each period, the volume was measured manually every minute and averaged over the 30-min period to determine mean volume.

Statistics

Results are indicated as means ± SE. Data were compared using Student's t-test for paired values, factorial ANOVA, and the Mann-Whitney U-test. A P value <0.05 was considered significant.

RESULTS

Histology

In the H. pylori-negative group, all subjects had normal gastric mucosa except one with mild inflammation in the antrum and fundus. The results of histology in the H. pylori-positive group are indicated in Fig. 1. In this group, gastritis was observed in all subjects but one. Gastritis was present in six subjects at the cardia (carditis) and the fundus and in seven subjects in the antrum. No intestinal metaplasia was observed, whereas atrophy was present in the antrum in three subjects. Lymphoid follicles were present in one, two, and six subjects at the cardia, fundus, and antrum, respectively.

Mucosal Cytokines

The mucosal concentrations of proinflammatory cytokines are reported in Fig. 2. Regardless of the area considered, mucosal concentrations of cytokines were dramatically higher in H. pylori-positive than in H. pylori-negative biopsy specimens, although the difference was not always statistically significant.

Gastric Barostat

The pain threshold pressure determined during graded intermittent gastric distension was similar in H. pylori-negative and -positive groups (Table 1). Moreover, because the pressure-volume relationship (reflecting gastric compliance) was similar in the two groups (Fig. 3), the distension pressure chosen for continuous gastric distension was also similar (Table 1).

During continuous gastric distension, CCK-8S infusion significantly increased mean intragastric bag volume in the 16 subjects (659 ± 42 vs. 545 ± 43 ml; P = 0.0001). Intragastric volumes during the two gastric distension periods in H. pylori-negative and -positive subjects are indicated in Table 1. CCK-8S infusion significantly increased bag volume in both groups (no significant between-group difference).

LES Motility

During the baseline period, resting LESP and TLESR rate were not significantly different between H. pylori-negative and -positive subjects (Figs. 4 and 5). Similarly, neither LESP nor TLESR rate was affected by gastritis during baseline.

The effects of gastric distension on LES motility in the 16 subjects are reported in Figs. 4 and 5. Compared with the baseline period, resting LESP was not affected by gastric distension alone, whereas it was significantly decreased by distension combined with CCK infusion (20.7 ± 1.5, 22.4 ± 1.4, and 16.6 ± 1.0 mmHg, respectively).
respectively; $P = 0.0001$; Fig. 3). Gastric distension significantly increased the TLESR rate (3.9 ± 0.3 vs. 1.2 ± 0.3/30 min; $P = 0.0001$). Moreover, CCK infusion further increased the TLESR rate (5.8 ± 0.4 vs. 3.9 ± 0.3/30 min; $P = 0.0001$; Fig. 5).

When *H. pylori* status was considered, the effects of gastric distension alone and with CCK infusion in each subgroup of subjects were similar to those described for the whole group, but no significant difference was observed between *H. pylori*-negative and -positive subjects in terms of resting LESP (Fig. 4) or TLESR rate (Fig. 5).

TLESR rates relative to gastritis in the antrum, fundus, and cardia are reported in Table 2. The TLESR rate was increased by gastric distension alone and with CCK infusion but not by gastritis, regardless of the gastric area considered.

As indicated in Table 3, TLESR rates were not correlated with mucosal concentrations of cytokines, regardless of the period considered. Similarly, mucosal concentrations of cytokines were not correlated with resting LESP (data not shown).

**DISCUSSION**

Our results show that the TLESR rate induced by gastric distension alone and with CCK was not affected by *H. pylori* infection in healthy subjects. Moreover, no correlation was found between mucosal levels of proinflam-
-inflammatory cytokines and the TLESR rate. Finally, gastric compliance and sensitivity to gastric distension were not affected by *H. pylori* status or inflammation (including carditis).

The interaction between *H. pylori* infection and the mechanisms causing gastroesophageal reflux is complex and poorly understood. Until recently, *H. pylori* was considered to be either an innocent bystander, an aggravating agent, or a protective factor (25). *H. pylori* eradication may increase gastric acidity and therefore increase gastroesophageal reflux (14) as well as reflux esophagitis, at least in duodenal ulcer patients (24). Even though the pathogenesis of GERD always includes an attack on esophageal mucosa by acid and pepsin present in reflux material, many other factors are involved, and it is widely established that GERD is primarily a motility disorder caused by an incompetent antireflux barrier (16). In healthy subjects, as well as in the great majority of patients with GERD, most reflux episodes occur during TLESRs (9, 11, 31).

Because no data were available, we investigated LES function, especially TLESR occurrence, in infected and noninfected healthy subjects. Two different stimuli were used to trigger TLESRs, gastric distension alone (21) and gastric distension in combination with CCK-8S infusion, which was recently reported to increase the TLESR rate (5). Gastric distension-induced TLESRs are thought to be neurally mediated by the involvement of a vago-vagal reflex (27) due to stimulation of gastric mechanoreceptors (15). Exogenous CCK increased the TLESR rate induced by gastric distension in dogs (4) and healthy human subjects (5), probably through activation of CCK-A receptors located on peripheral vagal afferent fibers (2, 4). In our study, this model performed well because 1) gastric distension significantly increased the TLESR rate compared with baseline, 2) gastric distension with CCK infusion further increased the TLESR rate compared with gastric distension alone, and 3) resting LESP was significantly increased.
decreased by CCK but not by gastric distension alone. However, the same pattern of results was observed irrespective of *H. pylori* status.

Our study rules out several of the mechanisms possibly involved in interaction between *H. pylori* infection and LES function. First, it has been suggested that *H. pylori* could affect gastric wall compliance and therefore increase the gastroesophageal pressure gradient (25). However, our study confirms a previous report (32) indicating that both proximal gastric compliance and pain threshold pressure, as determined during graded intermittent gastric distension, are similar in *H. pylori*-positive and -negative subjects. Gastric distension volumes have also been shown to correlate with the number of TLESRs (5). As expected (5, 33), CCK infusion increased barostat bag volume during continuous gastric distension, but this increase and that in the TLESR rate were similar for the two groups. Second, it has been presumed that gastritis is a factor in LESP reduction through the release of numerous inflammatory mediators, including cytokines (25). Our results show that resting LESP was not affected by gastritis, especially at the gastroesophageal junction (carditis). Moreover, gastritis was associated with enhanced mucosal levels of cytokines but showed no correlation with LES motility. Third, we hypothesized that gastric distension-induced TLESRs may be increased through sensitization ofafferent fibers by mucosal inflammation. However, the TLESR rate in our study was not affected by gastritis, especially in the proximal stomach (cardia and fundus), where mechanoreceptors involved in TLESR triggering are mainly located. Similarly, the role of mucosal cytokines has been suspected because IL-1β was shown to interact with gastric vagal nerve activity in anesthetized rats (6). In fact, IL-1β, when administered intravenously, sensitizes the response of the gastric vagal afferent to CCK (6), partly via stimulation of CCK-A receptors (23). However, in our study, the rate of distension-induced TLESRs was not correlated with mucosal cytokine levels, even when exogenous CCK was superadded to gastric distension. This apparent discrepancy may be explained by the fact that the levels of cytokines released in *H. pylori*-associated gastritis are not high enough to reproduce the pharmacological effects of cytokines administered systemically. Indeed, *H. pylori*-induced inflammation of gastric mucosa is usually considered to be mild, especially in healthy subjects.

An important issue to consider is whether our results, obtained in asymptomatic subjects, could be extrapolated to GERD patients. In fact, TLESRs represent the main mechanism underlying gastroesophageal reflux in healthy subjects as well as in patients with GERD, and many studies on either pathophysiology (5, 9, 20, 36) or pharmacology (3, 26, 28) of TLESRs have been conducted in healthy subjects. Moreover, our model (i.e., the study of TLESRs in healthy volunteers) offers the advantage of specifically addressing the role of *H. pylori*-associated inflammation without any interference with other factors potentially involved in a multifactorial disease like GERD. Finally, we cannot totally exclude that a more pronounced inflammation, e.g., in a subgroup of subjects infected by strains of higher virulence, may affect LES function because the level of gastric inflammation was relatively low in our healthy subjects. However, gastritis, especially in the corpus (13), is considered to be rather protective against the development of reflux esophagitis. Therefore, our study strongly suggests that *H. pylori*-associated inflammation, especially carditis, does not affect the motor events involved in the pathogenesis of GERD.

Because our results showed that *H. pylori* infection did not affect LES motility, we did not perform additional experiments after *H. pylori* eradication. However, further similar studies involving GERD patients are needed to definitely rule out the role of *H. pylori* infection in eliciting TLESRs.

### Table 2. TLESR rates according to presence of histological gastric mucosal inflammation

<table>
<thead>
<tr>
<th>Gastric Distension</th>
<th>n</th>
<th>Baseline</th>
<th>Alone</th>
<th>CCK infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No gastritis</td>
<td>10</td>
<td>1.5 ± 0.5</td>
<td>3.6 ± 0.4</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td>Gastritis</td>
<td>6</td>
<td>0.7 ± 0.3</td>
<td>4.5 ± 0.3</td>
<td>6.0 ± 0.7</td>
</tr>
<tr>
<td>Fundus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No gastritis</td>
<td>11</td>
<td>1.4 ± 0.5</td>
<td>3.7 ± 0.4</td>
<td>5.6 ± 0.4</td>
</tr>
<tr>
<td>Gastritis</td>
<td>5</td>
<td>0.8 ± 0.4</td>
<td>4.4 ± 0.4</td>
<td>6.0 ± 0.9</td>
</tr>
<tr>
<td>Antrum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No gastritis</td>
<td>8</td>
<td>1.5 ± 0.6</td>
<td>3.8 ± 0.5</td>
<td>5.8 ± 0.6</td>
</tr>
<tr>
<td>Gastritis</td>
<td>8</td>
<td>0.9 ± 0.3</td>
<td>4.1 ± 0.4</td>
<td>5.8 ± 0.6</td>
</tr>
</tbody>
</table>

Results are means ± SE; n, no. of subjects. Gastric distension alone and gastric distension with CCK infusion increased the transient LES relaxation (TLESR) rate with or without gastritis.

### Table 3. Correlation coefficients between TLESR rates and mucosal concentrations of cytokines

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Gastric Distension</th>
<th>Gastric Distension with CCK</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardia</td>
<td>0.10</td>
<td>0.17</td>
<td>0.19</td>
</tr>
<tr>
<td>Fundus</td>
<td>0.12</td>
<td>0.03</td>
<td>0.28</td>
</tr>
<tr>
<td>Antrum</td>
<td>0.14</td>
<td>0.09</td>
<td>0.35</td>
</tr>
<tr>
<td>IL-8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardia</td>
<td>0.24</td>
<td>0.25</td>
<td>0.41</td>
</tr>
<tr>
<td>Fundus</td>
<td>0.09</td>
<td>0.08</td>
<td>0.23</td>
</tr>
<tr>
<td>Antrum</td>
<td>0.07</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>TNF-α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardia</td>
<td>0.02</td>
<td>0.40</td>
<td>0.09</td>
</tr>
<tr>
<td>Fundus</td>
<td>0.02</td>
<td>0.26</td>
<td>0.13</td>
</tr>
<tr>
<td>Antrum</td>
<td>0.13</td>
<td>0.14</td>
<td>0.35</td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardia</td>
<td>0.10</td>
<td>0.25</td>
<td>0.10</td>
</tr>
<tr>
<td>Fundus</td>
<td>0.12</td>
<td>0.14</td>
<td>0.17</td>
</tr>
<tr>
<td>Antrum</td>
<td>0.10</td>
<td>0.04</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Values are correlation coefficients, calculated with the TLESR rate obtained during baseline, gastric distension alone, and gastric distension with CCK infusion. Mucosal concentrations of cytokines (ELISA) were obtained from the cardia, fundus, and antrum.
REFERENCES


