Inhibition of gastric emptying in response to intestinal lipid is dependent on chylomicron formation

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1Center for Ulcer Research and Education/Digestive Diseases Research Center, Departments of Physiology and Medicine, University of California Los Angeles School of Medicine, Veterans Affairs Medical Center West Los Angeles, Los Angeles, California 90073; 2Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710; and 3Department of Pathology, University of Cincinnati, Cincinnati, Ohio 45267

Raybould, Helen E., James H. Meyer, Yuri Tabrizi, Rodger A. Liddle, and Patrick Tso. Inhibition of gastric emptying in response to intestinal lipid is dependent on chylomicron formation. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1834–R1838, 1998.—Lipid in the intestine initiates feedback inhibition of proximal gastro-intestinal function and food intake. In rats and humans, inhibition of gastric emptying is mediated, at least in part, by cholecystokinin (CCK)-A receptors, and in rats there is evidence for involvement of an intestinal vagal afferent pathway. The mechanism by which luminal lipid acts to release CCK or activate vagal afferent nerve terminals is unclear. The role of chylomicron formation in this sensory transduction pathway has been investigated using the hydrophobic surfactant Pluronic L-81 that inhibits chylomicron formation. Gastric emptying of liquids was measured in awake rats fitted with a Thomas gastric fistula and a duodenal cannula. Intestinal perfusion of lipid induced a dose-dependent inhibition of gastric emptying (6, 12, and 39% inhibition for 25, 50, and 100 mg lipid, respectively). Perfusion of lipid with Pluronic L-81 (2.8% wt/vol) reversed the lipid-induced inhibition of gastric emptying. Pluronic L-63, a chemically similar surfactant that has no effect on chylomicron formation, had no effect on lipid-induced inhibition of gastric emptying. Perfusion of the intestine with lipid (100 mg) increased plasma levels of CCK from 1.9 ± 0.8 to 6.5 ± 1 pm. This increase was blocked by Pluronic L-81 but unaffected by L-63. These results provide evidence that chylomicron formation is important in the signaling of lipid in the intestinal lumen to CCK endocrine cells and to producing feedback inhibition of gastric emptying.

The presence of nutrients in the intestine is a potent stimulus for feedback regulation of proximal gastrointestinal function, including gastric emptying, acid secretion, intestinal motility, pancreatic secretion, and food intake. The different classes of macronutrients initiate the same repertoire of responses, but there is evidence that different macronutrients act via different neural and humoral pathways. However, responses to all nutrients seem to be, at least in part, dependent on the integrity of the extrinsic sensory nerves. Functional ablation of the vagal sensory pathway by perineural application of capsaicin to the vagus nerve markedly reduces the ability of nutrients to inhibit gastric emptying (8, 25), gastric acid secretion (17), and food intake (26) and to stimulate pancreatic secretion (13). There is evidence for a role for small intestinal afferent terminals in inhibition of gastric emptying in response to lipid, protein, and glucose (31), in stimulation of pancreatic secretion by protein (12), and in inhibition of food intake in response to lipid (28). Morphological evidence has demonstrated that the peripheral terminals of mucosal vagal afferents lie in the lamina propria of the duodenal villi with extensive terminal arborizations in the lamina propria beneath the epithelial cell layer (1). The sensory transduction mechanisms by which nutrients activate extrinsic afferents is unclear, but it is reasonable to hypothesize that the process involves the different cell types in the intestinal epithelium, including endocrine cells and absorptive enterocytes.

To further the understanding of the sensory transduction mechanisms, it is necessary to consider the pathways of digestion and absorption of each macronutrient. The major products of luminal lipid digestion are monoglyceride and fatty acids. These digestion products are absorbed into the enterocytes by diffusion and migrate from the site of uptake to the endoplasmic reticulum where the monoglyceride and free fatty acids are resynthesized into triglyceride (29). The triglyceride is packaged into chylomicrons and, removed from the enterocyte by exocytosis, diffuses through the lamina propria to the lacteals that are located centrally in the core of the lamina propria (29). Fatty acids of chain length greater than C10 are absorbed via chylomicron formation into the lymph, whereas fatty acids of chain length less than C10 simply diffuse out of enterocytes and pass predominantly into portal blood bound to albumin (29). It has been known for many years that lipids with chain lengths of C10 or higher inhibit gastric emptying (9). Ingestion of lipid increases plasma levels of cholecystokinin (CCK) in the human, dog, and rat (5, 6, 8, 11, 14, 15). In humans, there is evidence that only fatty acids with chain lengths greater than
C10 are effective in releasing CCK (20). Thus there is a correlation between the pathways of absorption and the ability of fats to initiate feedback responses and release CCK.

In the present study, we tested the hypothesis that chylomicron formation is an obligatory step in the ability of lipid to initiate feedback inhibition of gastric emptying. In a well-established model of intestinal feedback inhibition of gastric emptying in the awake rat, we previously showed that ~60% of the lipid-induced inhibition of gastric emptying is mediated by CCK-A receptors and the vagal afferent pathway (8). In the present study, we used Pluronic L-81, a nonionic hydrophobic surfactant shown by Tso et al. (30) to inhibit the formation of chylomicrons by enterocytes (23, 30), to assess the role of chylomicrons in signaling lipid content to the sensory nerve terminals. In addition, we determined whether inhibition of chylomicron formation is an important step in the lipid-induced release of CCK from endocrine cells.

METHODS

Animals. Experiments were performed using awake Sprague-Dawley rats (Harlan, San Diego, CA) of initial weight 240–280 g maintained on regular laboratory chow. Rats were fasted overnight but allowed water ad libitum before all surgical and experimental procedures.

Materials. Pluronic L-81 and L-63 were obtained from BASF (Wyandotte, MI) and were dissolved in ethanol and sonicated with lipid emulsion for 5 min immediately before use at a final concentration of 2.8% wt/vol. Lipid emulsion (Intralipid; Kabi Pharmacia, Clayton, NC) was diluted with 0.9% saline.

Surgical procedures. The procedure has been described in detail elsewhere (17). Briefly, rats (n = 19) were anesthetized with pentobarbital sodium (50 mg/kg ip; Nembutal, Abbott Laboratories, North Chicago, IL). A small stainless steel Thomas cannula was inserted into the forestomach, exteriorized through the abdominal wall, and capped. The duodenal cannula (PE-90, Intramedic; Clay Adams, Parsippany, NJ) was inserted into the duodenum 1–2 cm distal to the pylorus. Rats were allowed to recover for 2 wk before being used in experiments and were used for a period of up to 3 mo after surgery.

Rats (n = 30) for experiments to measure plasma levels of CCK were fitted with a duodenal cannula only and allowed to recover for at least 7 days before being used in experiments (8).

Measurement of gastric emptying. In the period during which rats were recovering from surgery, they were accustomed to light restraint in Bollman cages. The duodenal cannulas were flushed daily with 0.9% saline and plugged with petroleum jelly. On experimental days, fasted rats were placed in Bollman cages, the gastric cannula was opened, and any residual gastric contents were flushed out with warm 0.9% saline. The stomach was then allowed to drain freely for 30–45 min. Three milliliters of 0.9% saline containing the nonabsorbable marker phenol red (60 mg/ml) was instilled into the stomach, and the cannula was closed. After 5 min, the contents of the stomach were collected and the volume was measured and centrifuged. The concentration of phenol red was determined in the instilled and recovered fluid, and the rate of gastric emptying was calculated using the method of Hunt and Spurrell (10), which calculates the volume emptied from the stomach including any gastric secretions.

Experimental protocols. Gastric emptying was measured in two consecutive control periods with no perfusion of the duodenum, with 10 min between procedures. Perfusion of the duodenum with lipid (5, 10, or 20% Intralipid; total amounts 25, 50, and 100 mg) at a rate of 0.05 ml/min was started and continued for 10 min, and gastric emptying was measured in the 5–10 min period and again at 20–25 min and 35–40 min after duodenal perfusion with lipid. Only one test perfusion was performed on each day, and animals were used no more than every third day. Rats were randomized for dose of lipid and treatment. Rats were divided into two experimental groups: 1) intestinal perfusion of 25, 50, and 100 mg lipid with and without Pluronic L-81 (n = 12) and 2) 50 and 100 mg lipid perfused with or without Pluronic L-81 (2.8% wt/vol) and the effects compared with Pluronic L-63 (2.8% wt/vol) (n = 7). Not all rats received each treatment or dose of lipid because the full protocol could not be completed with some rats that were euthanized because of leaking gastric fistulas or duodenal cannula (n = 3).

Measurement of plasma levels of CCK. Rats were perfused (0.05 ml/min for 30 min) with either saline, lipid (100 mg), or lipid containing either Pluronic L-81 or L-63 prepared as described in Experimental protocols. Immediately at the end of the perfusion period, animals were deeply anesthetized with pentobarbital sodium (100 mg/kg ip). After a midline incision, blood was taken from the inferior vena cava and placed into chilled test tubes containing heparin and centrifuged, and the plasma was removed. Within 30 min of collection of blood, the plasma was flowed through Sep-Pak cartridges (Sep-Pak; Waters, Milford, MA), and then the Sep-Paks were stored at −70°C. CCK levels were measured using a bioassay as previously described (14).

Data analysis. Data for gastric emptying are expressed, as percent liquid emptied after 5 min, as mean ± SE. Values were compared using Student’s t-test (paired and unpaired) and were considered significantly different if P < 0.05.

RESULTS

Effect of Pluronic L-81 on lipid-induced inhibition of gastric emptying. Perfusion of lipid into the intestine produced a dose-dependent inhibition of gastric emptying. Under control conditions (n = 12 rats), 71 ± 2% of the volume placed in the stomach emptied over 5 min. During duodenal perfusion of lipid at 25, 50, and 100 mg, gastric emptying was 68 ± 5, 48 ± 7, and 44 ± 7%, respectively (n = 8 in each group; not significant, P < 0.05, and P < 0.01, respectively, for control vs. lipid) (Fig. 1). Inhibition of gastric emptying returned to control levels 10 min after intestinal perfusion for 25- and 50-mg doses of lipid was stopped, but the 100-mg dose of lipid produced a persistent inhibition of gastric emptying for up to 30 min (Table 1).

Perfusion of Pluronic L-81 completely abolished the ability of lipid to inhibit gastric emptying (Fig. 1). Gastric emptying during perfusion of lipid containing Pluronic L-81 was 79 ± 3, 79 ± 3, and 74 ± 4% (n = 9–11 in each group; P < 0.05 lipid vs. lipid + Pluronic L-81; not significantly different from control). Pluronic L-81 blocked the effect of 100 mg lipid at all time periods after intestinal perfusion.

Effect of Pluronic L-81 compared with L-63 on lipid-induced inhibition of gastric emptying. In the second group of rats (n = 7), control gastric emptying was 72 ± 3% (n = 7). Duodenal perfusion with 50 or 100 mg of
lipid inhibited gastric emptying to 45 ± 7 and 39 ± 9%, respectively (P < 0.01 control vs. lipid). Gastric emptying during infusion of lipid in the presence of Pluronic L-81 completely abolished the inhibitory effect of lipid on gastric emptying (Fig. 2). The amount of gastric emptying was 69 ± 7 and 80 ± 6% for 50 and 100 mg lipid, respectively (P < 0.01 vs. lipid alone). In contrast, gastric emptying during perfusion of Pluronic L-63 was not significantly different from lipid alone (percent gastric emptying 42 ± 6 and 38 ± 8%, respectively).

Effect of Pluronic L-81 and L-63 on plasma levels of CCK. Infusion of lipid into the intestine increased plasma levels of CCK from 1.9 ± 0.8 to 6.5 ± 1 pM (n = 4 in each group; P < 0.01, Fig. 3). Perfusion of lipid containing Pluronic L-81 produced a plasma level of CCK of 1.7 ± 0.9 (n = 12; not significant vs. saline perfusion); perfusion of the intestine with lipid containing Pluronic L-63 increased plasma CCK to 7.8 ± 1.9 pM (n = 10, P < 0.01 vs. lipid + Pluronic L-81).

DISCUSSION

The present study shows for the first time that chylomicron formation is required for fat in the intestinal lumen to signal feedback inhibition of gastric emptying and to increase circulating levels of CCK. Addition of Pluronic L-81, a nonionic, hydrophobic detergent that blocks formation of chylomicrons, to the perfused lipid completely abolished the effectiveness of lipid to inhibit gastric emptying of nonnutrient liquids in awake rats. We have previously shown in this model that lipid-induced inhibition of gastric emptying is mediated in part by CCK-A receptors and a vagal sensory pathway (8). Functional ablation of capsaicin-sensitive vagal, but not spinal, sensory innervation to the gastrointestinal tract attenuated by 57% lipid-induced inhibition of gastric emptying. In intact rats, administration of devazepide significantly attenuated by 66% the response to lipid. Administration of devazepide in vagal capsaicin-treated rats did not reduce the response to lipid any further, suggesting that CCK was acting via the vagal capsaicin-sensitive pathway. The mechanism of action of the remaining portion of the response has not been identified. The present data, showing a complete reversal of lipid-induced inhibition of gastric emptying by Pluronic L-81, suggests that whatever the multiple pathways involved, the initial packaging of digestive products of lipid into chylomicrons is a required step in the signaling pathway. Chylomicrons once released from enterocytes have to

Table 1. Time course of inhibition of gastric emptying by intestinal perfusion of lipid

<table>
<thead>
<tr>
<th>Lipid, mg</th>
<th>+10 min</th>
<th>+25 min</th>
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<tr>
<td>25</td>
<td>68 ± 5</td>
<td>72 ± 5</td>
<td>73 ± 5</td>
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<td>50</td>
<td>48 ± 5</td>
<td>64 ± 6</td>
<td>67 ± 5</td>
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<tr>
<td>100</td>
<td>44 ± 7</td>
<td>46 ± 6</td>
<td>44 ± 7</td>
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Values are means ± SE. Times are time after start of lipid infusion. Mean control gastric emptying is 71 ± 2% (n = 12).

Fig. 1. Duodenal perfusion of lipid inhibited gastric emptying, which was reversed by perfusion with Pluronic L-81, a nonpolar hydrophobic detergent that inhibits chylomicron formation. Duodenum of awake rats was perfused with Intralipid for 10 min, and gastric emptying was measured over the 5- to 10-min period. Results are expressed as means ± SE. *P < 0.05, **P < 0.01 control vs. lipid; #P < 0.05 lipid vs. lipid + Pluronic L-81.

Fig. 2. Inhibition of gastric emptying in response to duodenal lipid infusion is blocked by administration of Pluronic L-81 but not L-63, a chemically similar detergent that has no effect on chylomicron formation. *P < 0.05 control vs. lipid; #P < 0.05 lipid vs. lipid + Pluronic L-81.

Fig. 3. Duodenal perfusion of lipid (100 mg) increases circulating plasma levels of cholecystokinin (CCK); this increase is abolished by administration of Pluronic L-81 but not L-63. Results are expressed as means ± SE; *P < 0.01 saline vs. lipid and #P < 0.01 Pluronic L-81 vs. L-63.
pass through the lamina propria to the lacteals. Therefore, some property or constituent of chylomicrons could act on any number of targets in the lamina propria, including endocrine cells or sensory nerve terminals. On the basis of the present findings, we hypothesize that chylomicrons act on CCK endocrine cells to release CCK. CCK acts via a paracrine mechanism of action on CCK receptors located on vagal afferent nerve terminals, which then initiates a vago-vagal reflex inhibition of gastric motility and inhibition of gastric emptying. Recent anatomical studies show that vagal afferent nerve terminals were at distances of 10–100 µm from CCK-immunoreactive cells, suggesting a paracrine mechanism of action (2).

The fate of lipid after absorption into enterocytes depends on the chain length of the fatty acid. Fatty acids of chain length C10 or below pass out of enterocytes by diffusion in the form of very-low-density lipoproteins. In contrast, fatty acids of chain length greater than C10 are packaged into chylomicrons and removed via exocytosis from enterocytes by an energy-requiring process that utilizes calcium. It has been known for many years that long chain fatty acids [long chain triglycerides (LCTs)] are more potent to produce inhibition of gastric emptying (9). This was originally demonstrated by Hunt and Knox (9) in the dog and has been confirmed in the cat (21). Duodenal perfusions with LCTs inhibited the basal electrical rhythm of the antrum significantly, whereas medium chain triglycerides (MCTs) and short chain triglycerides (SCTs) were without effect. In addition, other physiological responses initiated by lipid in the intestine are stimulated more potently by LCTs than MCTs and SCTs. In humans, meals containing LCTs produced a more brisk and sustained gall bladder contraction than those containing MCTs (11), and LCTs were more effective in inhibiting gastrin-stimulated gastric acid secretion (18). The lipid used in the present study, Intralipid, contains an emulsion of predominantly LCTs from soybean oil, with small amounts of lecithin and glycerol. Therefore, the main constituents of Intralipid, LCTs, would be expected to be packaged and removed via exocytosis from enterocytes in the form of chylomicrons and, importantly, to initiate feedback inhibition of gastric emptying. The total amount of lipid required to inhibit gastric emptying represents a small fraction of that consumed by a rat in a normal meal.

In addition, it has been shown that LCTs release CCK more effectively than MCTs (11, 18, 20). In a study in humans in which the physicochemical differences in the fatty acids of differing chain length were controlled, only chain lengths of C12 or greater increased plasma levels of CCK (20). In rats, the ability of fat to release CCK has been confirmed (5, 6, 8). In particular, studies using oleate (C18) have demonstrated significant release of CCK. The mechanism by which fat, or any nutrient, releases CCK from endocrine cells is unclear. The results of the present study suggest that chylomicron formation is required. It is not known if endocrine cells absorb fat or have the ability to synthesize apolipoproteins that are required for chylomicron formation. It is possible that the fat has to exit the enterocyte and then acts via the lamina propria to stimulate release of CCK from the basolateral aspect of endocrine cells. Alternatively, there may be some intracellular signaling associated with chylomicron formation that passes via gap junctions between endocrine cells and enterocytes to signal release of CCK. The recent observations that fat releases CCK from STC-1 cells (intestinal CCK-secreting cell line) suggests a direct effect on endocrine cells (19).

The present study has shown a role for chylomicrons but does not provide any evidence about which property of chylomicrons is important. Apolipoprotein A-I (apo A-I) is synthesized in response to lipid in the intestine, and there is evidence that it may be involved in signaling fat content in the intestine to other organ systems. Plasma levels of apo A-I increase within 30 min of lipid placed into the stomach (27). Apo A-I levels also increase in the cerebrospinal fluid after lipid ingestion. Intravenous or intracerebrovascular administration of apo A-I suppresses meal size in rats and has been suggested to be a physiological controller of food intake (7). In addition, apo A-I has been shown to inhibit gastric emptying (24). Whether the site of action of apo A-I to reduce meal size or inhibit gastric emptying is a central, peripheral, or combined effect remains to be determined.

It was postulated by Debas et al. (4) and later confirmed (16) that CCK is a physiological regulator of gastric emptying. Recent publications using specific and potent CCK receptor antagonists have proven this hypothesis (3, 22). The present study provided evidence for a role of chylomicron formation in the inhibition of gastric emptying and increase in plasma CCK in response to intestinal lipid. The mechanism or factors associated with chylomicron formation remain to be evaluated, but the present study provides evidence on the sensory transduction mechanism within the intestinal wall.

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