Endogenous cholecystokinin in postprandial lower esophageal sphincter function and fundic tone in humans

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Zerbib, Frank, Stanislas Bruley des Varannes, Carmelo Scarpignato, Veronique Leray, Massimo D’Amato, Claude Roze, and Jean-Paul Galmiche. Endogenous cholecystokinin in postprandial lower esophageal sphincter function and fundic tone in humans. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1266–G1273, 1998.—Transient lower esophageal sphincter (LES) relaxations (TLESRs) are the main underlying mechanism of gastroesophageal reflux. Although CCK acts through CCK-A receptors to increase the TLESRs induced by gastric distension, the respective roles of endogenous CCK and fundic tone in triggering postprandial TLESRs remain unknown. The aim of this study was to determine the effect of the CCK-A receptor antagonist, loxiglumide, on postprandial LES function and fundic tone in humans. LES motor events and fundic tone were simultaneously monitored in two groups of healthy volunteers. Recordings were performed during fasting and for 3 h after a liquid meal (200 ml/200 kcal) administered either orally or intraduodenally at a rate mimicking gastric emptying. Each subject received loxiglumide (10 mg·kg−1·h−1) or saline (control) in randomized order, which was started 40 min before the meal and maintained for 3 h thereafter. After the oral meal, loxiglumide significantly reduced TLESRs (P = 0.002) without significantly affecting LES pressure and fundic tone. After duodenal infusion of the meal, loxiglumide totally abolished the increase in TLESRs, reduced LES pressure fall (P < 0.02), and strongly inhibited fundic relaxation (P < 0.0001). We concluded that endogenous CCK is involved in the postprandial control of both LES function and fundic tone through activation of CCK-A receptors.

Although the pathophysiology of gastroesophageal reflux disease (GERD) is multifactorial, it is now clearly established that impaired lower esophageal sphincter (LES) function plays a crucial role (18). In normal subjects as well as patients with GERD, most reflux episodes result from transient LES relaxations (TLESRs) rather than from low resting LES pressure (LESP) alone (12, 13, 36, 43). TLESRs are complete and long-lasting relaxations of the LES that are not preceded by swallowing (31). The factors and mechanisms involved in their occurrence remain largely unknown. Several studies have shown that TLESRs are triggered by gastric distension (19, 29) through the involvement of gastric mechanoreceptors (17) located mainly in the subcardial region. Because TLESRs are short lived, it has been suggested that they are neurally mediated. Indeed, animal experiments (29) have shown that they are abolished by cooling of the vagus nerve, thus indicating the involvement of a vago-vagal mechanism. Both TLESR and reflux occur mainly after a meal (13, 20, 36, 43), which is followed by fundic relaxation (2, 40). Meal-induced changes in fundic volume and tone may therefore represent a trigger for TLESRs. To gain better insight into the relationships between these physiological parameters, we recently developed and validated a technique for simultaneous assessment of LES function and fundic tone after food ingestion in humans (48).

Although gastric distension has been clearly identified as one of the triggers for TLESRs, the role of other factors such as nutrients or gastrointestinal (GI) peptides is still unknown. Fatty foods have been reported to worsen GERD symptoms (33) by lowering LESP (32). However, several studies have reported conflicting results for the effect of oral or intraduodenal fat on TLESRs (21, 35) and did not provide adequate investigation into the role of fat-induced release of GI peptides.

Among the different GI peptides, CCK, which functions as both a neuropeptide and a gut hormone (27), is a likely mediator for TLESR control. It is released during the postprandial state in which gastroesophageal reflux occurs more frequently (13, 20, 36, 43). Both exogenous (7, 16, 39) and endogenous (24) CCK can reduce LESP, and CCK also delays gastric emptying (42) through various mechanisms involving a reduction of intragastric pressure (15, 30), thus leading to prolonged gastric distension. Although conflicting results have been published (26), two recent studies reported that exogenous CCK produced a significant increase in distension-induced TLESRs in dogs (5) and humans (6). However, it is still not known how endogenous CCK may be involved in the occurrence of TLESRs in humans.

An early study in cats (37) suggested that different CCK receptor subtypes exist on LES neurons and muscle, and a recent investigation in dogs (5) reported that CCK is involved in the occurrence of TLESRs via stimulation of peripheral CCK-A receptors. Among the CCK-A antagonists under development for potential clinical applications, loxiglumide has been extensively studied (11) and appears to be a useful tool for investigating CCK receptor interactions in peripheral organs.

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Loxiglumide was therefore used in the present investigation to determine the involvement of endogenous CCK in both LES function (i.e., LESP and TLESR rate) and fundic tone after ingestion of a mixed meal. Because gastric emptying is delayed by CCK (thereby increasing intragastric volume and distension) and accelerated by loxiglumide (42), the same meal was also infused intraduodenally to exclude the food-related gastric distension in the observed motor events.

METHODS

Subjects and Experimental Design

Studies were performed on 31 healthy volunteers who had given their informed written consent to the protocol. All subjects were within 15% of ideal body weight, and no subjects were taking medications or had a history of 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 Numéro 2, Région des Pays de Loire, France).

A preliminary study was conducted on six subjects (3 men, 3 women, mean age 26.0 yr) to determine whether the liquid test meal (200 ml/200 kcal), which had previously been shown to produce significant fundic relaxation (8, 40), was also capable of increasing the TLESR rate and stimulating significant release of CCK into the bloodstream.

The main investigation to determine the relative contribution of endogenous CCK and fundic tone to LES function consisted of two separate studies, each conducted according to a randomized, double-blind, placebo-controlled, cross-over design (Fig. 1). Twelve healthy volunteers (7 men, 5 women, mean age 25.4 yr) participated in study 1 (in which the test meal was given orally), and 13 volunteers (10 men, 3 women, mean age 25.2 yr) participated in study 2 (in which the meal was given intraduodenally).

Preliminary Study

After an overnight fast, a carefully folded barostat bag was introduced into the stomach through the mouth, slowly unfolded by gently injecting 300 ml of air, and then completely deflated. An esophageal motility catheter was subsequently introduced through the nose. Resting LESP and TLESRs were first monitored for 1 h during fasting, and intrabag pressure was then set to 1 mmHg above minimal distending pressure, as previously described (8, 40). Recordings of fundic tone (volume) were performed while subjects were in a sitting position. After a 10-min control period, subjects drank the meal through a straw at a rate of 100 ml/min. LES function (i.e., LESP and TLESRs) and fundic tone were then recorded for 3 h. Blood samples for CCK measurement were also obtained before (−15 min) and after (0, 5, 10, 15, 30, 60, 120, and 180 min) the test meal.

Main Investigation

In study 1, LES motility was recorded during fasting, and LES motility and fundic tone were monitored 3 h after oral ingestion of the meal. Subjects were randomly and blindly assigned to receive loxiglumide (compound marked CR-1505, Rotta Research Laboratorium, Monza, Italy) or saline (infused intravenously) on two separate days. At least 3 days were allowed to elapse between experiments. The CCK-A receptor antagonist was injected at a dose of 30 mg·kg⁻¹·h⁻¹ for the first 10 min; subsequent injections were at 10 mg·kg⁻¹·h⁻¹. The infusion was started 40 min before the meal and continued throughout the postprandial period (3 h). At the dose used, loxiglumide has been shown to produce complete and selective blockade of CCK-A receptors (34).

The design of study 2 was similar to that of study 1. However, the test meal was instilled directly into the duodenum to avoid direct stimulation of gastric mechanoreceptors by intragastric food. Accordingly, a polyurethane feeding tube (Sherwood Medical, Tullamore, Ireland) was introduced into the duodenum through the nostril the night before the experiment, and its position was checked by fluoroscopy just before the start of the experiment. The meal was infused at a rate (5.5 ml/min during the first 18 min and then 1.7 ml/min for 60 min) designed to mimic normal gastric emptying of the same meal, as previously determined under the same experimental conditions (40).

Test Meal

The test meal (200 ml/200 kcal) consisted of partially skimmed milk (130 ml), 12 g of liquid protein solution (Alburone, Clintec Nutrition Clinique, Sèvres, France), 30 g of dextrin-maltose (Nutripharm Elgi, Levallois, France), and 50 ml of water. The total energy content was 1 kcal/ml, provided as 20% fat, 60% carbohydrate, and 20% protein.

Evaluation of LES Function

Pharyngoesophageal motility was monitored using a motility catheter fitted with a 6-cm Dent Sleeve (Arndorfer Medical Specialties, Milwaukee, WI). The catheter was introduced through a nostril and positioned so that pressures could be recorded from the LES (sleeve), fundus (side hole 2 cm below the sleeve), esophageal body (side holes 4, 7, and 10 cm proximal to the sleeve), and pharynx (side hole 28 cm proximal to the sleeve, to detect swallows). The catheter was perfused at a flow rate of 0.5 ml/min with distilled water using a pneumohydraulic perfusion system (Arndorfer Medical Specialties), connected to external pressure transducers (Gould P23D, Gould Instruments, Ballainvilliers, France). Signals from the pressure transducers were recorded on a polygraph (Gould ES 1000, Gould Instruments) running at a speed of 2.5 mm/s.
Monitoring of Fundic Tone

Fundic tone was monitored by means of an electronic barostat according to a previously described technique (8, 40). The barostat records the volume of air contained within an intragastric bag maintained at a low and constant pressure by an electronic feedback mechanism. Consequently, when the stomach contracts, the barostat aspirates air from the bag to maintain a constant pressure, and bag volume decreases. Conversely, when the stomach is relaxing, air is injected and the bag volume increases. Highly compliant polyethylene bags (with a capacity of 750–800 ml) were used and connected to the pressure transducer and injection pump via a 16F single-lumen tube. Bag volume changes were charted on a potentiometric recorder (Servotrace, IPE Sefram, Paris, France) running at 10 mm/min.

CCK Bioassay

Plasma concentrations of CCK were measured using the specific and sensitive bioassay described by Liddle et al. (28) with slight modifications (45). This method is based on the ability of CCK to stimulate amylase release from isolated rat pancreatic acini. Blood samples were centrifuged at 3,500 rpm for 10 min at 4°C. The plasma was applied onto Sep-Pak C18 cartridges (Waters Associates, Milpore Milford, Mol- sheim, France) previously activated with 10 ml of acetonitrile, 10 ml of methanol, and 20 ml of water. After washing with 20 ml of water, CCK was eluted with 1 ml of acetonitrile-water (1:1). This eluate was then lyophilized for 72 h, and the lyophilized extract was kept at −20°C until assayed.

Data Analysis

Eosophageal manometry. Resting LESP was measured every 3 min and averaged over 15-min intervals. Mean resting LESP was defined as the average of the 1-h fasting period and was used to determine the postprandial variation of resting LESP (ΔP; results are expressed in mmHg). TLESRs were defined according to Holloway et al. (22) as the absence of a pharyngeal swallow signal for 4 s before and 2 s after onset of LES relaxation, 2) an LESP decrease of ≥1 mmHg/s, 3) a time from onset to complete relaxation of ≥10 s, 4) a nadir pressure of ≤2 mmHg, and 5) an LESP decrease to ≤2 mmHg for >10 s (excluding multiple rapid swallows).

Barostat bag volume. Analysis was performed as previously described (8, 40). Briefly, volume was measured every minute and averaged over 15-min intervals. The following variables were calculated, as previously described (40): “control bag volume,” representing mean bag volume during the 10-min control period preceding the meal, “volume change” (ΔV), referring to volume variations over the control, “beginning of the response,” i.e., the time at which bag volume differed by 30 ml from control volume, “maximal response,” i.e., the maximal and uniform (variations <30 ml) changes in volume observed after the meal, and “end of response,” i.e., the time when bag volume reached a level within 30 ml of control volume. On the basis of these definitions, latency, maximal response (ΔVmax), rate of volume change, and response duration were determined.

Statistical Evaluation of Data

Results are indicated as means ± SE. Comparison of means [postprandial variations of LESP (ΔP) and gastric bag volume (ΔV)] was performed through ANOVA for repeated measures. The number of TLESRs and all paired and unpaired data were compared using Student’s t-test. Statistical analysis was performed using BMDP Statistical Software, version 1990 (BMDP Statistical Software, Los Angeles, CA), on an IBM PC. A P value <0.05 was considered significant.

RESULTS

Preliminary Study

LES motility. Ingestion of the meal induced a significant increase in the TLESR rate during the first and second postprandial hours. The number of TLESRs changed from 0.7 ± 0.5/h during fasting to 4.8 ± 0.6/h during the first postprandial hour (P = 0.004), to 3.2 ± 0.8/h during the second (P = 0.02), and to 1.3 ± 0.3/h during the third (P = 0.2). As expected, a significant decrease in LESP was observed during the postprandial period. After a maximum drop (ΔP = 8.2 ± 1.2 mmHg at 15 min), the pressure increased slowly, reaching fasting values 145 ± 27 min after the meal.

Fundic tone. In all six subjects studied, meal ingestion was followed by an increase in bag volume indicative of fundic relaxation. The maximal response (ΔVmax = 280 ± 32 ml) occurred 14 ± 4 min after the meal and lasted for 94 ± 30 min.

CCK plasma levels. Consumption of the test meal (20% fat, 60% carbohydrate, and 20% protein) induced a significant, though short-lived, increase in plasma CCK levels, with a peak of 3.2 ± 1.0 pM 10 min after ingestion (Fig. 2).

Main Investigation

LES function and fundic tone after an oral meal. Eleven subjects completed the study, since one subject experienced severe nausea and vomiting during saline infusion and was excluded. Ingestion of the test meal significantly (P < 0.01) decreased LESP (ΔP = 7.6 ± 1.4 mmHg). This effect was not modified by loxiglumide (Fig. 3A), although the maximum drop in LESP was slightly but significantly (P = 0.03) delayed (45 ± 7 vs. 30 ± 5 min).

The number of TLESRs increased significantly during the first and second postprandial hours (Fig. 3B). Loxiglumide significantly (P = 0.002) reduced, but did...
not abolish, the meal-induced increase in the TLESR rate, whereas mean TLESR duration was not affected by CCK-A receptor blockade (21.0 ± 0.8 vs. 23.5 ± 1.2 s).

Test meal consumption was followed by a significant increase in intragastric bag volume, indicative of fundic relaxation (Fig. 3C). Intravenous infusion of loxiglumide did not change fasting barostat pressure and control bag volume (Table 1) or have a significant effect on the time course of postprandial fundic tone (Fig. 3C). However, $\Delta V_{\text{max}}$ ($P = 0.02$) was slightly delayed, and the mean duration of relaxation was reduced ($P = 0.03$ (Table 1).

There was a significant correlation ($P = 0.002$) between changes in intragastric bag volume [expressed as area under the curve (AUC)/h] and TLESRs in the postprandial period. Similarly, variations in fundic tone correlated well ($P = 0.0001$) with postprandial changes of LESP (data not shown).

LES function and fundic tone after an intraduodenally administered meal. Twelve subjects completed the study, since one subject experienced severe nausea and vomiting during saline infusion and was excluded.

Intraduodenal infusion of the test meal resulted in a decrease of LESP ($\Delta P_{\text{max}} = 9.1 ± 1.4$ mmHg) (Fig. 4A). In these experimental conditions, loxiglumide significantly ($P < 0.02$) reduced the postprandial fall of LESP ($\Delta P_{\text{max}}$ was 5.2 ± 0.7 mmHg and 9.1 ± 1.4 mmHg, during loxiglumide and saline, respectively).

The TLESR rate was significantly increased by the intraduodenal meal, and this increase was completely prevented during loxiglumide infusion (Fig. 4B). Again, mean TLESR duration was virtually the same during saline or loxiglumide infusion (21.9 ± 0.8 and 23.5 ± 1.2 s, respectively).

Intraduodenal infusion of the test meal, like that of oral ingestion, was accompanied by significant fundic relaxation that lasted 74 ± 6 min (Fig. 4C). Loxiglumide blocked the meal-induced decrease of fundic tone markedly and significantly ($P < 0.0001$) (Table 2). Fundic relaxation was virtually suppressed in 8 of 12 subjects (Fig. 5). Again, a significant correlation was found between variations in fundic tone and either TLESRs ($P = 0.0001$) or LESP ($P = 0.0001$) throughout the postprandial period (data not shown).

**DISCUSSION**

Our investigations indicated that a significant relaxation of the gastric fundus, a decrease of LESP, and a

![Fig. 3. LES function and fundic tone after an orally ingested meal.](image)

**Table 1. Effects of loxiglumide on fasting and postprandial fundic tone after an orally ingested 200 ml/200 kcal meal in 11 healthy subjects**

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Loxiglumide</th>
<th>$P$</th>
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<tbody>
<tr>
<td>Fasting</td>
<td>Barostat pressure, mmHg</td>
<td>7.3 ± 0.7</td>
<td>7.7 ± 0.6</td>
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<tr>
<td></td>
<td>Control bag volume, ml</td>
<td>117 ± 9</td>
<td>119 ± 6</td>
</tr>
<tr>
<td>Oral meal</td>
<td>$\Delta V_{\text{max}},$ ml</td>
<td>299 ± 27</td>
<td>301 ± 17</td>
</tr>
<tr>
<td></td>
<td>AUC, ml/h</td>
<td>252 ± 31</td>
<td>230 ± 20</td>
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<tr>
<td></td>
<td>Response latency, min</td>
<td>2.8 ± 0.8</td>
<td>2.0 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Time of $\Delta V_{\text{max}},$ min</td>
<td>12.5 ± 1.4</td>
<td>20.5 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>Rate of volume change, ml/min</td>
<td>34.5 ± 5.8</td>
<td>19.5 ± 2.9</td>
</tr>
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<td></td>
<td>Response duration, min</td>
<td>93 ± 8</td>
<td>83 ± 6</td>
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Values are means ± SE. $\Delta V_{\text{max}}$ represents the maximal response (maximal volume variation of the barostat bag). Response latency is time interval between the beginning of the meal and beginning of the response. Rate of volume change relates to period between the beginning of response and occurrence of maximal response. AUC, area under curve; NS, not significant.
A concomitant increase in the TLESR rate occurred following either oral ingestion or direct duodenal delivery of the same test meal. Complete blockade of CCK-A receptors induced by the competitive, selective, and potent agonist, loxiglumide, significantly reduced and virtually abolished the meal-induced increase in TLESRs after oral and duodenal instillation, respectively. During intraduodenal infusion of the meal, however, loxiglumide also inhibited food-induced fundic relaxation and the postprandial decrease in LESP, whereas these two parameters were unaffected after oral ingestion.

TLESRs are now recognized as the main underlying mechanism of gastroesophageal reflux (12, 13, 36, 43) and probably represent the best-identified target for future drug development in GERD (23). Gastric distension triggers TLESRs (5, 6, 19, 29) probably through stimulation of mechanoceptors in the proximal stomach. In dogs (5, 29) and humans (6, 19), these data were obtained through rather unphysiological procedures, since gastric distension was performed using gas, air, or an intragastric bag, so that the physiological relaxation of the proximal stomach seen after food ingestion (2, 8, 40) was not reproduced. In the present investigation, a mixed meal was used to mimic more closely postprandial gastric distension so that the relationships between fundic tone and TLESRs could be studied. This experimental procedure had previously proved reliable and quite reproducible (48). In particular, the presence of the intragastric barostat bag did not modify the TLESR rate during fasting and postprandial periods in our experimental conditions (48). Because CCK induces a relaxation of the proximal stomach (15, 30), we investigated the role of endogenous CCK in the occurrence of postprandial TLESRs. On the basis of preliminary experiments, we selected a test meal ca-

**Table 2. Effects of loxiglumide on fasting and postprandial fundic tone after duodenal infusion of a 200 ml/200 kcal meal in 12 healthy subjects**

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Loxiglumide</th>
<th>P</th>
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<tbody>
<tr>
<td><strong>Fasting</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Barostat pressure, mmHg</td>
<td>8.3±0.5</td>
<td>8.5±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Control bag volume, ml</td>
<td>104±8</td>
<td>107±9</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Intraduodenal meal</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ΔVmax, ml</td>
<td>312±27</td>
<td>41±19</td>
<td>0.0001</td>
</tr>
<tr>
<td>AUC, ml/h</td>
<td>192±31</td>
<td>13±7</td>
<td>0.0001</td>
</tr>
<tr>
<td>Response latency, min</td>
<td>1.9±0.3</td>
<td>10.5±3*</td>
<td>0.01</td>
</tr>
<tr>
<td>Time of ΔVmax, min</td>
<td>13.7±1.5</td>
<td>20.3±5.8*</td>
<td>NS</td>
</tr>
<tr>
<td>Rate of volume change, ml/min</td>
<td>30.3±5.7</td>
<td>15.7±2.9*</td>
<td>NS</td>
</tr>
<tr>
<td>Response duration, min</td>
<td>73±6</td>
<td>42±12*</td>
<td>0.02</td>
</tr>
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Values are means ± SE. *Means calculated on values available only when a response was observed (n=4).

**Fig. 4. LES function and fundic tone after duodenal infusion of the test meal.** LES function and fundic tone were simultaneously assessed after duodenal infusion of a liquid test meal in 12 healthy subjects receiving loxiglumide or saline in randomized order. Results are expressed as means ± SE. A: 3-h postprandial ΔP compared with fasting levels. *P < 0.02, **P < 0.01 vs. loxiglumide. B: no of TLESRs during fasting and at 3-h postprandial. *P = 0.0001 vs. saline; **P = 0.02 vs. saline (paired Student’s t-test); C: postprandial fundic tone represented by ΔV of the intragastric bag connected to the barostat. *P < 0.0001; **P < 0.001; #P < 0.01; ###P < 0.05 vs. loxiglumide.

**Fig. 5. Effect of loxiglumide on fundic tone after duodenal infusion of the test meal.** Individual recordings of fundic tone obtained with a barostat in a healthy subject are shown. With saline (A), duodenal infusion of a 200 ml/200 kcal meal resulted in an increase in bag volume lasting ~90 min (barostat pressure 8 mmHg, maximal ΔV = 425 ml), reflecting a decrease in fundic tone. B: loxiglumide totally abolished fundic relaxation (barostat pressure 7 mmHg).
pable of relaxing the gastric fundus and releasing endogenous CCK.

The relative contribution of gastric distension and CCK was evaluated by infusing the meal directly into the duodenum to release endogenous CCK without inducing meal-related gastric distension. In fact, compared with orally administered meals, we cannot completely exclude the possibility that higher CCK plasma levels occurred after direct duodenal infusion. However, this hypothesis is unlikely because 1) the duodenal meal was infused at a rate mimicking normal gastric emptying of the same meal administered orally and 2) the LES motility pattern, i.e., the fall of LESP and the increase in TLESRs, was exactly the same after both the orally and duodenally administered meals.

As expected, intraduodenal nutrients elicited significant gastric relaxation (1) and also increased TLESRs. In a recent study, Holloway et al. (21) were unable to detect any change in the TLESR rate after infusion of intraduodenal fat into both healthy subjects and patients with GERD, although the number of TLESRs associated with reflux was increased in these patients. The different experimental conditions used (recording time and composition of the duodenal perfusate) may account for the discrepancies in these findings.

Experimental (5) and clinical (6) studies have recently shown that CCK increases the rate of TLESRs triggered by gastric distension through stimulation of CCK-A receptors. In our experimental conditions, blocking this receptor subtype by loxiglumide infusion significantly reduced (after oral ingestion) or virtually abolished (after intraduodenal administration) the meal-induced increase of TLESRs. These data are in line with recent results showing that loxiglumide can reduce postprandial TLESRs in obese (14) and GERD (46) patients as well as those induced by fundic distension (via gastric air insufflation) in healthy volunteers (3). Taken together, these findings strongly suggest an involvement of endogenous CCK in the control of postprandial TLESRs.

Our results showing that loxiglumide can significantly reduce the fall of LESp after an intraduodenal meal are in line with previous findings (24) and also confirm that CCK is an important regulator of postprandial LES tone. However, the unexpected lack of any effect of the CCK-A receptor antagonist on LESp after oral ingestion of the test meal is difficult to explain. Another striking difference in the results of the two experiments is a lack of loxiglumide inhibition of fundic relaxation, as revealed by a comparison of Figs. 3 and 4. Moreover, significant correlations were found between postprandial variations of fundic tone and LESp in both experiments. It is thus tempting to speculate that meal-induced relaxation of the proximal stomach may have been at least partly responsible for the postprandial drop in LESp.

Azpiroz and Malagelada (1) used the electronic barostat in a dog model to demonstrate the existence of an enterogastric mechanism controlling gastric tone. Gastric tone is currently considered to be a dynamic process that is extremely sensitive to specific nutrients in the small bowel lumen. Intestinal nutrients induce gastric relaxation via a nonadrenergic, noncholinergic mechanism, and this reflex is mediated by fibers contained in the vagus nerve (1). However, the specific neurotransmitters involved have not yet been elucidated, at least in humans.

CCK-A receptors are known to exist on afferent neurons in the abdominal vagus (10, 47), and the gastric relaxant effect of exogenous CCK in animals has proved to be dependent on intact vagal pathways (38). These data, as well as the role of the abdominal vagus in intestinal control of gastric tone, suggest that CCK is one of the transmitters involved in vagally mediated gastric relaxation by intestinal nutrients. Our results showing that loxiglumide markedly reduced the significant decrease of gastric tone induced by an intraduodenal meal are in line with previous findings (15) and lend support to the above hypothesis. These findings provide further evidence that CCK is a physiological regulator of gastric emptying of meals containing fat (4).

However, after oral ingestion of the same test meal in our study, the CCK-A receptor antagonist slightly delayed (by 8 min) the time of maximal fundic relaxation and reduced (by 10 min) the relaxation period. Although these changes seem to indicate that endogenous CCK is involved in the postprandial fundic relaxation, they appear to be minor compared with the overall time course of postprandial fundic tone. The different gastric relaxation responses to loxiglumide induced by an intraduodenal or orally ingested meal parallel the behavior of gastric compliance after ingestion of intraduodenal (15) or intragastric (30) lipids during CCK-A receptor blockade. It is difficult to explain why loxiglumide had no effect on postprandial gastric relaxation. However, in the more physiological conditions of orally ingested food, the interaction of multiple factors (either nervous or hormonal) should be taken into account. In our experimental conditions, deglutition (our volunteers ingested the liquid test meal by sipping it through a straw, and sipping is usually followed by multiple swallows) and intragastric distension may have activated neural inhibitory reflexes that relaxed the proximal stomach (25). When the meal was ingested, the neural influence on gastric tone may have predominated over hormonal and/or paracrine (e.g., CCKergic) control. Conversely, after intraduodenal instillation of the same test meal, the release of CCK from the small intestine would probably represent the major mechanism responsible for food-induced fundic relaxation. Thus, although blockade of CCK-A receptors could have counterbalanced the hormonally mediated (by intraduodenal nutrients) decrease in gastric tone, it would hardly have affected neurally triggered (by orally ingested food) fundic relaxation.

After an orally or intraduodenally administered meal, postprandial variations in fundic tone were significantly correlated with the TLESR rate. Moreover, after an intraduodenally administered meal, loxiglumide inhibited both postprandial gastric relaxation and the increase of TLESRs. These results strongly suggest the
role of both endogenous CCK and fundic relaxation in triggering postprandial TLESRs. After an orally ingested meal, loxiglumide induced minor changes of fundic relaxation but significantly reduced the postprandial increase in TLESRs. This shortened fundic relaxation could have resulted in a reduced stimulation of mechanical receptors and could thus explain the observed decrease in number of TLESRs. However, in our opinion, a 10-min reduction in duration of relaxation can hardly explain a 50% reduction of the rate of TLESRs. Moreover, there was no significant effect of loxiglumide on AUC, a parameter that takes into account both amplitude and duration of relaxation. The inability of CCK-A receptor blockade to abolish the meal-induced increase of TLESRs may have been related to residual mechanical fundic stimulation due to the presence of intragastric contents (meal and gastric secretions). Therefore, the apparent dissociation between the responses of the gastric fundus and esophageal function to loxiglumide suggests that inhibition of gastric tone is not a necessary prerequisite for blocking TLESRs. This pharmacological suppression of the TLESRs may represent a novel and rational approach in view of the well-recognized relationship between GERD and TLESRs.

Although our results clearly show an involvement of CCK-A receptors in CCK-mediated effects, they do not indicate whether the receptors involved are peripheral or central. However, the poor bioavailability of loxiglumide to the central nervous system [the drug largely crosses the blood-brain barrier (44)] and the fact that intracerebroventricular administration of devazepide (another CCK-A receptor antagonist) failed to affect TLESRs induced by gastric distension in dogs (5) strongly suggest that these receptors are peripheral.

In summary, oral intake or intraduodenal administration of nutrients is followed by a significant relaxation of the gastric fundus, a decrease of LESP, and a concomitant increase in the TLESR rate. Competitive blockade of CCK-A receptors significantly reduces or actually abolishes the meal-induced increase in TLESRs. The ability of loxiglumide to counterbalance meal-induced LES incompetence (via its effects on TLESRs and LESP) may well reduce postprandial gastroesophageal reflux. Because this drug is also able to accelerate gastric emptying (4, 42), which is delayed in a substantial number of patients with GERD (41), it may reduce esophageal exposure to acid by decreasing the volume of gastric contents available for reflux. A potential role for CCK-A receptor antagonists in the treatment of GERD must be, however, considered in the context of possible deleterious effects due to the long-term inhibition of gallbladder emptying, although no evidence of gallstone formation in humans has been reported so far after oral treatment for up to 12 mo with loxiglumide (9). Thus CCK-A receptor antagonists (loxiglumide in particular) may represent a more pathophysiological approach to GERD therapy.

We are grateful to Dr. Sophie Pueyo (Department of Biostatistics and Epidemiology, University of Bordeaux II Victor Segalen) for help in statistical analysis and to the Institut National de la Santé et de la Recherche Médicale for financial support (CRI no. 95 08).

F. Zerbib is a recipient of an ASTRA-France fellowship research grant.

Preliminary results were presented at Digestive Disease Week, in Washington DC, May 1997, and published in abstract form (Gastroenterology 112: A857, 1997).

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Received 3 J une 1998; accepted in final form 26 August 1998.

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