Gastrointestinal satiety signals
II. Cholecystokinin

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Cholecystokinin is found both within the brain and the gastrointestinal tract. In the brain, it is widely distributed, with high concentrations in the cortex, amygdala, hippocampus, and septum (2). Significant amounts of CCK are also found in the thalamus, hypothalamus, basal ganglia, and dorsal hindbrain. In the brain, CCK functions as a neurotransmitter (6). It is present in neurons, can be found at the level of the cell body and terminals where it is localized to synaptic vesicles, and is released in response to depolarization. As with other neurotransmitters, the actions of CCK in the brain depend on where it is released. Roles for central CCK include such diverse behaviors and states as anxiety, sexual behavior, learning, and memory, spontaneous activity, satiety, and pain (6).

Within the gastrointestinal tract, CCK is found in both secretory and neural tissues. CCK is present in high concentrations with the duodenal and jejunal mucosa, where it is localized to enteroeccrine cells (14). Endocrine cells containing CCK, classified as I cells, have a roughly triangular shape with the apical surfaces oriented toward the intestinal lumen and secretory granules containing CCK concentrated around the base (5). This orientation allows the I cells to be stimulated by intestinal nutrients and release their contents into the blood and/or surrounding tissue. CCK is also found in enteric nerves. CCK-containing neurons are most abundant in the ileum and colon (14). Neurons containing CCK are found within the circular muscle as well as in the myenteric and submucosal plexus.

CCK is found in multiple molecular forms (30). All forms are derived from a single CCK gene by posttranslational or extracellular processing. The complementary DNA structures for preprocholecystokinin have been determined for multiple species, demonstrating a great deal of conservation among mouse, rat, and human CCK gene structure. The 345-nt mRNA encodes a 115-amino acid precursor consisting of a 20-amino acid signal peptide, a 25-amino acid spacer peptide, CCK-58, and a 12-amino acid extension at the carboxyl terminus. This carboxyl terminus is the biologically active portion of the hormone. In several species, multiple biologically active forms of CCK are found in the brain, intestine, and blood with CCK-58, CCK-33, and CCK-8 being the dominant forms (30).

CCK receptors

Two types of CCK receptors have been identified. Specific binding for radiolabeled CCK in brain and pancreatic homogenates was first demonstrated in the early 1980s. Early pharmacological characterization of CCK binding demonstrated different profiles for rat cortex and pancreas. In the pancreas, receptor affinity depended on the presence of the sulfated tyrosine. Sulfated CCK was up to 1,000 times more potent in displacing radiolabeled CCK than was unsulfated CCK, and this profile matched with the ability of sulfated and unsulfated CCK to stimulate pancreatic amylase secretion. In homogenates from cerebral cortex, sulfated and unsulfated CCK had roughly equivalent affinities for CCK binding sites (12). These differing profiles suggested the existence of two CCK receptor subtypes. Studies using receptor autoradiography in which nuclear distinctions in the pharmacological profiles of CCK receptors in the brain could be made demonstrated that both binding profiles could be found in brain. The majority of brain receptors, however, demonstrated the same pharmacological profile as had been shown for the cortical binding sites (27). These profiles led to the nomenclature of CCK_A for the peripheral or alimentary sulfate-dependent binding subtype and CCK_B for the central or brain nonspecific binding subtype (27). Specific antagonists are now available for the two receptor subtypes allowing clear determinations of the receptor subtypes mediating the various actions of CCK.

Both CCK receptors have now been isolated, and their DNA sequences have been cloned (13, 29). Both receptors are members of the seven transmembrane G protein-coupled family of receptors. In the rat, analysis of the deduced amino acid sequences has indicated that there is 48% homology between the CCK_A and CCK_B receptor subtypes. Although pharmacological studies had indicated multiple binding sites with different affinities within the rat pancreas and other tissues, molecular studies have now demonstrated that there is a single CCK_A receptor protein expressed in rat pancreas and gallblad-
GASTROINTESTINAL CCK RELEASE

CCK release from the intestine, as measured by increases in plasma CCK levels, is stimulated by the intraluminal presence of nutrient digestive products. Basal plasma CCK concentrations are ~1 pM in most species and rise to 5–8 pM following meal ingestion (17). Levels gradually increase over 10–30 min after meal initiation and then gradually fall, although remaining elevated for as long as 3–5 h after eating. Dietary fat and protein, and ultimately their digestive products, are the most potent stimulators of CCK release, with carbohydrates being weak stimulants (17). Studies in animals and humans suggest that in order for fat to stimulate CCK secretion, triglyceride must be hydrolyzed to fatty acids. Additionally, fatty acid stimulation of CCK is dependent on acyl chain length; saturated fatty acids below an acyl chain length of 12 carbon atoms do not induce CCK secretion in humans (19).

Whereas, in most species, digestion of protein is required to effectively stimulate CCK release, it is not required to do so in the rat (16). In fact, in the rat, intact proteins are more potent stimulators of CCK release than hydrolyzed proteins or amino acids (10). This is likely due to the ability of intact protein to serve as a substrate for proteases that degrade CCK-releasing factors.

The presence of CCK-releasing factors was first evidenced by experiments in the 1970s in which inactivation or removal of proteolytic activity from the proximal small intestine via trypsin resulted in a significant increase in pancreatic exocrine secretion. Additionally, intraduodenal infusion of trypsin in the absence of bile-pancreatic juice in the intestine suppressed pancreatic enzyme secretion (9). Since that time, two CCK-releasing factors have been identified and chemically characterized: luminal CCK-releasing factor (LCRF), which is of intestinal origin and is present in the gut under basal conditions (33), and diazepam-binding inhibitor (DBI), which is also secreted from the proximal intestine (11). Intraduodenally infused LCRF significantly elevates plasma CCK levels (34). DBI similarly increases plasma CCK levels, and antiserum to DBI abolishes peptone-induced elevations in plasma CCK (15).

In addition to CCK-releasing factors, there exists a 61-amino acid protein purified from pancreatic juice that stimulates CCK secretion when introduced into the lumen of the rat intestine. Monitor peptide, also called pancreatic secretory trypsin inhibitor-I is produced by pancreatic acinar cells and secreted into pancreatic juice. It acts to monitor the intraduodenal environment for protein digestion and stimulates CCK secretion on reaching the intestine (20).

It is important to note that measurements of plasma CCK might not fully reflect all nutrient-induced CCK release. As will be discussed, although some actions of CCK are mediated through endocrine mechanisms, there is evidence that locally released CCK can activate vagal afferent fibers in the absence of significant plasma CCK elevations.

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<th>Table 1. Locations of CCK receptors</th>
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<tr>
<td>Gastrointestinal tract</td>
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<td>Pancreas</td>
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<td>Gallbladder</td>
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<td>Brain</td>
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<td>Nucleus tractus solitarius</td>
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<td>Area postrema</td>
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<tr>
<td>Interpeduncular nucleus</td>
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<tr>
<td>Median raphe</td>
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<td>Dorsal medial hypothalamus</td>
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<td>Nucleus accumbens</td>
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CCK has now been shown to inhibit food intake across multiple test situations and in multiple species, including humans, and CCK’s actions in food have been well characterized. CCK affects food intake rapidly, and the duration of inhibition is relatively brief. Thus administration of CCK at the beginning of the rat’s active cycle reduced the size of the initial meal but does not affect overall daily intake. West et al. (37) demonstrated that meal onset-contingent CCK administration resulted in consistent decreases in the size of the meal but in no overall decrease in daily food intake. In response to the decreases in meals size, rats compensated by increasing the number of meals consumed.

The availability of potent and specific CCK agonists and antagonists facilitated the identification of the CCK receptor subtype mediating the satiety actions of CCK and allowed an assessment of the physiological relevance of endogenous CCK in the control of food intake within meals. Specific CCK<sub>A</sub> agonists compounds potently inhibit food intake (1), whereas nonsulfated CCK and other peptide fragments with high affinity for the CCK<sub>B</sub> receptor do not. These data suggest that the feeding inhibitory actions of CCK are mediated through interactions with the CCK<sub>A</sub> receptor subtype. Antagonist experiments confirm this conclusion. Specific CCK<sub>A</sub> receptor antagonists dose dependently block the ability of CCK to inhibit intake, whereas CCK<sub>B</sub>-selective antagonists are ineffective (22).

Experiments examining pancreatic CCK binding and the effects of CCK on pancreatic acinar function had identified two functional affinity states for CCK<sub>A</sub> receptors: a high-affinity state, which, when activated, stimulated amylase secretion and a low-affinity state, which, when stimulated, inhibited secretion. Compounds were developed that had differential activity at the two sites, allowing a differentiation of whether the satiety actions of CCK were mediated through activation of high- or low-affinity CCK<sub>A</sub> receptors. CCK-JMV-180, a heptapeptide CCK analog, was shown in pancreatic acinar cell studies to be an agonist at the high-affinity site but an antagonist at the low-affinity site. In feeding studies, CCK-JMV-180 was shown to lack agonist activity (it did not inhibit food intake following peripheral administration) but acted as an antagonist in that it blocked the feeding inhibitory action of exogenously administered CCK (36). These findings pointed to the low-affinity CCK<sub>A</sub> receptor site as the critical site for CCK satiety. This conclusion has an important implication. Postprandial plasma CCK levels likely do not attain sufficient concentrations to interact with the low-affinity CCK<sub>A</sub> receptors, suggesting that the inhibition of food intake by CCK, if it is a physiological action of meal-stimulated CCK release, is not an endocrine action of the peptide but must reflect higher CCK concentrations at local sites of action.

Administration of potent and selective CCK antagonists has provided compelling evidence for the hypothesis that the actions of exogenously administered CCK in inhibiting food intake were mimicking a physiological role for the endogenous peptide in satiety. Systemic administration of CCK<sub>A</sub> receptor antagonists results in dose-related increases in food intake. As demonstrated in Fig. 1, the CCK<sub>A</sub> receptor antagonist devazepide increased 4-h daily food intake in rhesus monkeys (21). The increase was maximal at the 100 μg/kg dose, resulting in a 40% increase in food intake for that day. The major effect was on the size and duration of the first meal, although the increases were sustained throughout the feeding period consistent with the long duration of action of this antagonist. Administration of a specific CCK<sub>B</sub> receptor antagonist was without effect in this paradigm. Similar findings have been demonstrated in a variety of species, including humans, indicating that endogenous meal-elicited CCK is important in limiting meal size.

Consistent with the ability of lipids to stimulate CCK release, a variety of studies in animals and humans have demonstrated that endogenous CCK plays a role in the ability of intestinal lipids to affect food intake. Administration of a CCK<sub>A</sub> antagonist in human studies has been shown to abolish both the fat-induced decreases in food intake and a fat-induced decrease in reported hunger sensations (18). These data further demonstrate the role of endogenous CCK as a feedback mediator important for meal termination.

CCK has both direct and indirect actions in inhibiting food intake, and these are likely mediated through different sites. CCK directly activates vagal afferent fibers. CCK-induced activation has been demonstrated in both gastric and duodenal mechanoreceptive fibers and in duodenal chemosensitive fibers (31). In mechanoreceptive fibers, CCK mimics and adds to the actions of distention and sensitizes fibers to subsequent distention. These actions of CCK have been shown to be mediated through CCK’s interaction with low-affinity CCK<sub>A</sub> receptors, a finding consistent with the receptor subtype mediating the satiating effects of CCK. CCK also interacts with low-affinity CCK<sub>A</sub> receptors localized to circular muscle cells in the pyloric sphincter. Activation of these CCK receptors results in pyloric contraction, inhibition of transpyloric flow, and the slowing of

![Fig. 1. Effects of the specific CCK<sub>A</sub> receptor antagonist devazepide on daily food intake in Rhesus monkeys. Doses are expressed as micrograms per kilogram. Devazepide increased intake in a dose-related manner. The effect was maximum at a dose of 100 μg/kg.](http://ajpgi.physiology.org/DownloadedFrom/10.1152/ajpgi.01003.2004)
gastric emptying. Roles for actions of CCK at both of these sites in satiety have been supported by results demonstrating that lesions of the two receptor subpopulations eliminated different aspects of the CCK-satiety dose-response curve. As shown in Fig. 2, a specific vagal afferent lesion eliminated the ability of low doses of CCK to inhibit food intake, right shifting the dose-response curve, in that the 8 μg/kg dose was still effective in inhibiting intake. In contrast, surgical removal of the pyloric sphincter produces a truncation of the dose-response curve. Low doses of CCK are effective, but the ability of higher doses to provoke a further intake suppression is eliminated. We have interpreted these findings as indicating that low doses of CCK directly activate vagal afferent fibers providing a negative feedback signal to the brain leading to feeding inhibition. The actions of higher CCK doses in food intake are indirect, secondary to activation of pyloric CCK receptors, and dependent on CCK’s ability to inhibit gastric emptying.

Further evidence for roles for CCK in the controls of food intake has come from work with genetic models in which there is a defect in CCK signaling. Otsuka Long Evans Tokushima Fatty (OLETF) rats were originally identified as a spontaneous development of diabetes and obesity in an outbred colony of Long Evans rats. Through selective breeding, a line of rats was established that was characterized by obesity, hyperglycemia, and eventual transition from non-insulin-dependent to insulin-dependent diabetes. In characterizing their pancreatic function, it was discovered that they lacked a pancreatic exocrine response to CCK, and further work demonstrated a lack of CCK binding to pancreatic acinar cells, suggesting that OLETF rats lacked CCKA receptors. This turned out to be the case, because it was demonstrated that OLETF rats had a 64-kb deletion in their CCKA receptor gene that included the promoter region and the first and second exons. Although the regions upstream and downstream of the deletion were intact, the deletion completely prevented protein expression, resulting in a CCKA receptor knockout rat (7).

We demonstrated that OLETF rats were insensitive to the feeding inhibitory actions of peripherally administered CCK, providing a functional confirmation of the CCKA receptor deficit and then providing a characterization of their spontaneous food intake in a variety of paradigms (23). Maintaining OLETF and control Long Evans Tokushima Otsuka (LETO)
rats on access to 45-mg chow pellets in computer-monitored cages allowed us to follow their feeding patterns over multiple days in both the dark and light components of the light-dark cycle. As shown in Fig. 3, OLETF rats were hyperphagic, with increased intake in both the dark and the light. Their hyperphagia was characterized by a significant increase in the size of their average meals. Meal sizes were almost doubled, and this was reflected throughout the dark-light cycle. In response to this increase in meal size, the total number of meals dropped, but the decreases were not sufficient to compensate for the increased meal size, resulting in their hyperphagia and obesity. Similar results were observed when the OLETF rats were maintained on a balanced liquid diet. Average meal size was consistently elevated, and the resulting decrease in meal frequency did not adequately compensate for this elevation. Examination of microstructure of licking during these meals demonstrated that there were no differences in the initial rates of licking within a meal between OLETF and LETO rats, but the rate at which lick rate declined in the OLETF rats was significantly slower. Furthermore, consistent with their lacking, a functional CCK satiety pathway, OLETF rats were less sensitive to the feeding inhibitory effects of gastric or intestinal nutrient infusion and failed to rapidly compensate for the increased caloric density of a high-fat diet, resulting in an exaggeration of their hyperphagia and obesity (32).

The OLETF rat has also revealed a potential action of CCK in longer-term energy balance (3). When OLETF rats were pair fed to amounts consumed by LETO controls, they failed to become obese. Examinations of patterns of hypothalamic gene expression in ad libitum-fed OLET F and LETO rats and pair-fed OLET F rats demonstrated that pair-fed OLET F rats had significantly elevated levels of NPY expression in the dorsomedial hypothalamus (DMH) relative to both the ad libitum-fed OLET F and ad libitum-fed LETO rats. This result suggested that these rats might have a primary deficit in central neuropeptide Y (NPY) signaling that contributed to their failure to compensate for their increased meal sizes. Consistent with this view, young, preobese OLETF rats displayed the same deficit, increased DMH NPY expression. When allowed ad libitum access to food, OLETF rats become obese, and the NPY mRNA expression normalized. Interestingly, the DMH is one of the few hypothalamic sites in the rat that contains CCKα receptors (see Table 1) and the DMH has been demonstrated to be the brain site at which local CCK injection produces the greatest intake reduction (4). This pattern of findings suggests that CCK, acting through CCKα receptors in the DMH, plays a role in suppressing DMH NPY. In the absence of functional CCKα receptors, this inhibition of NPY is removed, and rats fail to compensate for their increased meal size. These ideas require further work, but they do suggest an important role of brain CCK in food intake control beyond that of peripheral CCK in limiting the size of individual meals.

In summary, CCK is an important feedback signal for the control of meal size. CCK is released from intestinal endocrine cells both locally and into the plasma in response to the intraluminal presence of nutrient digestion products. CCK acts to coordinate the digestive process through both endocrine and paracrine/neurocrine actions by stimulating pancreatic and gallbladder secretions, inhibiting gastric emptying and modifying intestinal motility. CCK also plays a major role in satiation. CCK acts at CCKα receptors on vagal afferent fibers and on pyloric circular muscle to inhibit ingestion through both direct and indirect actions. The OLETF rat provides a novel model for examining multiple actions of CCK. In the absence of intact CCK signaling, meal sizes increase. The obesity in the OLETF rat, lacking CCKα receptors, suggests a role for brain CCK in modulating hypothalamic NPY expression and affecting overall energy balance.

REFERENCES


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