Cholecystokinin

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**Abstract**

In 1928, Ivy and Oldberg discovered that intestinal extracts prepared after instilling weak acid or fats into the proximal duodenum, elicited gallbladder contraction in dogs, cats, and guinea pigs (33). Based on this biological property, the hormone was named cholecystokinin (CCK). In addition to gallbladder contraction, CCK was later shown to stimulate pancreatic secretion (55) and to delay gastric emptying by its effect on the lower esophageal sphincter (80). CCK was the first hormone shown to influence satiety and cause reduction in food intake (23). Due to this discovery and the implications of CCK’s therapeutic potential for eating disorders, considerable attention has focused on the study of this hormone.

In the gastrointestinal tract, CCK is secreted by discrete enteroendocrine cells (EECs) which contain intermediate-size secretory granules (I cells) (95). CCK-producing cells are primarily located in the proximal small intestine (duodenum and jejunum, *Figure 1*), and their numbers decrease significantly towards the distal end (ileum and colon) (**Figure 2**). CCK cells are often flask-shaped with the narrow apical edge facing the gut lumen. The basolateral membrane often contains one or more basal process(es) named neuropods that run alongside or project into the lamina propria (6, 10). Neuropods contain neuronal markers and have been shown to interact with enteric nerves suggesting that in addition to secretion to the blood, CCK can be released directly adjacent to enteric nerves (**Figure 3**). CCK immunoreactivity is abundant in the pyloric region of mouse stomach (45), cerebral cortex, dopaminergic neurons projecting to the limbic forebrain and ventromedial hypothalamus, peripheral nerves of the gastrointestinal tract, celiac plexus, and vagus nerve (2, 48). CCK has been shown to function both as a hormone and a neurotransmitter and belongs to the ‘brain-gut’ family of peptides. In addition to intestinal and neuronal expression, CCK is also expressed in other tissues such as the urogenital tract and heart (78). The structure of CCK and its function, pertaining to its role in the gastrointestinal tract, is discussed in this review.
Figure 1. Transverse section of mouse duodenum showing CCK cells (green). Nuclei are stained with DAPI (blue).

Figure 2. The number of CCK cells is highest in the proximal small intestine of the mouse and decreases exponentially towards the distal end (ileum). CCK antibody (8) used for immunostaining sections did not react with gastrin. Number of cells in 5 sections spread over 1 inch in length were counted (R. Chandra, unpublished data).

Figure 3. Transverse section of CCK-EGFP mouse duodenum showing EGFP positive CCK cells (green) and enteric nerves immunostained for pan-neuronal marker PGP9.5 (red). Two CCK cells from the left panel, Cells A and B, are shown at higher magnification on the right. Cell A has three short whisker-like neuropods and its basolateral surface is in contact with enteric nerves. Cell B has 2 thin neuropods (arrows). The longer of the two neuropods terminates in a bulb and is in contact with a nerve.

1. General

CCK is present in all vertebrates from fish to mammals. A CCK-like peptide has been found in the protostegate Ciona intestinalis, suggesting that the CCK/gastrin family probably arose 500 million years ago (37). Based on the phylogeny of CCK and gastrin genes in protostegates versus cartilaginous fish such as Squalus acantias and amphibians, it is proposed that gene duplication occurred 350 million years ago during the appearance of cartilaginous fish (37, 38). In humans, the CCK gene is present on chromosome 3, spans 7 kb, and consists of three exons, the first of which is noncoding (87, 88). The mouse Cck gene is similar in structure to the human gene and is present on chromosome 9 in a syntenic cluster (21).

CCK polypeptides of various lengths have been described in the literature (Figure 4). Although there are four known transcripts of the human CCK gene, only a single preprocholecystokinin
A polypeptide of 115 amino acids is synthesized. After proteolytic excision of the signal peptide by signal peptidase, prochollecystokinin of 94 amino acids is generated. This is again cleaved on both the N (24 amino acids) and C (12 amino acids) termini by endopeptidase and proprotein convertase 1 respectively, to generate a mid-section polypeptide of 58 amino acids known as CCK-58, which is the largest known circulating form of the hormone (19). It contains a carboxyl-amidated phenylalanine and O-sulfated tyrosine residue, which is responsible for increasing its biological activity by approximately 100-fold (18, 76, 77). CCK-58 undergoes subsequent endopeptidase cleavage at single or double basic residues to generate shorter peptides, CCK-39, CCK-33, CCK-22, CCK-12 and CCK-8 (3, 84). CCK-8 is the smallest peptide which exhibits complete biological activity and is used most often in experiments for assessing CCK function. The five C-terminal residues of CCK are identical to gastrin, and as a result these two hormones display some functional similarities. This sequence identity complicated the measurement of CCK in the blood, as many antibodies against CCK-8 cross react with gastrin which is present at much higher concentrations in the blood (5).

**Regulation of CCK secretion**

CCK is released from EECs in response to entry of food into the duodenum. Plasma levels of CCK increase from basal levels of 0.5-1 pM to peak levels of 5-15 pM within a few minutes of food ingestion. In rodents, peak plasm levels are usually attained within 20 minutes of oral gavage. In humans, postprandial levels remain elevated for 3-5 hours until food empties from the stomach into the duodenum (57). Therefore, gastric emptying affects CCK secretion. Plasma CCK levels decline once food passes from the proximal small intestine. The half-life of CCK in the plasma is very short; in dogs the half-life of CCK-58 was 4.4 ± 0.6 minutes and that of CCK-8 was shown to be 1.3 ± 0.1 minutes (32). CCK is cleared from the circulation as it passes through the liver and by neutral endopeptidases in capillary endothelial cells (71). CCK secretion is stimulated by ingested fats, proteins, and amino acids, whereas carbohydrates such as glucose cause only a brief, transient increase in circulating CCK levels (57).

**Figure 4.** Amino acid structure of the human CCK precursor and the different forms of CCK produced by processing.
The apical surface of CCK-producing cells is exposed to the intestinal lumen and receptors located on the apical surface can be stimulated by food molecules present in the lumen. Aromatic L-amino acids such as phenylalanine and tryptophan (but not non-aromatic amino acids such as alanine) stimulate CCK release through a Ca\(^{2+}\)-dependent mechanism mediated by the calcium-sensing receptor (CaSR) (61, 94) while L-phenylalanine, L-leucine, and L-glutamic acid mediate CCK release through umami taste receptors T1R1 – T1R3 (14). In addition to amino acids, medium to long chain fatty acids (C12 and longer) also stimulate CCK release (65). The long chain fatty acid receptor GPR40 also known as free fatty acid receptor 1 (FFAR1), mediates some fatty acid-dependent CCK secretion (62). Fat mediated CCK-stimulation was completely eliminated in immunoglobulin-like domain containing receptor 1 (ILDR1) knockout mice suggesting that ILDR1 plays a role in CCK release (13). ILDR1-mediated CCK release occurred only in the presence of both high density lipoprotein (HDL) and fatty acids, suggesting a novel pathway in which uptake of HDL and/or fatty acid from the basolateral membrane could play an important role in CCK release.

Evidence is accumulating that cell surface receptors linked to hormone secretion may be located on the basolateral surface of the CCK cell. ILDR1-mediated CCK release requires both fatty acids and HDL which are most likely secreted onto the basolateral surface of the intestinal epithelium suggesting that CCK cells respond to absorbed nutrients (13). In addition, bile acid receptors have also been localized to the basolateral surface of EECs (8).

CCK secretion is under the control of negative feedback regulation by pancreatic proteases and bile acids (25, 56, 70). In most species, including humans, it has been shown that food-stimulated CCK secretion is suppressed by release of pancreatic proteases (31, 34, 54). This effect appears to be mediated by an endogenous protease-sensitive CCK-releasing peptide in the intestinal lumen (30, 50, 85). In addition to proteases, bile acids in the intestine affect CCK secretion (26, 55). In rats, luminal administration of taurocholate inhibited pancreatic enzyme secretion as well as CCK (90). In humans, single bile acids did not cause a decrease in CCK release, however, under conditions in which endogenous release of bile acid was inhibited by the CCK1 receptor antagonist loxiglumide, addition of a mixture of bile acids to a test meal prevented the increase in CCK release suggesting that bile acids play an important role in downregulating CCK secretion (43).

CCK released from EECs can act locally via a paracrine mechanism or enter the enteric bloodstream and exert effects on distant target organs through hormonal mechanisms. There is evidence for neural activation of vagal afferents in the intestinal mucosa which express CCK1 receptors and terminate in the lamina propria (74). Although the effect of CCK on the vagus nerve was believed to be paracrine or hormonal action, recently, CCK cells have been shown to be in direct contact with neurons (7, 12, 53) and this connection may provide a direct neural link between the gut and brain.

**Cholecystokinin Receptors**

The action of CCK on tissues is mediated by two G protein-coupled receptors, CCK1 and CCK2, formerly known as CCK-A (for alimentary) and CCK-B (for brain) (17). CCK1 receptors are mainly located in the gastrointestinal tract, myenteric plexus, and vagal afferents and bind sulfated CCK with 1000-fold higher affinity than gastrin or nonsulfated CCK (16). CCK2 receptors are present in the stomach and the brain and have similar affinity for sulfated or nonsulfated CCK and for gastrin; hence this receptor is also known as the gastrin receptor. Development of receptor knockout mice have demonstrated that CCK1 receptors are important in regulation of CCK-mediated satiety responses (44) while CCK2 receptors are primarily
involved in maintaining gastric morphology and acid secretion (47). A double CCK1/CCK2 knockout mouse displayed brain development abnormalities (67).

2. Actions of CCK

CCK Induces Gallbladder Contraction

CCK mediates bile release into the intestine by the dual action of stimulating gallbladder contraction and relaxing the sphincter of Oddi which allows bile to flow into the duodenum. In humans, infusion of CCK-8, decreased gallbladder volume by 80% and increased bilirubin output by 8 to 10-fold (82). These effects are mediated by CCK1 receptors that are located on both the smooth muscle layer of the gallbladder as well as cholinergic nerve terminals (81). The CCK1 receptor antagonist loxiglumide blocked bile release and CCK1 receptor knockout mice showed increased gallbladder volumes with enhanced susceptibility for gallstone formation compared to wild type mice (93).

CCK Stimulates Exocrine Pancreatic Secretion

Along with gallbladder contraction, the effects of CCK on pancreatic secretion were demonstrated in the first half of the 20th century when this hormone was also known as pancreozymin (29). CCK is one of the most important stimulants of pancreatic secretion (11). Physiological levels of exogenous CCK-33 administered to humans induced a trypsin output profile similar to that seen after a standardized test meal (40). The effect of CCK on human pancreatic enzyme output was partially blocked by the CCK1 receptor antagonist loxiglumide and completely inhibited by atropine suggesting that cholinergic activation is the major mechanism of CCK action (1, 22). In support of this finding, human acinar cells did not respond to CCK agonists and were shown to lack CCK1 and CCK2 receptors even though adenoviral mediated expression of CCK receptors on human acinar cells resulted in stimulation with CCK agonists (35). In contrast to humans, pancreatic secretion in rodents is mediated by direct activation of CCK1 receptors located on acinar cells as well as on vagal afferents (49, 83, 92).

CCK Stimulates Pancreatic Ductal Secretion

In addition to stimulation of protein by acinar cells, CCK promotes bicarbonate and fluid secretion from pancreatic ductal cells. Intraduodenal administration of corn oil in dogs led to elevated CCK levels along with protein and bicarbonate secretion (36). Secretion of bicarbonate and fluid was dependent on activation of CCK1 receptors, but not CCK2 receptors, and could be mimicked by a CCK1 receptor agonist (86).

Trophic effect of CCK on the pancreas

Several studies have demonstrated that CCK can increase pancreatic size (96). Exogenous administration of CCK increased pancreatic mass in hamsters and rats (27, 28, 68). In the rat, CCK1 receptor but not CCK2 receptor agonists increased pancreatic mass by increasing the number of cells comprising the exocrine pancreas (72). Since the human pancreas lacks CCK1 receptors, administration of ximelagatran, which has protease inhibitor activity and stimulates pancreatic growth in rats, did not have a significant effect on pancreatic growth in humans (56). Despite the lack of hypertrophic effects observed in human studies, it was recently shown that pancreatic atrophy resulting from total parenteral nutrition (TPN) could be reversed by CCK in rodents. When sulfated CCK-8 was infused in rats on TPN or an oral food diet, an increase in pancreatic mass was observed compared to non-CCK infused rats. The increase in pancreatic mass was somewhat less in rats on TPN suggesting that other factors may be involved in atrophy of the pancreas during TPN (98).

CCK Delays Gastric Emptying
CCK has been shown to have a pronounced effect in delaying gastric emptying in fish, rodents, dogs and humans (15, 69) by both relaxation of the proximal stomach and contraction of the pylorus (99). Administration of physiological levels of CCK-8 delayed gastric emptying in humans (52). In addition, this effect was dose-dependent and blocked by the CCK1 receptor antagonist loxiglumide (42, 52). Both vagal and splanchnic nerve pathways mediate the effect of CCK on gastric relaxation. In rats, bilateral cervical vagotomy partially reduced CCK-8-induced gastric relaxation but this response was completely eliminated when vagotomy was coupled with splanchnic nerve section (75).

**CCK Induces Satiety**

A seminal paper published in 1973 showed that administration of exogenous CCK-8 reduced food intake in rats (24). Similar effects have been noticed in a number of species including humans, where CCK-8 or CCK-33 infusion limited meal size and frequency (41, 59). CCK mediates satiety through its effects on CCK1 receptors located on vagal afferent nerves which provide negative feedback to the dorsal hind brain limiting food intake. The CCK1 receptor antagonist devazepide reduced the effects of CCK on satiety (4, 73) and increased hunger in humans (97). Moreover, Otsuka Long Evans Tokushima Fatty (OLETF) rats which lack CCK1 receptors (due to a spontaneous deletion of the promoter and exons 1 and 2 of the CCK1 receptor gene) were insensitive to reduction of feeding after administration of exogenous CCK (66). However, a CCK1 receptor specific agonist, GI191771X, which delayed gastric emptying, did not cause reduction in body weight of obese patients (BMI ≥30 or ≥27 kg/m²) in a 24-week double-blind trial, suggesting that regulation of this pathway by CCK was insufficient for controlling obesity (9, 39).

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<tr>
<th>Influence on Tissue</th>
<th>Gall Bladder</th>
<th>Pancreas</th>
<th>Stomach</th>
<th>Brain</th>
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<tr>
<td>Physiological effects</td>
<td>1. Stimulation gall bladder contraction</td>
<td>1. Augmentation of trypsin output from acinar cells</td>
<td>1. Relaxation of the proximal stomach</td>
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<td></td>
<td>2. Relaxation sphincter of Oddi</td>
<td>2. Increase in bicarbonate and fluid secretion from ductal cells</td>
<td>2. Contraction of pylorus</td>
<td></td>
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<tr>
<td>Action mediated by</td>
<td>CCK1 Receptor</td>
<td>CCK1 Receptor</td>
<td>CCK1 Receptor</td>
<td>CCK1 Receptor</td>
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<tr>
<td>Net Effect</td>
<td>Bile flow into duodenum</td>
<td>Digestion of food</td>
<td>Delayed gastric emptying</td>
<td>Induction of satiety</td>
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**Figure 5.** Physiological targets for CCK action.
In summary, CCK plays an important role in the regulation of postprandial gallbladder contraction, pancreatic secretion (enzyme, bicarbonate and fluid), as well as gastric emptying which optimizes the lumenal environment (pH) and regulates digestion of food in the gastrointestinal tract (Figure 5).

3. Tools for study of CCK

a. Peptide:
CCK-8 (DYMGWMDF-NH$_2$) peptide retains full biological activity and is available in powder form from several vendors (Anaspec, Sigma Chemical Company, Tocris). Biologically active CCK-8 is sulfated on its tyrosine residue. De-sulfated CCK-8 peptide is often used as a control. Longer forms of CCK can be purchased as recombinant proteins.

b. Antibodies:
Antibodies that detect CCK on western blots or in tissues are available from numerous sources (Abcam Cat# ab134713, Sigma Chemical Company Cat# C2581, LSBio Cat# LS-C293314). In our lab we generated a rabbit polyclonal to amino acids 19-36 of human CCK and affinity purified the antiserum over a peptide column (10). It should be noted that antibodies generated against CCK-8 peptide may also detect gastrin due to sequence identity of the last four amino acids.

c. cDNAs:
Human and rodent codon-optimized CCK cDNAs for expression in *E. coli* and mammalian cells are commercially available. Myc-tagged CCK cDNAs are also available.

d. Viral vectors:
Lentiviral particles of CCK tagged with myc or EGFP are available. Adenoviruses containing either the human or rodent CCK gene can also be purchased.

e. Assay:
ELISA kits with sensitivity in the range of 10 -1000 pg/mL are available from several vendors to measure CCK concentration. Kits from the following manufacturers are cited in literature: RayBiotech (100), Cloud-Clone Corp. (46), Phoenix Pharmaceuticals (20, 89). In addition, radioimmunoassays (RIA) are also used to quantitate CCK (60, 64, 91). In our laboratory we routinely use a CCK bioassay to measure CCK concentrations in human or rodent plasma samples (51, 58, 79). For the CCK bioassay, trunk blood is collected from three mice (1 ml total serum) per data point.

f. Mouse models:
CCK knockout mice (expressing lacZ reporter in cells where CCK is knocked out) and transgenic mice expressing EGFP in CCK cells are available from Jackson Labs and Mutant Mouse Resource and Research Centers (MMRRC) respectively. These mice have been characterized in various publications in the literature (10, 63).

g. Clinical Testing:
CCK-8 intravenous bolus injections (Sincalide 0.02 mcg/kg) are given to patients to perform clinical assessments: 1) Measurement of gallbladder contractility prior to cholecystectomy (https://clinicaltrials.gov/ct2/show/record/NCT02748525); 2) Evaluation of the composition of pancreatic secretion; and 3) Diagnosis of intestinal disorders by barium sulfate imaging; administration of CCK-8 accelerates the transit of barium through the GI tract. (https://www.rxlist.com/kinevac-drug.htm#description).
4. References:


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