Duodenal motility in fasting dogs: humoral and neural pathways mediating the colonic brake

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Wen, J., E. Luque-de Leon, L. J. Kost, M. G. Sarr, and S. F. Phillips. Duodenal motility in fasting dogs: humoral and neural pathways mediating the colonic brake. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G192–G195, 1998.—We have previously described a negative feedback loop that inhibits duodenal motility when nutrients are infused into the ileum and colon. In this study, we examined role of extrinsic innervation and plasma levels of peptide YY (PYY) in inhibiting this phenomenon. We perfused a similar loop (n = 5 dogs) or extrinsically denervated (n = 6 dogs) isolated loops of proximal colon with isomolar NaCl or a mixed-nutrient solution at 2 and 6 ml/min for 4 h during fasting or for 2 h beginning 15 min after a meal. Both rates of infusion with NaCl prolonged the cycle length of the duodenal migrating motor complex (MMC) in the group with neurally intact loops but not in the group with extrinsically denervated loops. Nutrient infusions increased the MMC cycle length in both groups. Integrated plasma concentrations of PYY were increased by nutrients but not by NaCl in both groups. These data suggest that increased volumes and unabsorbed nutrients in the proximal colon alter proximal small bowel motility. Volume-induced effects are mediated via extrinsic nerves, whereas nutrient-induced effects may be mediated by humoral factors, such as plasma PYY with inhibition of the duodenal MMC in response to ileal nutrients (26). Moreover, exogenous PYY at a physiological dose mimicked the effects of ileal nutrients on upper gut motility. In these experiments, colonic perfusion of nutrients also inhibited duodenal MMCs and increased plasma PYY. However, our experimental preparation did not preclude ileal reflux of colonic infusates (26).

The aim of the present experiments was twofold: first, to further document the effects of colonic nutrients and distension on small bowel motility and second, to explore the mechanisms of colonic feedback control by utilizing dogs with extrinsically denervated colonic loops.

MATERIALS AND METHODS

Preparation of animals. Eleven healthy female mongrel dogs, weighing 19–22 kg, were divided into two groups. Enterically isolated colonic loops with the extrinsic nerves intact were created in five dogs (group 1); enterically isolated and extrinsically denervated isolated loops were fashioned in the other six dogs (group 2). While the dogs were under anesthesia induced with pentobarbital sodium and maintained with Fluthane, a midline celiotomy was performed. Atropine (0.1 mg/kg im) was given to prevent shortening of the bowel and to standardize the test segments. Isolated colonic loops of 50 cm in length were created, beginning immediately distal to the ileocolic junction. The proximal end of the loop was oversewn, and a polyvinyl catheter (1 mm ID, 2 mm OD) was inserted immediately beyond the proximal end to serve as a site for colonic perfusion. The distal end was brought to the abdominal wall as an end colostomy. Intestinal continuity was restored by an end-to-end ileocolic anastomosis. Four manometric catheters (0.5 mm ID, 1.5 mm OD) were implanted in the small bowel, with two in the duodenum (10 and 20 cm distal to the pylorus) and two in the ileum (20 and 10 cm proximal to the anastomosis).

In group 2, a similar loop was prepared. In addition, all nerves, lymphatics, and connective tissue accompanying the middle colonic vessels to the colonic loop were transected and ligated under optical magnification. Also, the middle colic artery and vein were stripped of their adventitia for 2 cm. The only connection between the colonic loop and the dog was through this artery and vein (19). As described before (19), for comparable extrinsic denervation of the canine jejunum and ileum, levels of catecholamines in the bowel wall were measured subsequently on tissues removed at the time of death and frozen immediately.

Animals were allowed 2–3 wk to recover from the operation, during which they were trained to stand in a Pavlov sling. The study was approved by the Institutional Animal Care and Use Committee of the Mayo Clinic. Surgical procedures and experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892].

Experimental procedures. Manometric catheters perfused with deionized water (0.1 ml/min) by a low-compliance perfu-
tion system using a nitrogen pressure of 10 psi were connected to strain gauge transducers (DT-XX, Viggo-Spectramed, Oxnard, CA). The signals were simultaneously displayed on a MFE 1600 chart recorder and collected by an IBM XT computer at a sample rate of 10 Hz for further analysis. Before each experiment, dogs were fasted for at least 18 h but were allowed free access to water.

The duodenum was monitored manometrically, and the isolated colonic loops were perfused immediately after a duodenal phase III contraction was recorded. Infusions were at rates of 0 (unperfused control), 2, and 6 ml/min and lasted for two complete MMC cycles or 240 min. Studies were performed on different days and in random order. Colonic infusates were 0.9% NaCl (volume control, to determine the effects of colonic distension) and Ensure Plus (Ross Laboratories, Columbus, OH), diluted in water to an osmolality of 300 mosmol/kg and at a pH of 7.4. This mixed nutrient perfusate contained 67 cal/100 ml, 55% carbohydrate, 28% fat, and 17% protein.

In another set of experiments, the colon was perfused postprandially. After an overnight fast, the saline and Ensure were infused at rates of 0 and 6 ml/min. Fifteen minutes after a phase III contraction was recorded in the duodenum, a 900-kcal meal (Alpo Petfoods, Lehigh Valley, PA) was given and the motility was monitored for 2 h.

To measure plasma concentrations of PYY, blood samples were collected immediately before saline or Ensure was infused (0 min) and at 15, 30, 45, 60, 90, 120, 150, 180, 210, 240 min thereafter. The samples were immediately separated, and the plasma was stored at −20°C for assay of PYY according to a previously described radiomunoassay (17).

Data analysis. For fasting studies, duodenal and ileal manometric tracings were assessed visually to detect the phasic periodicity of the MMCs. After infusions began, MMC cycles were sought for 240 min; if phase III contractions had not returned during this period, the cycle length of the MMC was expressed as >240 min and calculated statistically as 240 min. For postprandial studies, duodenal and ileal motility was analyzed for 120 min after the meal by a computer program and expressed as the motility index: \( \log(e) \) (number of peaks × sum of peak amplitudes + 1).

 Plasma levels of PYY were expressed individually at each sampling time and also as an integrated response (21). All dogs were studied twice on separate days. For each dog, an average for each parameter was calculated from the two studies and used for statistical analysis. Statistical tests included paired or unpaired t-tests, using a statistical analysis program in Microsoft Excel. The data are shown as means ± SE, and P < 0.05 was considered significant.

**RESULTS**

All dogs developed diarrhea and experienced slight weight loss (5-10%) during the first 2 wk postoperatively. Stools were loose for 2–4 wk but thereafter were firm. After this recovery period, dogs remained healthy throughout the study with good appetites and stable body weights.

Under basal conditions (without colonic perfusion), the cycle lengths of duodenal and ileal MMCs were not different in innervated and denervated loops (Table 1). The duration of duodenal (16 ± 1 min) and ileal (7 ± 1 min) phase III contractions and their qualitative features were also not different.

<p>| Table 1. Cycle length of duodenal and ileal MMCs during perfusion of colonic loops |
|----------------------------------|----------------|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Unperfused</th>
<th>Saline (2 ml/min)</th>
<th>Ensure (2 ml/min)</th>
<th>Saline (6 ml/min)</th>
<th>Ensure (6 ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>107 ± 5</td>
<td>138 ± 13*</td>
<td>152 ± 15</td>
<td>148 ± 11*</td>
<td>188 ± 15*†</td>
</tr>
<tr>
<td></td>
<td>(2 ml/min)</td>
<td>(2 ml/min)</td>
<td>(2 ml/min)</td>
<td>(6 ml/min)</td>
<td>(6 ml/min)</td>
</tr>
<tr>
<td></td>
<td>(114 ± 11</td>
<td>145 ± 9</td>
<td>148 ± 28</td>
<td>130 ± 12</td>
<td>191 ± 26*</td>
</tr>
<tr>
<td></td>
<td>107 ± 8</td>
<td>117 ± 10</td>
<td>123 ± 12</td>
<td>126 ± 6</td>
<td>149 ± 18*</td>
</tr>
</tbody>
</table>

Values are means ± SE in min; n = no. of dogs. MMCs, migrating motor complexes. Nos. in parentheses are duodenal ranges. *P < 0.05 vs. unperfused control. †P < 0.05 vs. saline control.

Nonnutrient infusions. Saline at 2 and 6 ml/min significantly delayed duodenal MMC cycles in the innervated loops (Table 1); variability in the length of ileal cycles was such that comparable quantitative changes were not significant. Durations and characteristics of phase III contractions in the duodenum and ileum were not altered by saline perfusions.

Perfusion of extrinsically denervated loops with saline at both rates did not delay duodenal and ileal MMCs (Table 1).

Nutrient infusions. Ensure delayed the appearance of phase III contractions in the duodenum and ileum of both innervated and extrinsically denervated loops. A trend toward a dose response with the 6 ml/min rate caused more delay. At the faster rates of perfusion, duodenal MMCs were delayed more by nutrient than by saline (Table 1).

Effects of infusions on plasma concentrations of PYY. Saline at 2 or 6 ml/min did not increase plasma PYY concentrations in dogs in group 1 or 2 (Figs. 1 and 2). Perfusion with Ensure increased blood levels of PYY, and peak increases occurred at 60–90 min after infusions began (data not shown). The integrated PYY responses to Ensure at 2 and 6 ml/min were increased above the saline perfusion levels in every dog. Re-
plasma concentrations of PYY. Values are means ± SE; n = 5 dogs. *P = 0.06.

responses to 2 and 6 ml/min Ensure were increased significantly in both groups (Figs. 1 and 2). However, linear regressions between delays of MMCs and PYY responses were not significant.

Effects of infusions on postprandial motility. Postprandial motility indexes in the duodenum and ileum were calculated for three dogs in each group for the different solutions and rates. None of the infusates significantly altered postprandial duodenal or ileal motility indexes.

Tissue concentrations of catecholamine. Catecholamines were measured in colonic tissue from three dogs in group 1 and four dogs in group 2. Tissue levels were markedly depleted in segments of colon that were extrinsically denervated, but were not different between groups in the ileum or the rectosigmoid, regions that were not denervated (Table 2).

**DISCUSSION**

We have reported that colonic perfusion with a mixed nutrient solution (Ensure) prolonged the cycle length of the duodenal MMC (26), implying the presence of a colonic brake in dogs. We also tested individual nutrients, with protein hydrolysates being the most active. A mechanism for this phenomenon, through the release of PYY, was suggested by the finding of elevated circulating levels of PYY in parallel with negative feedback to the duodenum. Finally, intravenous infusions of PYY in physiological amounts mimicked the effects of colonic perfusion. However, the canine preparation (26) featured an enterically isolated ileocolic loop, and reflux of colonic infusates into the ileum could not be excluded. The preparations we used here were fully isolated loops of colon, and the present data clearly demonstrate that the colon participates in feedback control of proximal motility. Moreover, the data also demonstrate that unabsorbed nutrients reaching the proximal large bowel are a probable stimulus.

It is likely that the colonic brake phenomenon is important physiologically. Numerous common items of food are digested and absorbed completely in the small intestine (2, 11, 24); indeed, the term "unavailable (dietary) carbohydrate" has been used. It is of potential clinical interest that unabsorbed dietary residues reaching the colon could alter the functions of the proximal gut. For example, carbohydrate residues infused into the colon (2, 11, 24) and their metabolic end products (short-chain fatty acids) have been shown to change gastric tone and compliance in humans (18). The amount of dietary residues infused was relevant to normal digestion and absorption, and even more so, to malabsorption states (18). Mechanisms such as these, whereby the colon and the proximal bowel communicate, could therefore be relevant to a variety of symptoms.

We proposed earlier that PYY participated in the ileal brake, as monitored by the duodenal MMC (26). This concept, which has been suggested by several groups (3, 7, 10, 22), received additional support by the finding that immunoneutralization of PYY reversed the inhibitory consequences of ileal infusions of lipid (12). The colon per se has received less attention. By using enterically isolated colonic loops, we confirmed the presence of a canine colonic brake and were able to implicate PYY as a mediator in the response to nutrients, just as was noted in the ileum (26). The observation was not unexpected, since canine ileal and colonic mucosa contain equally high concentrations of PYY (25). Comparing our experimental systems, the colon was somewhat less sensitive than the ileum; the faster infusions of Ensure (6 ml/min) inhibited duodenal motility to lesser degrees than did 2 ml/min perfusion of the ileum (26), although circulating levels of PYY were similar between the two sets of experiments. In the present experiments, we were again unable to show a correlation between levels of circulatory PYY and MMC delays, but our numbers are small. We did not measure glucagon-like peptide-1 (GLP-1) (5) in the present experiments, since our earlier observations indicated that this peptide was probably of secondary importance in our model. The role of GLP-1 in humans is still unclear (10, 18).

These results also have implications as to possible separate mechanisms whereby feedback signals from colonic distension and nutrient stimulation are mediated. Availability of colonic loops that had been extrinsically denervated was extremely useful in this regard. Thus perfusion of the colonic loops with saline was

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**Table 2. Catecholamine concentrations in canine bowel**

<table>
<thead>
<tr>
<th>No.</th>
<th>Ileum</th>
<th>PC</th>
<th>MC</th>
<th>DC</th>
<th>Rectosigmoid</th>
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</thead>
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<tr>
<td></td>
<td>Innervered loop</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>117</td>
<td>286</td>
<td>334</td>
<td>556</td>
<td>267</td>
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<tr>
<td>2</td>
<td>307</td>
<td>246</td>
<td>403</td>
<td>634</td>
<td>202</td>
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<td>3</td>
<td>370</td>
<td>10</td>
<td>10</td>
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<tr>
<td>Mean</td>
<td>213</td>
<td>262</td>
<td>310</td>
<td>420</td>
<td>423</td>
</tr>
<tr>
<td></td>
<td>Denervated loop</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>170</td>
<td>10</td>
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<td>365</td>
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<td>314</td>
</tr>
<tr>
<td>Mean</td>
<td>303</td>
<td>13</td>
<td>40</td>
<td>10</td>
<td>434</td>
</tr>
</tbody>
</table>

Values given in ng/g tissue; value of 10 = background. The ileum and rectosigmoid were not extrinsically denervated. PC, proximal colon; MC, midcol; DC, distal colon.
considered a distension stimulus. Saline inhibited duodenal motility when the colon was neurally intact, and this negative feedback occurred without a detectable rise in circulating PYY levels. When extrinsically denervated colonic loops were perfused with saline, there was also no PYY response, but duodenal motility was not inhibited, suggesting that an extrinsic neural mechanism mediated the response to colonic distension. In our earlier experiments (26), ileocolic loops were perfused with saline at 2 ml/min only. There was no inhibition of duodenal MMCs.

By contrast, extrinsically denervated colonic loops responded to nutrient infusions no differently than did the neurally intact bowel. In both groups, Ensure evoked release of PYY that was associated with inhibition of the duodenal MMC, suggesting humoral mediation of the response to nutrients (together with perfusion and distension) in contrast to the neural mechanism for stimuli generated by distension alone.

The model of in situ neural isolation of the colonic loop used here has been employed extensively and successfully for the stomach and small intestine (19). Though the middle colic vessels were not severed, all other tissues to the colonic loop were cut, and these mesenteric vessels were skeletonized for a length of 2 cm. Catecholamines disappeared from denervated tissues, although not from adjacent segments of neurally intact bowel, confirming the selective state of extrinsic denervation and allowing us to be confident about our mechanistic interpretations.

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