Annu. Rev. Physiol. 1997. 59:221–42

CHOLECYSTOKININ CELLS

Rodger A. Liddle
Department of Medicine, Duke University Medical Center and Durham VA Medical Center, Durham, North Carolina 27710

KEY WORDS: gastrointestinal hormone, radioimmunoassay, bioassay, cell culture

ABSTRACT

Cholecystokinin (CCK) is an important hormonal regulator of the digestive process. CCK cells are concentrated in the proximal small intestine, and hormone is secreted into the blood upon the ingestion of food. The physiological actions of CCK include stimulation of pancreatic secretion and gallbladder contraction, regulation of gastric emptying, and induction of satiety. Therefore, in a highly coordinated manner, CCK regulates the ingestion, digestion, and absorption of nutrients. CCK is produced by two separate cell types: endocrine cells of the small intestine and various neurons in the gastrointestinal tract and central nervous system. Accordingly, CCK can function as either a hormone or a neuropeptide. This review focuses on the physiology of the CCK cell in the intestine and, in particular, on how the CCK cell is regulated to secrete its hormone product. The effects of ingested nutrients on the CCK cell and the intracellular messenger systems involved in controlling secretion are reviewed. A summary is provided of recent studies examining the electrophysiological properties of CCK cells and newly discovered proteins that act as releasing factors for CCK, which mediate feedback pathways critical for regulated secretion in the intact organism.

Introduction

Cholecystokinin (CCK) was discovered in 1928 by Ivy & Oldberg based on the ability of intestinal extracts to stimulate gallbladder contraction when infused into dogs (1). In 1943, Harper & Raper recognized that similar intestinal extracts also stimulated pancreatic enzyme secretion and proposed the name pancreozymin (2). It was not until the active substance was purified and the amino acid sequence determined that CCK and pancreozymin were proven to be the same hormone that now goes by the name cholecystokinin (3).

In addition to the two biological actions described above, CCK has several other important activities. Among the most notable is its ability to induce

satiety and reduce food intake in experimental animals and humans (4–6). It also inhibits gastric emptying and gastric acid secretion and stimulates intestinal peristalsis. Defining the physiological actions of CCK has been greatly facilitated by the development of specific and potent antagonists of the CCK-A (A for alimentary origin) receptor. Each of the above actions ascribed to CCK arises from endogenous CCK, as shown in studies by which the response to a normal meal can be reversed by specific CCK-A receptor antagonists.

CCK release is stimulated by ingestion of food, with fats and protein the most potent secretagogues. CCK secretion is initiated when food leaves the stomach and enters the small intestine, and secretion continues until proteins, fats, and their metabolites have passed the upper small intestine.

CCK receptors have been identified on the gallbladder, pancreas, and stomach (7). Recent evidence indicates that specific CCK-A receptors are also present on peripheral autonomic afferent nerves that enable CCK to initiate certain neural reflexes. The specific site on which CCK acts to affect organ responses is still somewhat unclear. The best evidence for a purely endocrine role for CCK is regulation of gallbladder contraction, whereas other effects may be either neural or a combination of endocrine and neural actions. Nevertheless, the importance of CCK in regulating a variety of digestive processes must not be underestimated. CCK secreted locally or into the blood binds to specific receptors on the gallbladder, pancreas, stomach, or various nerves to stimulate gallbladder contraction, pancreatic enzyme secretion, delay gastric emptying, and regulate satiety (7). Therefore, in a highly regulated fashion, CCK coordinates the ingestion, digestion, and disposal of nutrients (8, 9).

Peptide Structure

CCK was originally purified from porcine intestine as a 33-amino acid peptide (3). The hormone possesses an amidated carboxy-terminal pentapeptide, Gly-Trp-Asp-Met-Phe-NH_2, that is identical to that of gastrin. The carboxyl terminus of CCK is the biologically active portion of the hormone, and because of sequence similarity between CCK and gastrin, each hormone can interact with the receptor of the other (see receptor characterization below). Therefore, gastrin has slight CCK-like bioactivity and CCK possesses some, albeit weak, gastrin-like activity. The homology shared by the two peptides explains why antibodies raised against CCK often cross-react with gastrin. This overlapping cross-reactivity has been a considerable problem in developing radioimmunoassays for CCK, particularly because gastrin circulates in the blood at concentrations 10–100 times greater than those of CCK (10).

Since the discovery of CCK, multiple molecular forms have been identified in intestine, brain, and the circulation of multiple species (11–20). The octapeptide of CCK (CCK-8), consisting of the carboxy-terminal 8 amino acids of CCK,

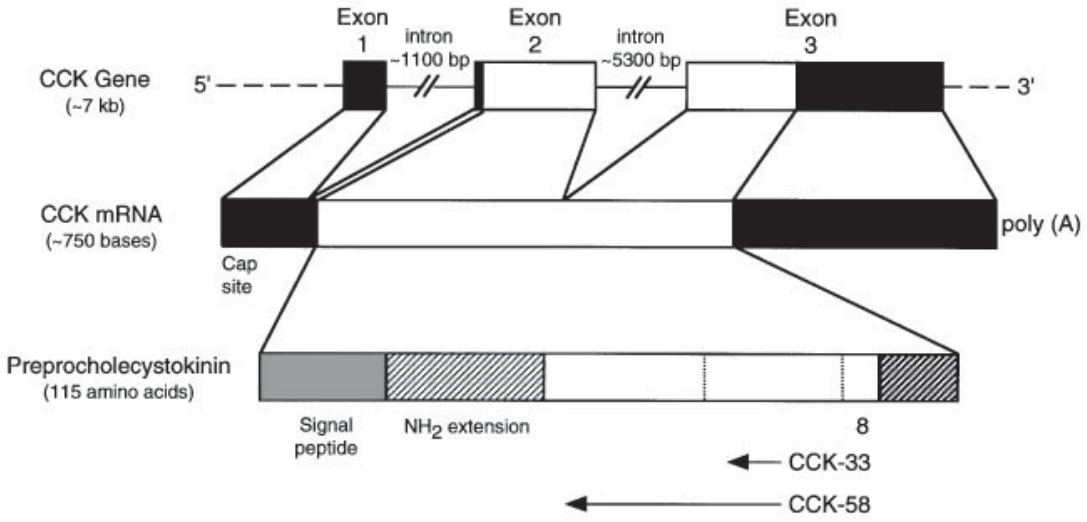

Figure 1 Structure of the CCK gene, complementary DNA and preprohormone. (Modified from Reference 8.)

is the most biologically potent small peptide of CCK that has been isolated. However, amino-terminal extended forms have been extracted from brain and intestine of the pig, dog, rat, and human. Using techniques to minimize protein degradation, recent studies have demonstrated that the most abundant molecular form of CCK in these species is CCK-58. Intermediate-sized peptides of 39, 33, 25, 22, 18, 8, 7, 5, and 4 amino acids have been isolated from several species.

CCK Gene Structure and Expression

The complementary DNA structures have been determined for rat, mouse, pig, and human CCK (21–24). The structure of the rat cDNA is shown schematically in Figure 1. The 345-nucleotide 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.

The genes for CCK isolated for mouse, rat, and human reveal remarkable conservation (22–25). Each consists of ~7 kilobases containing three exons, the second and third of which encode the prepropeptide. In all species, only a single gene encodes CCK. The human CCK gene is located on chromosome 3 in the 3q12-3pter region (26, 27).

The mature CCK mRNA is ~750 bases. It is expressed in brain and intestine in mature animals in nearly equivalent amounts. CCK mRNA is most abundant in the cerebral cortex and duodenum. In the mouse, at birth, CCK mRNA levels are relatively high in the intestine but very low in brain. Over the first two weeks of life, levels decrease in the gut and increase in brain. These

changes correlate with levels of immunoreactive CCK protein in the respective tissues. Intestinal expression of CCK mRNA is modified by diet. CCK mRNA levels decline in either fasting rats or rats fed a diet that does not stimulate CCK secretion (28). CCK mRNA expression increases with feeding under conditions that also stimulate CCK secretion. However, it is possible to stimulate CCK secretion without affecting CCK gene expression by administration of exogenous bombesin (29). Somatostatin has been shown to inhibit CCK gene expression (30).

Structure-Activity Relationships

To function as a CCK molecule, a peptide must have the sequence Trp-Asp-Met-Phe-NH_2. However, because this sequence is identical to the the carboxyl terminus of gastrin, this peptide does not confer CCK-like specificity. In order to bind specifically to CCK receptors, CCK peptides must be extended to 7 amino acids. Full potency is not achieved unless the tyrosine residue at position 7 from the carboxyl terminus is sulfated (31). Although sulfation is unusual for hormones, it is critical for biological potency of CCKs. The unsulfated form of CCK is ~1000-fold less active than its sulfated counterpart. In contrast, sulfation occurs only 50% of the time in gastrin biosynthesis and is not important for its biological activity.

Molecular forms ranging in size from CCK-8 to CCK-33 appear to be of similar biological potency on pancreatic acinar cells (32). However, CCK-58 is somewhat less potent on a molar basis than CCK-8 and is less immunoreactive with CCK antibodies (33). This diminished activity is likely due to the structure of CCK-58, whereby the amino-terminal end shields the biologically active carboxy-terminal end of CCK from interacting with CCK receptors or C-terminal directed antibodies. Cleavage of CCK-58 with trypsin restores both the full biological potency and immunoreactivity to that of CCK-8 (34). Although CCK-58 is the major molecular form in intestine, brain, and the circulation of many species, identification of multiple smaller forms of CCK in circulation, even under conditions that preserve CCK-58, suggests that intracellular processing of CCK occurs to produce small forms of CCK that are secreted into the blood.

CCK Receptors

CCK exerts its biological effects by binding to specific receptors on its target tissues. Originally receptors were characterized on pancreatic acinar cells, islets, gallbladder, and brain by radioligand binding and autoradiography (31). In the pancreas and gallbladder, CCK bound with an affinity that was ~1,000-fold greater than that of either unsulfated CCK or gastrin. These receptors were termed CCK-A. In the brain, however, CCK and gastrin bound with similar

affinities. These receptors became known as CCK-B (B for brain origin). Along with these two types of CCK receptors, a third related type, "gastrin receptor," which exhibited binding properties similar to those of the brain CCK receptor, was believed to transmit the biological actions of gastrin in the stomach. However, with recent cloning and expression of the CCK receptor cDNAs, it has become clear that there are only two CCK receptors, A and B, and that the gastrin receptor is identical to the CCK-B receptor.

The CCK-A receptor complementary DNA was cloned following purification of the receptor protein from rat pancreatic acinar cells (35). The cDNA encodes a seven transmembrane protein typical of G protein-coupled receptors. It consists of 444 amino acids, which when expressed in transfected cells, demonstrates high affinity for sulfated CCK and much lower affinity for unsulfated CCK or gastrin.

The CCK-B receptor cDNA was identified by expression cloning from canine gastric parietal cells (36). When expressed in transfected cells, the CCK-B receptor binds CCK and gastrin with similar affinities and demonstrates a ligand-induced increase in intracellular calcium. When the cDNAs for the rat and human receptors were cloned, it was demonstrated that CCK receptor cloned from brain was identical to the gastrin receptor in the stomach (37). The deduced amino acid sequences of the rat CCK-A and canine CCK-B receptors are ~50% homologous.

CCK Cells

As is typical of many gastrointestinal hormones, CCK is produced by endocrine cells of the intestinal mucosa, which are concentrated in the duodenum and proximal jejunum. A gradient of cell density exists such that CCK cell abundance is greatest in the proximal small intestine and less in the distal intestine. Endocrine cells containing CCK are flask-shaped, with their apical surfaces oriented toward the lumen of the gut (38, 39) (Figure 2). In this position, microvillus-like processes come in contact with lumenal contents. Secretory granules, which are ~250 nm in size and contain CCK, are concentrated around the basolateral surface of the cell. It is this orientation that allows food or other factors within the intestinal lumen to interact with the apical surface of the CCK cell and initiate a series of as yet unknown intracellular signaling events that ultimately result in secretion of CCK from the basal surface of the cell into the blood. According to the Wiesbaden classification, CCK cells, by their ultrastructural characteristics, have been officially named I cells (40). Where examined in experimental animals or humans, I cells have not been shown to contain other gut hormones. Outside of the intestinal tract, CCK is synthesized by a subpopulation of pituitary and adrenal meduallary cells (41, 42). The function of CCK in these locations is unknown.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.