Nutrient Tasting and Signaling Mechanisms in the Gut
I. Sensing of lipid by the intestinal mucosa*

HELEN E. RAYBOULD
CURE: Digestive Diseases Research Center, West Los Angeles Veterans Affairs Medical Center, Departments of Medicine and Physiology, University of California School of Medicine, Los Angeles, California 90095

Raybould, Helen E. Nutrient Tasting and Signaling Mechanisms in the Gut. I. Sensing of lipid by the intestinal mucosa. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G751–G755, 1999.—It is well recognized that lipid in the intestine is a potent inhibitor of gastric secretomotor function. Progress has been made in the identification of the “sensor” for lipid in the intestinal wall. Long-chain free fatty acids are the stimulus both for release of CCK and for the production of functional effects. Long-chain triglyceride requires chylomicron formation for absorption, and there is strong evidence that the postabsorptive products of long-chain triglyceride absorption, including chylomicrons and apolipoproteins, are involved in sensory transduction in the intestinal wall.

long-chain fatty acids; chylomicrons; cholecystokinin A receptors; vagal afferents

One of the characteristics of the intestinal phase of a meal is feedback inhibition of gastric secretory and motor function together with stimulation of pancreatic secretion, gallbladder contraction, and relaxation of the sphincter of Oddi. These processes are tightly regulated in the postprandial phase to match the digestive and absorptive capacities of the intestine with the entry of food from the stomach. Food intake is also regulated; inhibition of food ingestion will also serve to limit entry of nutrients into the intestine. Typical postprandial responses can be initiated in experimental situations by infusion of meal nutrients (carbohydrate, lipid, and protein or amino acids) into the distal and proximal small intestine and the cecum and the colon. The existence of these feedback and feedforward responses implies the existence of “sensors” that can detect the presence of nutrients. There is good evidence that these sensors are located in the gut wall, but they may also exist at extraintestinal sites such as lymph vessels, portal veins, liver, pancreas, and the central nervous system.

Much of the work to characterize the sensors that are activated by nutrients has involved functional analysis that uses inhibition of gastric motility or acid secretion as a measure of activation of sensors. This approach has yielded useful information about the nature of the mechanisms by which nutrients are sensed by the intestinal wall. It is clear that nutrients act separately from any osmotic or mechanical effects, that all macronutrient groups alter gastric secretomotor function and food intake (suggesting the effect is not secondary to detection of caloric content), and that each macronutrient group acts via activation of separate and distinct mechanisms and pathways.

This discussion will focus primarily on the identity of the chemoreceptor involved in the detection of lipid in the intestinal wall. To further the understanding of sensory transduction, it is helpful to consider the pathways of digestion and absorption of each macronutrient group. Recent evidence from studies using this approach has pointed to a possible role for postabsorptive products of lipid digestion and has provided a new perspective on nutrient sensing in the gut.

Functional Effects of Long-Chain Triglyceride on Gastric Emptying

Although it has been known since the last century that lipid in the intestine could inhibit gastric motor and secretory function, the work of Hunt and Knox in the 1960s (13) clearly demonstrated that the effective stimulus was not triglyceride but free fatty acids. Importantly, they also demonstrated that free fatty acids of chain length C12 or greater were the most effective, much more effective than C10. This work has since been confirmed in different species and experimental paradigms. The observation that long-chain fatty acids, in contrast to both short- and medium-chain fatty acids, initiate intestinal feedback implies the existence of a specific “receptor” for long-chain fatty acids in the intestinal wall. Alternatively, there may be some element in the pathway of absorption and processing of long-chain fatty acids in the intestinal wall that is involved in the detection of lipid.

Intestinal absorption of long-chain triglyceride, and its subsequent secretion from enterocytes, is a complex event that includes the coordination of synthesis of apolipoproteins and lipids and their intracellular assembly into mature lipid-containing particles (28). The major digestive products of triglyceride are monoglycerides and free fatty acids. These are absorbed into the...

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enterocytes via passive diffusion and transported within the enterocyte to the endoplasmic reticulum (ER), where biosynthesis of complex lipids to form triglyceride takes place. The precursors of chylomicrons, termed prechylomicrons, are synthesized in the ER and Golgi apparatus. Lipoproteins are made in the ER and then transported to the Golgi. The chylomicrons then migrate to the lateral membrane of enterocytes and are exocytosed, and the triglyceride-rich lipoproteins are discharged into the intercellular space. From there, the products of long-chain fatty acid absorption diffuse through the lamina propria and pass into lymph. The chylomicron is a heterogeneous globular particle 100–500 nm in diameter whose composition consists mainly of triglyceride, some cholesterol, cholesterol esters, and phospholipids. The surface of the chylomicron contains both exchangeable apolipoproteins (apoA, apoC, apoE) and nonexchangeable apolipoprotein (apoB). Recent attention has focused on the role of chylomicrons and apolipoprotein A-I (apoA-I) in signaling the lipid content of the gut to other organ systems.

**ROLE OF CHYLOMICRONS IN INTESTINAL FEEDBACK INHIBITION**

The hypothesis that chylomicron formation is an obligatory step in the ability of lipid to initiate feedback inhibition of gastric emptying was tested using Pluronic L-81, a nonionic hydrophobic surfactant shown to inhibit the formation of chylomicrons in enterocytes (19). In awake rats, intestinal perfusion with a triglyceride emulsion in physiological amounts (25–100 mg) induces a dose-dependent inhibition of gastric emptying that occurs within 5 min after the start of perfusion (12). Perfusion of lipid together with Pluronic L-81 reversed the lipid-induced inhibition of gastric emptying, whereas Pluronic L-63, a chemically similar detergent that has no effect on chylomicron formation (22), had no effect. Similarly, chylomicron formation is required for the effectiveness of long-chain fatty acids to inhibit short-term food intake in rats (26). The complete reversal of these lipid-induced responses by Pluronic L-81 suggests that the initial packaging of products of lipid digestion into chylomicrons is a required step in the signaling pathway. Therefore, it is likely that activation of the sensor by long-chain free fatty acids does not occur in the lumen of the intestine but requires entry into enterocytes and translocation to the ER or Golgi and either assembly of chylomicrons or their exocytosis out of enterocytes.

A possible interpretation of the data obtained with inhibition of chylomicron formation suggests that sensors are located outside of the intestinal wall, for example in the lymph vessels or liver. However, considerable functional evidence supports the concept that the sensor is located in the wall of the small intestine (12, 23). 1) Intestinal perfusion of lipid is sufficient to elicit functional responses regardless of any other stimuli from the stomach or other parts of the intestinal tract. 2) The latency for inhibition of gastric function or food intake following the start of lipid infusion into the intestine is very short (several minutes). 3) Prior treatment of the intestinal mucosa with a local anesthetic or with the sensory neurotoxin capsaicin attenuates functional responses to intestinal lipid perfusion. Application of capsaicin to mucosal surfaces, for example the cornea or nasal mucosa, has been shown to result in a loss of chemoceptive function. Perfusion of the small intestine with capsaicin significantly attenuated the lipid-induced inhibition of gastric emptying by ~50%; the treatment was judged to be effective because duodenal acid-induced inhibition of gastric emptying was completely reversed (30). 4) Lipid is less potent (compared with intestinal perfusion) or is ineffective when administered intravascularly. This evidence points to the existence of chemoceptors located in the intestinal wall. Some property or constituent of chylomicrons or associated apolipoproteins could act on any number of targets in the brush border or lamina propria, including endocrine cells or primary afferent nerve terminals.

**POSSIBLE IDENTITY OF SENSORS FOR LONG-CHAIN FATTY ACIDS IN THE INTESTINAL WALL**

Primary afferent nerve terminals. The importance of the extrinsic innervation to the intestine in mediating functional responses to lipid is well established. Inhibition of gastric emptying and acid secretion in response to intestinal long-chain triglycerides is reduced by 60–80% by functional ablation of the vagal afferent pathway with the sensory neurotoxin capsaicin; the spinal afferent innervation does not seem to play a role (12). Studies on extrinsic afferent nerve responsiveness to nutrients have demonstrated that mucosal afferent nerve endings respond to mechanical and chemical stimulation of the gut wall. All the major macronutrients, including lipid, have been reported to activate mucosal afferent nerve fiber discharge (see Ref. 9). In the cat, recordings from the cell bodies of vagal afferents innervating the small intestine revealed two populations of afferents responding to lipid. One population was sensitive to short-chain fatty acids and glycerol, and the other was selectively sensitive to long-chain fatty acids; the latter did not respond to other nutrients such as glucose or to mechanical stimulation (17).

Does this apparent chemosensitivity represent a direct effect of lipid on extrinsic nerve terminals? Small molecules such as protons might penetrate the mucosa to act directly on afferent nerve terminals. Recently, it has been shown that proton-sensitive channels are expressed by some primary afferents supporting this concept. However, the receptive unit could be extrinsic afferent nerve terminals themselves or an intrinsic primary afferent neuron or other cell types located in close proximity to the terminals of vagal afferents. Detailed studies of the terminations of vagal afferents have shown terminals in the submucous and myenteric nerve plexi and within the longitudinal and circular smooth muscle layers of the proximal small intestine (2). Individual afferents collateralize extensively within the submucosa, producing terminal arborizations in the mucosa that cover large areas with endings around
the crypts and within the lamina propria of the villi. The densest innervation is at the proximal part of the small intestine into which the stomach empties its contents. Vagal afferent fibers in the crypts and villous lamina propria were found to be in intimate anatomic contact with fibrocyte-like cells (possibly interstitial cells of Cajal) and with small granular cells (possibly granulocytes or histocytes); the functional significance of this association is not clear. Importantly, no vagal fibers can be seen to penetrate between epithelial cells or protrude into the lumen; vagal terminal branches come into close contact with the basal lamina but do not seem to make direct contact with epithelial cells. This suggests that luminal content may signal to afferent nerve terminals via an indirect interaction with a cell in the epithelium. In addition, intrinsic primary afferent neurons have been shown to respond directly to acid and short-chain fatty acids, but delayed in the response to luminally applied stimuli suggests that the response depended on an intermediary cell (4). The most likely intermediary is the enteroendocrine cell.

Enteroneuroendocrine cells. Triglyceride in the intestinal lumen releases a number of regulatory peptides from the intestine, including CCK, neurotensin, peptide YY, and proglucagon-derived peptides. In humans, dogs, and rats, infusion of long-chain fatty acids or long-chain triglyceride emulsions increase plasma levels of CCK (15). This response is remarkably rapid, occurring in awake rats as rapidly as 10 min after the start of perfusion of either intact triglyceride or free fatty acid (12). Intact fat requires hydrolysis to be effective (10). Inhibition of lipase will block the effect of intact triglyceride perfusion to secrete CCK, suggesting the lack of a lipase-sensitive feedback loop similar to that described for the trypsin-sensitive CCK-releasing peptide involved in protein-induced CCK release.

Studies that have specifically looked at chain length have verified that only long-chain fatty acids are stimulants for CCK secretion. Ingestion of medium-chain fatty acids (predominately C8 and C10) produced small changes in plasma levels of CCK; in contrast, equivalent amounts of long-chain fatty acids (predominately C16 and C18) were potent stimulants of CCK secretion. A careful study in which the physicochemical differences of fatty acids of different chain lengths were controlled clearly established that only fatty acids of chain length C12 or longer and not C11 or shorter increased plasma levels of CCK. The amount of saturation of long-chain fatty acid had no effect on CCK release (16).

The observation that free fatty acids of chain length C12 and above release CCK in vivo suggests that chylomicron formation may be involved, since these are the fatty acids that are absorbed via chylomicron formation. We examined the role of chylomicron formation on release of CCK in vivo; perfusion of an emulsion of long-chain triglyceride in the rat rapidly increases the plasma level of CCK. Perfusion of Pluronic L-81 with the lipid emulsion abolished the increase in plasma levels of CCK (22).

The cellular mechanisms mediating the effect of free fatty acids on the CCK endocrine cell is poorly understood. This is in part due to the difficulties in obtaining a preparation of pure native endocrine cells. In a study that used a primary culture of canine intestinal mucosa enriched for the enteroendocrine cell fraction, neurotensin was released by long-chain fatty acids; this was associated with increases in intracellular calcium (1). In a preparation of fetal rat intestine, long-chain fatty acids of C14 or greater stimulated release of proglucagon-derived peptides (24). However, because neither of these preparations is a pure enteroendocrine cell preparation, it is not clear if these responses represent direct effects of the nutrients on enteroendocrine cells. Recent advances have been made in understanding secretory mechanisms in intestinal endocrine cells using a number of different enteroendocrine cell lines, such as STC-1 (15). These cells secrete CCK in response to a number of stimuli including gastrin-releasing peptide, β-adrenergic agonists, L-phenylalanine, and glucose. With the use of these cells, a direct effect of long-chain fatty acids on stimulation of CCK release has been demonstrated (16).

However, the data from the experiments using inhibition of chylomicron formation suggest that the effect of lipid on CCK endocrine cells in vivo may not be solely direct; since it was shown to depend on chylomicron formation. It is not known if endocrine cells absorb fats or express fatty acid binding proteins, which have been postulated to be involved in the intracellular trafficking of lipid products in the cells and also for protection of cell organelles from the otherwise toxic effects of high concentrations of lipid. Also, it is not known whether endocrine cells synthesize chylomicrons or apolipoproteins. It is possible that release of CCK in response to lipid in vivo represents an interaction between enterocytes and endocrine cells, possibly involving the products of chylomicron formation. Lipid may have to exit the enterocyte (in the form of chylomicrons), the products of which then act on the basolateral aspect of the endocrine cells. Alternatively, there may be some intracellular signal formed by synthesis of chylomicrons that passes via gap junctions between enterocytes and endocrine cells.

CCK IN SENSORY TRANSDUCTION IN THE INTESTINAL WALL

CCK plays an obligatory role in the lipid-induced regulation of gastric emptying and secretion and is an important mediator of the intestinal phase of the meal. In the awake rat, lipid-induced inhibition of gastric emptying or gastric acid secretion is reduced by ~70% by administration of a specific CCK-A receptor antagonist (12, 18). In humans, administration of CCK-A receptor antagonist significantly accelerated gastric emptying of both liquid and solid components of a mixed meal and gastric relaxation in response to lipid was also abolished by the CCK-A receptor antagonist (6). Although this cannot be tested in humans, data from rats indicate that this action of CCK is mediated at type A receptors on vagal afferent nerve terminals.
Functional ablation of the capsaicin-sensitive vagal afferent pathway appears to mediate around 60–70% of the responses to both CCK and lipid (12); administration of the CCK-A receptor antagonist in capsaicin-treated rats had no additional effect, suggesting that the CCK-mediated part of the response is via the vagal capsaicin-sensitive pathway.

Both extrinsic and intrinsic neurons express CCK-A receptors and thus could be a target for a paracrine action of CCK (18, 27). Consistent with the finding that they express CCK A receptors, extrinsic primary sensory neurons do seem to be directly sensitive to CCK. As shown by electrophysiological recordings (5), extrinsic mucosal afferents innervating the intestine of ferrets and rats are extremely sensitive to exogenous CCK. The first evidence showing that endogenous CCK is involved in the response of vagal afferents to nutrients was obtained by Eastwood et al. (7). Luminal application of casein acid hydrolysate stimulated mesenteric nerve fiber discharge. Fibers sensitive to casein acid hydrolysates were also sensitive to CCK, and responses to both were abolished by the CCK-A receptor antagonist devazepide. This is the first direct electrophysiological evidence to support the concept that CCK plays a role in the sensory transduction of luminal nutrient signals. However, it should be noted that casein hydrolysate does not increase plasma levels of CCK. This supports the local release and action of CCK. In morphological studies, CCK-immunoreactive cells were localized 10 to 100 µm from vagal afferent axons with no synap 설정 all positions, suggesting that CCK acts on nerves in a paracrine manner (3). However, an alternative explanation is that CCK is released from intrinsic nerve terminals innervating the mucosa.

ROLE FOR APOLIPOPROTEIN A-IV

ApoA-IV is a 46,000-molecular-weight glycoprotein that in humans is only synthesized in the intestine but in rats is synthesized by both the intestine and the liver, although the intestine accounts for the major proportion of circulating apoA-IV (14). ApoA-IV has been postulated to play a role in lipid absorption, chylomicron formation, and cholesterol and lipoprotein metabolism. In response to a lipid-containing meal, apoA-IV is secreted into the intestinal lymph on chylomicrons. In plasma, apoA-IV dissociates from chylomicrons and circulates with the high-density lipoprotein and in the lipoprotein-free fraction of plasma (14).

ApoA-IV is the only lipoprotein that seems to be directly regulated by lipid in the intestine. Dietary lipid increases expression, synthesis, and release of apoA-IV, whereas another apolipoprotein, apo-1, is not so regulated (11). Intestinal lymphatic transport of apoA-IV increases in a dose-dependent manner following intestinal perfusion with triglycerides. It is possible that the increased apoA-IV synthesis and secretion may be due to either increased fat uptake or increased secretion of triglyceride-rich lipoproteins by enterocytes. The time course of apoA-IV release has recently been shown to be much more rapid at onset than previously suspected. In awake rats, apoA-IV in lymph increased as rapidly as 15 min after an intragastric load of mixed triglyceride and fatty acid (25). There is evidence that apoA-IV synthesis and secretion requires chylomicron assembly; this evidence comes mainly from data using the detergent Pluronic L-81, which inhibits chylomicron formation. The expected increase in lymph apoA-IV levels is abolished when lipid is perfused with Pluronic L-81 (11). Further evidence that apoA-IV synthesis and secretion requires chylomicron formation comes from experiments using fatty acids of different chain lengths. Intestinal infusion of fatty acids of chain length C14 and greater increased lymphatic triglyceride and lyphatic apoA-IV, whereas those with chain lengths of C8 and C10 were ineffective.

FUNCTIONAL EFFECTS OF APOLIPOPROTEIN A-IV IN THE GASTROINTESTINAL TRACT

There is growing evidence that apoA-IV can influence postprandial gastrointestinal function. Exogenous administration of apoA-IV to rats inhibits short-term food intake, gastric acid secretion, and emptying (8, 20, 26). Evidence from apoA-IV knockout mice suggests that apoA-IV may be physiologically involved in regulation of food intake (29). ApoA-IV knockout mice showed an increase in short-term food intake after an overnight fast, although their overall growth and ad libitum food intake were not significantly different from results in wild-type controls. The site or sites of action of apoA-IV are unknown. Intracranial administration of apoA-IV inhibits gastric emptying, suggesting that it may act, at least in part, via a central site of action (20). However, a peripheral site of action of apoA-IV cannot be ruled out, and apoA-IV may be involved in sensory transduction in the wall of the intestine.

CONCLUSIONS AND SPECULATIONS

Available evidence suggests that lipid, specifically long-chain fatty acid, is sensed in the wall of the intestine, releases CCK from enteroendocrine cells, and activates extrinsic vagal afferent nerve terminals. However, direct evidence demonstrating a role for CCK in activating vagal afferent fibers in response to lipid is lacking and can only come from direct recordings of vagal afferent fiber activity. It is also clear from recent studies that the products of chylomicron formation, including apoA-IV, are likely to be involved in this process. The rapid release of apoA-IV into the lamina propria and lymph, together with its actions on postprandial gastrointestinal function, suggests its involvement in the process of sensory transduction in the intestinal wall.

It is evident from the present discussion that several critical questions remain unanswered. One important question is whether postabsorptive products of lipid absorption and chylomicrons, such as apoA-IV, mediate the neural response to lipid. CCK is present in neurons innervating the gastrointestinal tract, and a neuronal source of CCK should also be considered. It is not known to what extent neurally released CCK might contribute to the physiological effects of CCK on gastrointestinal function. In addition, it is quite likely that CCK mediates its action locally; measurements can
only be made in peripheral plasma and are unlikely to reflect those close to peripheral nerve terminals in the intestinal wall. Observations showing that intestinal perfusion with carbohydrates, which do not increase intestinal wall. Observations showing that intestinal reflect those close to peripheral nerve terminals in the only be made in peripheral plasma and are unlikely to

REFERENCES


