Intestinal fat-induced inhibition of meal-stimulated gastric acid secretion depends on CCK but not peptide YY

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Zhao, Xiao-Tuan, John H. Walsh, Helen Wong, Lijie Wang, and Henry C. Lin. Intestinal fat-induced inhibition of meal-stimulated gastric acid secretion depends on CCK but not peptide YY. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G550–G555, 1999.—Fat in small intestine decreases meal-stimulated gastric acid secretion and slows gastric emptying. CCK is a mediator of this inhibitory effect (an enterogastrone). Because intravenously administered peptide YY (PYY) inhibits acid secretion, endogenous PYY released by fat may also be an enterogastrone. Four dogs were equipped with gastric, duodenal, and midgut fistulas. PYY antibody (anti-PYY) at a dose of 0.5 mg/kg or CCK-A receptor antagonist (devazepide) at a dose of 0.1 mg/kg was administered alone or in combination 10 min before the proximal half of the gut was perfused with 60 mM oleate or buffer. Acid secretion and gastric emptying were measured. We found that 1) peptide-induced gastric acid secretion was inhibited by intestinal fat (P < 0.0001), 2) inhibition of acid secretion by intestinal fat was reversed by CCK-A receptor antagonist (P < 0.0001) but not by anti-PYY, and 3) slowing of gastric emptying by fat was reversed by CCK-A antagonist (P < 0.05) but not by anti-PYY. We concluded that inhibition of peptide meal-induced gastric acid secretion and slowing of gastric emptying by intestinal fat depended on CCK but not on circulating PYY.

GASTRIC ACID SECRETION is inhibited by nutrients in the small intestine via mediators known as enterogastrones (14). Fat is the most potent nutrient for this response (7). Several mediators released by fat in the small intestine have been postulated as enterogastrones (8). With the use of devazepide, a CCK-A receptor antagonist, CCK has been shown to be an enterogastrone (13, 26). CCK antagonism did not, however, completely reverse fat-induced inhibition of acid secretion, suggesting contribution by other enterogastrones. Because fat in the small intestine releases peptide YY (PYY), either directly by contact with distal gut PYY-releasing cells (3, 12, 35) or indirectly (proximal gut fat) via a CCK-stimulated mechanism (16), and because PYY has been shown to inhibit gastric acid secretion when given intravenously (17), this peptide may also be an enterogastrone (1). However, the effect of PYY administered systemically may differ from the effect of this peptide released endogenously by intestinal fat, which can only be examined with peptide antagonism or peptide immunoneutralization. In this study, we tested the hypothesis that inhibition of meal-stimulated gastric acid secretion and emptying by fat in the proximal small intestine depended on CCK and PYY in fistulated dogs using CCK antagonist and PYY immunoneutralization techniques.

MATERIALS AND METHODS

General experimental design. Four dogs were equipped with gastric, duodenal, and midgut fistulas. Buffer or fat was confined to the proximal half of gut (between duodenal and midgut fistulas) while 8% peptone meal was instilled into the stomach via the gastric fistula to stimulate acid secretion. To test the role of PYY, nonspecific IgG or antibody to PYY (anti-PYY) was administered intravenously. To test the role of CCK, devazepide (CCK-A receptor antagonist) or vehicle was administered intravenously. Gastric acid output and gastric emptying were compared among the following five groups (described according to the following order of listing: perfusate, PYY antibody, CCK antagonist): 1) buffer perfusion with nonspecific IgG and vehicle (buffer group), 2) fat perfusion with nonspecific IgG and vehicle (buffer group), 2) fat perfusion with nonspecific IgG (fat group), 3) fat perfusion with specific IgG and devazepide (devazepide group), 4) fat perfusion with anti-PYY and vehicle (anti-PYY group), and 5) fat perfusion with anti-PYY and devazepide (anti-PYY and devazepide group). A randomization schedule was followed. Gastric acid secretion and gastric emptying were measured during a 2.5-h experiment in five 30-min peptone meal-stimulated periods.

Preparation of animals. The procedures used in this study were approved by the Institutional Animal Care and Use Committee at Cedars-Sinai Medical Center (Los Angeles, CA). Four mongrel dogs (22–25 kg) were each surgically equipped with chronic gastric, duodenal, and midintestinal fistulas (18). Modified Thomas cannulas (18) were placed into the fistulas located in the antrum and the small intestine at ~10 cm (duodenal fistula, distal to the biliary and pancreatic ducts) and ~160 cm (midintestinal fistula) from the pylorus. J ust distal to the intestinal fistulas, a length of Tygon tubing (diameter of 2 mm) was looped around the small intestine and fixed by suture through the visceral peritoneum to the intestinal wall. The length of tubing used was individualized to be as short as possible without causing a tightening effect on the lumen. All dogs were allowed to recover after surgery for 4 wk and underwent testing only after normal feeding behaviors were reestablished postoperatively. This preparation was well tolerated by all four dogs, with the animals remaining healthy and showing stable weights and unaffected demeanor during the entire experimental period.

Peptone meal and perfusates. Oleate (60 mM) (Fisher Scientific, Fair Lawn, NJ) was prepared as a solution of mixed micelles with monolein (Eastman Organic Chemicals, Rochester, NY) and 10 mM taurocholate (Sigma Chemical, St. Louis, MO) in pH 7.0 phosphate buffer (21). Peptone (Bacto Peptone, Difco, Detroit, MI) was dissolved in water to make an 8%
solution (wt/vol). The 8% peptone solution was adjusted to pH 5.5 with 6.25 N HCl. Phenol red (Mallinckrodt, Paris, KY) was added to the peptone meal to achieve a final phenol red concentration of 5%.

Experimental preparations. Dogs were deprived of food but not water for 18 h before each experiment. Thirty minutes before the start of each experiment, the gastric, duodenal, and midgut fistulas were uncurled, and the stomach and the openings of intestinal fistulas were rinsed with water so that a Foley catheter could be placed into the distal limb of each intestinal cannula. By inflating the catheter balloons with 10 ml of water and cinching the balloon up against the Tygon ring, a watertight seal was achieved at each intestinal fistula. With the use of this method, a 150-cm test segment was isolated between fistulas. The output of each fistula was allowed to drain freely by gravity. A buffer (no oleate) or 60 mM oleate was perfused into the 150-cm test segment beginning 10 min before the third peptone-stimulation period.

Measurement of peptone-stimulated gastric acid secretion. Two 15-min basal gastric secretions were collected by gravity drainage via the gastric fistula. A 100-µl sample of acid output was titrated with 0.1 N NaOH to pH 7.0 using an automatic titrator (Radiometer, Copenhagen, Denmark). After 30 min, 300 ml of 8% peptone (wt/vol) liquid meal was instilled via the gastric cannula, and acid output was measured by intragastric titration to pH 5.5 with 0.5 N NaOH following a previously described technique (21). The test meal was collected after 30 min through the gastric cannula, and the output volume was measured. A new meal consisting of 300 ml of 8% peptone was then instilled into the stomach. This procedure was repeated every 30 min during a 2.5-h experiment, resulting in five 30-min peptone-stimulated periods. The results are given as the acid output (in mmol) per 30 minutes.

Measurement of gastric emptying. A modification of the double-dye dilution technique was used to measure gastric emptying rate (26). The volume of gastric acid secretion and the fluid added for intragastric titration were all accounted for in the calculations, following the technique described by Lloyd et al. (26). The results were calculated as the mean rate of emptying (ml/min).

Intravenous test agents. The CCK-A receptor antagonist devazepide (a gift from Merck) at a dose of 0.1 mg/kg was dissolved in vehicle consisting of 2 ml PEG-400 (Sigma), 2 ml glycerol (Sigma), and 0.5 ml 0.15 M NaCl (6). In the control experiment, vehicle alone was administered.

A polyclonal antibody to PYY (anti-PYY) was provided by H. Wong of the Antibody Core Laboratory of CURE: Digestive Diseases Research Center (Los Angeles, CA) (4). Nonspecific IgG from normal rabbit was used for the control experiment.

Nonspecific IgG or anti-PYY at a dose 0.2 mg/kg was administered intravenously 10 min before the start of the third 30-min period for the five test groups are shown in Table 1. Acid outputs during the last 30-min peptone-stimulated period for the five test groups are shown in Table 1. Under buffer perfusion with intravenous nonspecific IgG and vehicle (buffer group), mean acid secretion in response to intragastric peptone increased from 0.6 ± 0.1 mmol/30 min at the end of the third 30-min period. Peptone-stimulated acid output then remained stable during the last three 30-min periods (Fig. 1).

Table 1. Gastric acid secretion during last 30-min period of peptone meal

<table>
<thead>
<tr>
<th>Condition</th>
<th>Perfusate in Proximal Half of Gut</th>
<th>Agent</th>
<th>Acid Output, mmol/30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>Buffer</td>
<td>Control</td>
<td>20.9 ± 1.3</td>
</tr>
<tr>
<td>Fat</td>
<td>60 mM oleate</td>
<td>Control</td>
<td>6.0 ± 1.1</td>
</tr>
<tr>
<td>Devazepide</td>
<td>60 mM oleate</td>
<td>Devazepide alone</td>
<td>21.4 ± 2.5</td>
</tr>
<tr>
<td>Anti-PYY</td>
<td>60 mM oleate</td>
<td>Anti-PYY alone</td>
<td>6.3 ± 1.6</td>
</tr>
<tr>
<td>Anti-PYY</td>
<td>60 mM oleate + devazepide</td>
<td>Anti-PYY + devazepide</td>
<td>22.3 ± 2.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4 dogs. Buffer or 60 mM oleate was perfused into proximal half of the gut at 2 ml/min, starting at the beginning of the third 30-min period of peptone meal. Vehicle and nonspecific IgG (0.5 mg/kg) were administered intravenously in control groups. Intravenous IgG (0.1 mg/kg) was administered as a bolus. Anti-peptide YY (PYY) (0.5 mg/kg) was given as a bolus. All 4 agents were given 10 min before the start of the third 30-min period of peptone meal.

RESULTS

Gastric acid secretion. The time courses of gastric acid secretion during basal and during the five 30-min peptone-stimulated periods are shown in Fig. 1. Acid outputs during the last 30-min peptone-stimulated period for the five test groups are shown in Table 1. Under buffer perfusion with intravenous nonspecific IgG and vehicle (buffer group), mean acid secretion in response to intragastric peptone increased from 0.6 ± 0.1 mmol/30 min at the end of the third 30-min period. Peptone-stimulated acid output then remained stable during the last three 30-min periods (Fig. 1).
When 60 mM oleate was perfused into the proximal gut with nonspecific IgG and vehicle (fat group), the mean acid output during the last 30-min period of peptone meal decreased to 6.0 ± 1.1 mmol/30 min, consistent with inhibition of gastric acid secretion by intestinal fat, and was significantly different from the buffer group result of 20.0 ± 0.6 mmol/30 min (P < 0.0001).

Fat-induced inhibition of acid secretion was totally reversed by intravenous devazepide (devazepide group), with a mean acid output of 21.4 ± 2.5 mmol/30 min during the last 30-min period, which was not different from the buffer group result (20.9 ± 1.3 mmol/30 min).

Intravenous administration of anti-PYY (anti-PYY group) at a dose of 0.5 mg/kg had no effect on fat-induced inhibition of peptone-stimulated acid secretion, having a mean acid output of 6.3 ± 1.6 mmol/30 min, which was not different from the control experiment (6.0 ± 1.1 mmol/30 min), whereas combining anti-PYY and devazepide produced the same effect as devazepide alone, resulting in a mean acid output of 22.3 ± 2.8 mmol/30 min (Table 1, Fig. 1).

Gastric emptying. The rates of gastric emptying during basal and the five 30-min peptone-stimulated periods are shown in Fig. 2. The gastric emptying rates during the last 30-min peptone-stimulated period were compared among the five test groups (Table 2). Under buffer group conditions, 8% peptone meals emptied at a constant rate throughout all five peptone periods (8.4 ± 0.4 ml/min).

The mean gastric emptying rate was reduced significantly to 6.2 ± 0.1 ml/min when 60 mM oleate was perfused into the proximal half of the gut (fat group) compared with that in the buffer group (P < 0.001) (Table 2 and Fig. 2).

The slowing of gastric emptying by intestinal fat was totally abolished by intravenous administration of devazepide either alone (8.2 ± 0.2 ml/min) or combined with intravenous administration of anti-PYY (8.4 ± 0.1 ml/min) (P < 0.01) vs. results for the fat group (6.2 ± 0.1 ml/min). However, intravenous anti-PYY alone (6.4 ± 1.0 ml/min) did not reverse the slowing of gastric emptying by intestinal fat (Table 2 and Fig. 2).

**DISCUSSION**

Intestinal fat potently inhibits gastric acid secretion as a part of the intestinal phase of the control of gastric acid secretion (8). In response to fat, inhibitory peptides known as enterogastrone (8) are released to inhibit gastric acid secretion. PYY has been considered a possible enterogastrone because of its fulfillment of the three requirements that must be fulfilled for a substance to be characterized as an enterogastrone: 1) release of the substance must achieve a plasma level above baseline in response to intestinal fat, 2) systemic administration of the substance in a dose that results in a physiological plasma level should inhibit gastric acid secretion, and 3) receptor antagonism or in vivo immunoneutralization of the substance should abolish the inhibitory effect of intestinal fat on gastric acid secretion. PYY has been considered a possible enterogastrone because of its fulfillment of the first two requirements. Specifically, after a fatty meal, the plasma level of PYY rises above baseline (36) and intravenous administration of PYY inhibits meal-stimulated gastric acid secretion (32).

Because the effect of PYY administered systemically may be different from that of the endogenous PYY released by intestinal fat, to fully test the role of PYY as

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**Table 2. Gastric emptying rate of last 30-min period of intagastric titration with peptone meal**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Perfusate in Proximal Half of Gut</th>
<th>Agent</th>
<th>Gastric Emptying, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>Buffer</td>
<td>Control</td>
<td>8.4 ± 0.4</td>
</tr>
<tr>
<td>Fat</td>
<td>60 mM oleate</td>
<td>Control</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td>Devazepide</td>
<td>60 mM oleate</td>
<td>Devazepide alone</td>
<td>8.2 ± 0.2</td>
</tr>
<tr>
<td>Anti-PYY</td>
<td>60 mM oleate</td>
<td>Anti-PYY alone</td>
<td>6.4 ± 1.0</td>
</tr>
<tr>
<td>Anti-PYY + devazepide</td>
<td>60 mM oleate</td>
<td>Anti-PYY + devazepide</td>
<td>8.4 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4 dogs. Buffer or 60 mM oleate was perfused into proximal half of the gut at 2 ml/min, starting at the beginning of the third 30-min period of peptone meal. Vehicle and nonspecific IgG (0.5 mg/kg) were administered intravenously in control groups. Intravenous devazepide (0.1 mg/kg) was administered as a bolus. Anti-PYY (0.5 mg/kg) was given as a bolus. All 4 agents were given 10 min before the start of the third 30-min period of peptone meal.
an enterogastrone, we intravenously administered a polyclonal antibody to PYY. We found that PYY immunoneutralization did not reduce the inhibition of gastric acid secretion or gastric emptying by intestinal fat. Thus PYY does not appear to be an enterogastrone.

The PYY antibody used in this study has been shown to be highly effective in an experiment that demonstrated complete reversal of fat-induced ileal brake (21). The efficacy of this exact batch of antibody was also shown in a separate experiment in which the PYY antiserum was highly effective in fat-induced jejunal brake (20). Thus this study investigated the role of PYY under conditions in which PYY release was stimulated strongly and the PYY antibody was given at a dose that maintained a circulating antibody titer at $>1:500$ dilution (21) so that immunoneutralizing amounts of antibody were present during the study.

In contrast to the role of PYY, the role of CCK as an enterogastrone was also confirmed, corroborating previous reports (11). We found that CCK-A receptor antagonist reversed the inhibition of gastric acid secretion and gastric emptying by intestinal fat. PYY may be released by fat in the upper small intestine via a CCK-dependent pathway (17). Previously, with the use of devazepide, a CCK-A receptor antagonist, Lloyd et al. (26) reported that CCK antagonism reversed fat-induced inhibition of meal-stimulated acid secretion, suggesting that CCK was an enterogastrone.

Because PYY may be released by CCK, the observed effect of the CCK antagonist may have been due to its indirect effect on PYY (by lowering PYY level) rather than a direct effect of CCK as an enterogastrone. To sort out these possibilities of a direct vs. indirect effect of CCK, we compartmentalized the small intestine in our dog model so that fat was confined to the proximal half of the gut. In this system, because PYY release would depend on CCK, by comparing the effect of devazepide or anti-PYY each alone and then together, we tested whether CCK has an inhibitory action on gastric acid secretion and gastric emptying independent of PYY. Our findings that the effect of the combination of devazepide and anti-PYY was not different from the effect of devazepide alone supported the idea that CCK was an enterogastrone with a direct inhibitory action independent of the release of PYY.

This effect of CCK on acid secretion cannot be explained by the amount of CCK in the circulation after a fatty meal. Specifically, this effect of CCK is abolished by a vagotomy (22), as supraphysiological doses of CCK inhibit acid secretion via the release of somatostatin by gastric D cells, but physiological release by fat does not produce the blood concentrations required for these effects (27). Further support of the idea that the action of CCK depends on a neural pathway was reported by Reidelberger et al. (33); here, a difference was found between neural CCK inhibiting food intake and the level of circulating CCK that stimulates pancreatic secretion.

CCK may not be the only enterogastrone responsible for the inhibition of acid secretion; secretin has also been shown in a number of different species to play that role. Specifically, secretin is released by fat in dogs (37). When intravenous secretin was administered to produce plasma levels observed with oleic acid infusion, acid secretion was inhibited. In anesthetized rats (34) and in conscious dogs (6), acid output was greater following an antiserum to secretin.

In this study, we tested for the role of endogenous PYY released by fat using the technique of peptide immunoneutralization. The polyclonal PYY antibody used in this study had been shown to have no cross-reactivity with rat or human pancreatic polypeptide or neuropeptide Y at a dilution of 1:30,000 (33). In our previous study in the same dog model using this polyclonal antibody (19), we also confirmed that adequate PYY antibody activity was achieved after intravenous administration of this antibody at the 0.5 mg/kg dose used in this study.

Peptone at pH 5.5 results in gastric levels similar to those produced during a protein-rich meal (10, 28). The acid stimulation obtained during peptone is about two-thirds that of the maximal response to gastrin or pentagastrin (15, 28, 35). We therefore worked with submaximal levels of stimulation of acid secretion during our testing for the role of PYY and CCK in the inhibition of acid secretion.

Our results are consistent with other published reports on the inhibition of gastric acid secretion by intestinal fat (2). Lloyd et al. (22) reported in dogs that devazepide alone nearly completely reversed the inhibitory effect of fat on meal-stimulated gastric acid secretion. In addition, this same group (25) reported that somatostatin levels rise with intraduodenal fat perfusion and blockade of CCK-A receptor with devazepide abolished this response. These observations suggest that inhibition of acid secretion by intestinal fat may depend on endogenously released CCK via somatostatin. Somatostatin may be the critical inhibitory mediator for the inhibition of gastric acid secretion by intestinal fat. Intravenous CCK has also been shown to increase plasma somatostatin level (24, 27). In a rat model, both neural and hormonal mechanisms were found to be responsible for the inhibitory effect of intestinal fat, with somatostatin identified as the dominant mediator of the hormonal mechanism (31). Thus CCK may inhibit gastric acid secretion and emptying via somatostatin rather than PYY. Indirect evidence is available to support our conclusion that inhibition of gastric emptying does not depend on PYY. In a human study of patients who have undergone jejunoileal bypass for obesity, PYY release was found to be abnormally elevated even in the setting of accelerated gastric emptying (29).

Our findings with PYY immunoneutralization contrast with published results using exogenous PYY injected systemically (32) or intracerebroventricularly (39). These studies have generally found systematically delivered PYY to be effective in suppressing gastric acid secretion (22, 32) and intracerebroventricularly delivered PYY was effective in stimulating acid secretions (39). Pappas et al. (32) found that exogenous PYY inhibited meal-stimulated gastric acid secretion. Wet-
tergren et al. (38) also reported that PYY suppression of acid secretion was even more effective when given with glucagon-like peptide-1. Vagally stimulated acid secretion was inhibited by intravenous PYY (24).

In contrast, Yang and Tache (39) reported stimulation of gastric acid secretion with PYY administered intracerebroventricularly. The differing effects of exogenous PYY and endogenous PYY are not surprising, since these studies differ not only in the route of administration but also supraphysiological doses of PYY were often used (30).

In this study, CCK-A receptor antagonism but not PYY immunoneutralization significantly abolished the slowing of gastric emptying by intestinal fat. The importance of endogenously released CCK in the inhibitory effect of intestinal fat is well supported by published reports showing similar response using the CCK receptor antagonist loxiglumide (2).

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