Modulation by colonic fermentation of LES function in humans

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Modulation by colonic fermentation of LES function in humans. Am J Physiol Gastrointest Liver Physiol 278: G578–G584, 2000.—Colonic fermentation of carbohydrate has been shown to influence gastric and intestinal motility. Our aim was to investigate the effects of colonic infusion of lactose and short-chain fatty acids (SCFAs) on lower esophageal sphincter (LES) function in humans. LES pressure (LESP), transient relaxations of LES (TLESRs), and esophageal pH were monitored over 6 h on 4 different days in 7 healthy volunteers. After 1 h of baseline recording, the effects of different colonic infusions (270 ml of isotonic or hypertonic saline, 30 g lactose, or 135 mmol SCFAs) were tested in fasting conditions and after a standard meal. Peptide YY (PYY) and oxyntomodulin (OLI) were also measured in plasma. Both lactose and SCFA infusions increased the number of TLESRs as well as the proportion of TLESRs associated with acid reflux episodes, but saline solutions did not. The postprandial fall of LESP was enhanced by previous SCFA infusion. Plasma PYY and OLI increased similarly after all colonic infusions. Colonic fermentation of lactose markedly affected LES function, and this effect was reproduced by SCFA infusion. Whether the mechanisms of this feedback phenomenon are of hormonal nature, neural nature, or both remains to be determined.

esophageal manometry; short-chain fatty acids; gastroesophageal reflux; peptide YY; oxyntomodulin

ALTHOUGH TRANSIENT LOWER ESOPHAGEAL sphincter relaxations (TLESRs) represent the main mechanism associated with the occurrence of reflux episodes (4, 5, 11, 19, 24), both in healthy subjects and in patients with gastroesophageal reflux (GER) disease, little is known about the factors involved in their occurrence. They can be triggered by gastric distension (11, 18) and through activation of mechanoreceptors located in the subcardial area (7) and are closely related to postprandial relaxation of the proximal stomach (32, 33).

Exposure of the distal gut to nutrients contributes to regulation of gastrointestinal motility in humans. This phenomenon was first referred to as the ileal brake, since the infusion of fatty acids (6, 10, 25, 26, 29) or complex carbohydrates (13, 16) into the ileum delayed gastric emptying (13, 16) and slowed transit time (6, 10, 26). Colonic fermentation is a likely regulator of gastrointestinal motility, since 2—20% of ingested starch escapes digestion in the small intestine under physiological conditions (27). Most carbohydrates are metabolized by colonic bacterial flora into short-chain fatty acids (SCFAs) and hydrogen. In healthy volunteers, we recently showed that colonic fermentation of ingested lactulose as well as direct colonic infusion of a mixture of SCFAs in the cecum resulted in a marked dose-dependent relaxation of the proximal stomach, as measured with an electronic barostat (22). The mechanisms involved in the ileocolonic brake remain largely obscure, nor is it known how the presence of SCFAs in the colon activates the feedback mechanism. Attention has focused on the possible role of digestive peptides such as peptide YY (PYY) and proglucagon-derived peptides [i.e., oxyntomodulin (OLI)] because they are colocalized and released from endocrine L cells of the distal small intestine. However, conflicting results have been reported so far (13, 21, 22).

Whether lower esophageal sphincter (LES) motility could also be affected by exposure of the colon to malabsorbed carbohydrates is presently unknown. We therefore hypothesized that a feedback mechanism could exist between colonic metabolic activity and LES motility.

The aims of the present study were 1) to investigate the motor activity of LES in response to colonic infusions of lactose, a frequently malabsorbed disaccharide, and of SCFAs, the main endproducts of lactose colonic fermentation, and 2) to determine whether circulating levels of PYY and OLI may be involved in the changes in LES motility.

METHODS

Subjects

Eight healthy volunteers (five men and three women; mean age 24.5 yr; age range 22–31) were studied on two separate
The lactose solution consisted of 111 g of D-lactose diluted in 1 l of isotonic saline. The osmolality of this solution was 600 osmol/kg. Therefore, the amount perfused (270 ml) corresponded to 30 g of D-lactose.

In the second set of experiments, hypertonic saline and a mixture of SCFAs were tested. The hypertonic saline solution consisted of NaCl 36 g/l, corresponding to 600 mmol/l. The osmolality of this solution was 1,200 osmol/kg, and the amount infused was 162 mmol. The SCFA solution consisted of a mixture of 500 mmol SCFAs diluted in 1 l of isotonic saline. The composition of the SCFA was 70% acetic acid, 20% propionic acid, and 10% butyric acid. The osmolality of this solution was 1,200 osmol/kg, and the amount infused was 135 mmol. The pH of solutions was kept constant (i.e., 5.6–6.1), as in the first set of experiments.

After 1 h of baseline pH/pressure recording, the tested solution was infused into the proximal colon for a 90-min period. The subjects sat up to eat a standard 324-kcal meal at 11 AM (i.e., 30 min before the end of colonic infusions) that consisted of an egg, 10 g of butter, 2 rusks, 1 slice of ham, 100 ml of orange juice and 100 ml of water. The volunteers were asked to eat the meal over a 20-min period. Esophageal motility and pH were then further monitored for four consecutive hours. At the end of the recording, the subjects were allowed to walk, and a starch-free meal was served at 8 PM. On day 2, the procedures were similar to those of day 1, except that the solution infused into the proximal colon was changed for the other one in a random fashion.

For analysis, the overall 6-h recording time was divided into three periods: the first hour corresponded to baseline fasting, the second to colonic infusions in the fasting state (i.e., infusion fasting), and the third to the 4 h after the meal (i.e., postprandial).

Assessments
LES motility. A standard motility catheter fitted with a 6-cm Dent sleeve (Arndorfer Medical Specialties, Milwaukee, WI) was used to monitor esophageal pressures. The assembly was swallowed and positioned so that pressures could be recorded from the LES (sleeve), fundus (2 cm below the sleeve), esophageal body (side holes 5 and 10 cm proximal to the sleeve), and pharynx (side hole 28 cm proximal to the sleeve to detect swallowing). The catheter was perfused at 0.5 ml/min with a low-compliance hydraulic capillary infusion system (Arndorfer Medical Specialties) driven by a pressure head of nitrogen. The infusion system was connected to pressure transducers (Gould P23D; Gould Instruments, Ballaigues, France), and the output was displayed on a multichannel pen recorder running at a speed of 2.5 mm/s (Gould ES 1000; Gould Instruments).

Resting LES pressure (LESP) was measured every 3 min and averaged over 15-min intervals. Mean resting LESP was defined as the average of the baseline period (i.e., baseline fasting), and was used to determine the variation of LES tone (ΔP) during fasting infusions and the postprandial period. The maximal decrease of LESP (ΔPmax) was the mean of individual values of maximal variation of the LESP. Results are expressed in mmHg.

TLESRs were defined according to Holloway et al. (12) as 1) a LES relaxation occurring in the absence of a pharyngeal swallow signal for 4 s before and 2 s after the onset of the LES relaxation, 2) a LESP decrease of ≥1 mmHg/s, 3) a time from

![Fig. 1. Study design. PYY, peptide YY; OLI, oxyntomodulin.](http://ajpgi.physiology.org/ by 10.220.33.2 on September 27, 2017)
Hormonal assays. Blood samples were collected in glass tubes containing EDTA plus aprotinin, centrifuged at 1,200 g for 6 min at 4°C within 10 min of venipuncture, and then stored at −30°C until assay. Immunoreactive plasma PYY levels were measured by a sensitive and specific radiommunoassay (8, 28). The antiserum (kindly provided by Dr. J. C. Cuber, INSERM U45, Lyon, France) was raised in New Zealand White rabbits immunized with unconjugated synthetic human PYY. The assays were performed in duplicate. The detection limit in plasma was ~3 fmol/ml. The antiserum cross-reacted 100% with human synthetic PYY-(1–36) and PYY-(3–36), whereas no significant cross-reaction occurred with bovine pancreatic polypeptide, human pancreatic polypeptide, and avian pancreatic polypeptide and only a slight cross-reaction with porcine neuropeptide Y. OLI determination was performed using an OLI COOH-terminal octapeptide-specific antibody, as described in detail elsewhere (15). The detection limit of the assay was 1 fmol/ml.

Statistical Analysis

Results are expressed as means ± SE. Postprandial variations of LESP and plasma levels of PYY and OLI were compared by ANOVA for repeated measurements. The number of TLESRs and reflux episodes were compared by one-way ANOVA and Fisher’s test. Percentages of TLESRs associated with reflux episodes were compared with a contingency table. Correlation studies were performed using linear regression. Statistical analysis was conducted using Statview version 4.01 (Brain Power, Calabasas, CA). A P value <0.05 was considered significant.

RESULTS

The eight subjects completed the first set of experiments, but one refused to participate in the second set. Most subjects experienced mild side effects during lactose or SCFA infusions (bloating in five and four cases and diarrhea in two and three cases, respectively). Three subjects also complained of bloating while on hypertonic saline during the second set of experiments.

LES Motility

LES pressure. Compared with baseline fasting, colonic infusions (i.e., infusion fasting) did not significantly change resting LESP. As expected, LESP fell after the meal, and this fall was significantly more pronounced after colonic infusion of SCFAs (area under the curve, P < 0.01) compared with saline and hypertonic saline solutions (Fig. 2). The maximal decrease in LESP (ΔP<sub>max</sub>) was observed after colonic infusion of SCFAs, and ΔP<sub>max</sub> occurred later after colonic infusion

Table 1. Effect of colonic infusion of short-chain fatty acids, lactose, saline, and hypertonic saline solutions on postprandial changes of lower esophageal sphincter pressure from baseline fasting

<table>
<thead>
<tr>
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<th>First Experiment, n = 8</th>
<th>Second Experiment, n = 7</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Lactose</td>
</tr>
<tr>
<td><strong>AUC, mmHg·min</strong></td>
<td>309 ± 138</td>
<td>933 ± 331</td>
</tr>
<tr>
<td><strong>ΔP&lt;sub&gt;max&lt;/sub&gt;, mmHg</strong></td>
<td>−5.4 ± 1.2</td>
<td>−7.4 ± 2.1</td>
</tr>
<tr>
<td>Time of ΔP&lt;sub&gt;max&lt;/sub&gt;, min</td>
<td>52.5 ± 12.7</td>
<td>101.2 ± 30.5††</td>
</tr>
</tbody>
</table>

Data are means ± SE. ΔP<sub>max</sub>, mean of maximal variation of lower esophageal sphincter pressure (LESP) for all individual subjects, irrespective of time this peak occurred. Interval before ΔP<sub>max</sub> (time of ΔP<sub>max</sub>) is expressed in minutes. *P < 0.01 vs. saline and hypertonic saline; †P = 0.03 and §P = 0.01 vs. hypertonic saline and short-chain fatty acids (SCFAs), respectively.
of lactose than after infusion of SCFAs (P = 0.01) or of both saline solutions (P < 0.05) (Table 1).

TLESRs. The rates of TLESRs during the different periods are shown in Fig. 3. The rate of TLESRs was not significantly different between baseline fasting and infusion fasting in each group. During infusion fasting, SCFAs significantly increased the rate of TLESRs compared with saline (4.4 ± 0.6 vs. 1.8 ± 0.5, respectively; P = 0.005), whereas lactose had no effect. The meal was followed by a significant increase in the rate of TLESRs, which was significantly greater after colonic infusion of SCFAs (17.4 ± 1.3) and lactose (15.8 ± 1.5) than after saline (11.1 ± 1.6; P < 0.01 and P < 0.05, respectively) and hypertonic saline (12.2 ± 1.3; P < 0.01 and not significant, respectively). At all times of the postprandial period, the rate of TLESRs was numerically higher after lactose and SCFA infusions than after saline, although the difference was only statistically significant during the third hour (Fig. 4). After meal ingestion, the peak number of TLESRs was observed later after colonic infusion of lactose than after SCFAs (Fig. 4).

GER episodes. Most GER episodes occurred during the postprandial period. The number of postprandial reflux episodes was numerically but not significantly increased by colonic infusion of both SCFAs and lactose (Table 2). The average postprandial esophageal acid exposure tended to be longer after colonic infusions of SCFAs (7.7 ± 2.1 min) and lactose (7.2 ± 3.3 min) than hypertonic saline (5.8 ± 1.0 min) or saline (3.8 ± 0.9 min), although the differences were not statistically significant.

Compared with baseline fasting, the number of TLESRs associated with a GER episode during the infusion fasting period was not significantly affected. In contrast, after the meal, colonic infusions of both lactose and SCFAs significantly (P < 0.05) increased the number of TLESRs associated with a GER episode, compared with saline (Fig. 5).

PYY and OLI Plasma Levels

Average plasma levels of PYY and OLI increased immediately after colonic infusions, and PYY rose more abruptly than OLI (Fig. 6). However, no significant difference was found between the different solutions infused. Conversely, meal ingestion did not produce a further increase in PYY and OLI plasma levels. No correlation was found between plasma PYY or OLI response and the number of TLESRs or LESP.

DISCUSSION

This study demonstrates that infusions of lactose or SCFAs into the colon markedly affected LES function in humans. The postprandial fall in LESP was more pronounced after colonic infusion of SCFAs, whereas the number of TLESRs increased after both lactose and SCFA colonic infusions. The proportion of TLESRs associated with reflux also increased.

Some methodological issues need to be considered first. For practical reasons, the experiments were per-

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Table 2. Effect of colonic infusion of SCFAs, lactose, saline, and hypertonic saline solutions on number of reflux episodes and esophageal acid exposure

<table>
<thead>
<tr>
<th></th>
<th>First Experiment, n=8</th>
<th>Second Experiment, n=7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time, min</td>
<td>Saline</td>
<td>Lactose</td>
</tr>
<tr>
<td>Baseline fasting</td>
<td>60</td>
<td>0.2±0.2</td>
</tr>
<tr>
<td>Infusion fasting</td>
<td>60</td>
<td>0.8±0.4</td>
</tr>
<tr>
<td>Postprandial</td>
<td>240</td>
<td>5.9±1.6</td>
</tr>
<tr>
<td>Eosophageal acid</td>
<td>Baseline fasting</td>
<td>60</td>
</tr>
<tr>
<td>Exposure, min</td>
<td>Infusion fasting</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Postprandial</td>
<td>240</td>
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Data are means ± SE. None of the differences between solutions was statistically significant.
formed on two separate occasions (i.e., saline vs. lactose in the first set of experiments and then hypertonic saline vs. SCFAs in the second set). Therefore, the four solutions were not administered in a completely random order. In fact, we were concerned about losing some subjects because of the relative invasiveness of the procedures. However, despite mild side effects, all subjects except one tolerated the procedure quite well, thus allowing crossover comparison for 7 of them. Moreover, the order of the two sets of experiments was not randomized since the rationale of the second one (SCFAs) was to explain the results obtained in response to lactose fermentation. In other words, the second set of experiments would not have been performed if the results of the first one had been completely negative.

The dose of lactose administered (30 g), although comparatively large, was calculated by reference to a previous work (22) in which infusion of 90 mmol SCFAs into the colon induced a profound relaxation of the proximal stomach. This amount roughly corresponds to the production of SCFAs resulting from complete fermentation of 20 g of disaccharides (30). In the present study, 30 g of lactose were considered to approximate the amount malabsorbed in lactase deficiency after consumption of 50 g of lactose orally (i.e., 1 l of cow’s milk). Hence, complete fermentation of 30 g of lactose would produce ~135 mmol of SCFAs, which corresponds to the quantity infused in the present experiments. Because of the mixing of this solution with colonic contents, the intracolonic concentrations of SCFAs were probably in the physiological or slightly supraphysiological range. The high proportion of subjects who experienced bloating and diarrhea further confirms that lactose infusion was consistently and effectively fermented by colonic flora.

The role of SCFAs in the observed effects is supported not only by the fact that exogenous SCFAs reproduced the effects of lactose but also by the delayed effect of lactose compared with SCFA infusion. Indeed, the peak number of postprandial TLESRs, and the peak decrease of LESP, occurred later after colonic infusion of lactose than after SCFAs. Together, these findings suggest that colonic infusion of lactose modulated LES function through the production of SCFAs after colonic fermentation of the disaccharide.

Although SCFAs typically reproduced the effects of lactose infusion, other factors, such as pH or osmolality, were also influenced by the fermentation process. In our experiments, the pH of the solution was kept constant but did not necessarily reflect intraluminal pH. Similarly, a subtle effect of osmolality cannot be entirely excluded, although it is noteworthy that the hypertonic saline solution (1,200 osmol/kg) was consis-
ently less effective than the less-hypertonic lactose solution (i.e., 600 osmol/kg). Therefore, it is likely that the effect (if any) of osmolality was of minor importance compared with that of SCFAs. Finally, it is also possible that gas production after lactose infusion might have stimulated mechanoreceptors sensitive to distension (17).

The mechanisms triggered by lactose fermentation and SCFA infusion can affect LES motility either directly or indirectly via an action on proximal stomach and/or gastric emptying. Among the different neurohormonal pathways, the role of intestinal regulatory peptides should be considered first. Indeed, some studies have suggested that PYY and proglucagon-derived peptides such as OLI are released by fat (14) and/or carbohydrates (13) into the ileum (14) and colon (22). These peptides may therefore play a role in the so-called ileocolonic brake. However, despite a rapid increase in both PYY and OLI plasma levels after colon infusion, this study confirms our previous results (22) suggesting that the release of these peptides is not related to specific nutrients but to mechanical stimulation of the colon. Other peptides that may play a major role in triggering TLESRs (e.g., CCK) were not specifically checked in this study because of the colonic site of infusion. Furthermore, the rapid onset of LES response to colonic infusion of SCFAs rather suggests a neural pathway. Interestingly, Azpiroz and Malagelada (1) showed in dogs that gastric relaxation induced by intestinal nutrients was mediated by fibers contained in the vagus nerves. Moreover, Gué et al. (9) observed that colonic distension-induced inhibition of gastric motility was suppressed by hexamethonium, suggesting that nicotinic ganglionic receptors are involved in the inhibitory cologastric pathway. They also showed that α-agonists such as fedotozine are able to block colonic distention-induced inhibition of gastric motility and emptying. In summary, it is conceivable that colonic exposure to SCFAs may influence LES function through a neural mediation.

Finally, TLESRs are triggered by gastric distension through a vago-vagal reflex involving nonadrenergic-noncholinergic neurons (2). Although a direct effect of colonic fermentation on LES motility through unidentified specific pathways cannot be completely excluded, several studies have suggested that gastric motility may play an important role. Indeed, we have shown that oral lactulose administration as well as colonic exposure to exogenous SCFAs markedly influenced gastric tone (22). Colonic exposure to SCFAs may also increase the number of reflux episodes by delaying gastric emptying. For example, Jain et al. (13) have reported that blockade of carbohydrate digestion by an amylose inhibitor induced colonic fermentation associated with slower gastric emptying. In addition, the gastric emptying of a second meal was delayed after ingestion of a first meal containing unabsorbed carbohydrates (16). We and others have also observed either more prolonged (20) or profound (34) relaxation of the proximal stomach in response to a liquid meal in patients with GER disease (34). It is conceivable that a more profound relaxation of the proximal stomach can affect the mechanisms triggering TLESRs. Finally, SCFA production may result in decreased gastric tone and delayed emptying, conditions known to be associated with GER disease (23, 34).

The relevance of our findings to clinical situations is unclear. Although our healthy volunteers did not report heartburn or regurgitation, extrapolation from these negative findings to patients with clinical GER disease is not feasible. Similarly, the role of LES dysfunction or gastric motor disturbances on symptoms observed in patients with lactase deficiency cannot be ascertained.

In summary, we have shown that colonic fermentation, through the production of SCFAs, exerts a controlled feedback on LES motor function. Whether the mechanisms of this phenomenon are of hormonal nature, neural nature, or both remains to be determined.


