How does cholecystokinin stimulate exocrine pancreatic secretion?
From birds, rodents, to humans

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Wang BJ, Cui ZJ. How does cholecystokinin stimulate exocrine pancreatic secretion? From birds, rodents, to humans. Am J Physiol Regul Integr Comp Physiol 292: R666–R678, 2007. First published October 19, 2006; doi:10.1152/ajpregu.00131.2006.—The field of cholecystokinin (CCK) stimulation of exocrine pancreatic secretion has experienced major changes in the recent past. This review attempts to summarize the present status of the field. CCK production in the intestinal I cells, the molecular forms of CCK produced and subsequently circulated in the blood, the presence or absence of CCK receptors on the isolated pancreatic acinar cells and the associated signaling for acinar cell secretion, and the actual circuits and sites of action for CCK regulation of exocrine pancreatic secretion in vivo are reviewed in different animal species with an emphasis on birds, rodents, and humans. Clear differences in the relative importance of neural and direct modes of CCK action on pancreatic acinar cells were identified. Rodents seem to be endowed with both modes of action, whereas in humans the neural mode may predominate. In birds, such as duck, the direct mode needs further assistance from putative adenylyl cyclase-activating peptide/VIP receptors. However, much further work needs to be directed to the neural mode to map out all sites of CCK action and details of the full circuits, and we foresee a major revival for this field of research in the near future.

Pancreatic acinar cells; vagal afferent nerves; plasma cholecystokinin concentration; species specificity

IT IS GENERALLY ACCEPTED THAT cholecystokinin (CCK) as a gut hormone is an important endogenous secretagogue in exocrine pancreatic secretion. CCK also stimulates gallbladder contraction and enhances growth of the exocrine pancreas (63, 115, 138). CCK is produced and released by the intestinal mucosal I cells (78). This source of CCK may travel through the circulation to target tissues that include the exocrine pancreas and gallbladder (65, 78). CCK peptides are also found in large quantities in neurons, but neuronal CCK contributes negligibly to CCK concentration in the circulation (118). This general picture has been changed drastically by the recent findings that CCK can also stimulate exocrine pancreatic secretion by the excitation of sensory nerves and vagovagal and enteropancreatic reflexes, and this may be the only pathway in humans (65, 105). In addition, major differences have been found in the traditional humoral pathway, depending on the animal species (35, 149). Therefore, the purpose of this review is to present the current status of CCK regulation of exocrine pancreatic secretion with a particular emphasis on species specificity as shown in birds, rodents, and humans.

GUT CCK-SECRETING CELL

CCK is produced by I cells of the intestinal mucosa, which in rodents are concentrated in the duodenum and proximal jejunum (31, 78). I cells are flask shaped, with their microvilli-in rodents are concentrated in the duodenum and proximal jejunum (31, 78). I cells are flask shaped, with their microvilli-oriented toward the intestine lumen (31, 78). Their secretory granules, which are ~250 nm in size and contain packaged CCK, are concentrated around the basolateral surfaces of the cell (13). Because CCK-secreting cells are diffusely scattered in intestinal mucosa, I cell studies are rather limited to immortalized cell lines (44). The neuroendocrine cell line STC-1 was derived from the murine gut and established as a model system for rodent I cell CCK secretion (120). In STC-1 cells, SNARE proteins syntaxin-1, synaptosome-associated protein of 25 kDa (SNAP-25), and vesicle-associated membrane polypeptide 2 (VAMP-2) have been found to be involved in Ca2+-induced CCK secretion (100), and the GTP-bound form of rab3A has been found to be a negative clamp for exocytosis (39).

The most potent stimulators of CCK secretion are digestive products of fats and proteins, with carbohydrates being less potent. Of triglyceride hydrolysis products, fatty acids with longer acyl chains (>12 carbon atoms) are potent secretagogues for CCK secretion; of protein breakdown products, tryptophan and phenylalanine are the most potent (79). Soybean agglutinin stimulates CCK release by opening L-type Ca2+ channels in cultured rabbit jejunal cells (62). Fatty acids elicit a marked increase in intracellular Ca2+ concentration ([Ca2+]i) and CCK secretion in STC-1 cells; both responses are blocked by nicardipine (88). In STC-1 and GLUTag cells, fatty acid induces both [Ca2+]i increases and CCK secretion (129). Sodium oleate has been found to stimulate CCK release in enriched rat mucosal cells and in STC-1 cells (18).

It was found early on that diversion of bile-pancreatic juice and infusion of trypsin inhibitors into the intestine of rats led to enormous increases in pancreatic secretion and CCK release (45); this formed the basis of negative feedback regulation of CCK release. The hypothesis postulated that trypsin-sensitive
intestinal-releasing factors were present in the small intestine lumen, and, when pancreatic proteases were absent, endogenously produced releasing factors are intact and would interact with the CCK cell to stimulate CCK release (79). This idea led to the discovery of luminal CCK-releasing factor (LCRF), isolated from rat intestinal washings (133), and diazepam-binding inhibitor (DBI), isolated from porcine intestine (47). Both LCRF and DBI are involved in the negative feedback regulation of CCK secretion because they are intact and functional in the absence of the proteadic proteases. However, such mode of CCK feedback regulation may be restricted to rats because there is no spontaneous release of LCRF in humans. In humans, the release of LCRF requires the stimulation of luminal amino acids and fatty acids (78).

A peptide isolated from rat pancreatic juice when infused into rat intestine also stimulated CCK secretion, and this led to the discovery of the monitor peptide (77, 78, 82). Monitor peptide, also known as pancreatic secretory trypsin inhibitor I-61, is produced by pancreatic acinar cells. Monitor peptide-binding sites were found in rodent small intestine mucosa (89). The monitor peptide monitors the status in the intestinal lumen, and its increased presence after pancreatic secretion stimulates CCK release from I cells to trigger more pancreatic secretion (77, 78, 82). Monitor peptide—peptide, also known as pancreatic secretory trypsin inhibitor I-61, is produced by pancreatic acinar cells. Monitor peptide—led to the discovery of luminal CCK-releasing factor (LCRF), where LCRF is secreted into intestinal lumen to stimulate CCK secretion. In dispersed human intestinal mucosal cells and in STC-1 cells, LCRF markedly stimulated CCK secretion, and this led to the discovery of luminal CCK-releasing factor (LCRF). (133), and diazepam-binding inhibitor (DBI), isolated from porcine intestine (47). The biologically active DBI fragment DBI(33–50) induces CCK release when administered intraduodenally in rat (47).

In addition to luminal factors, neuropeptide bombesin (101, 131, 136), β-adrenergic receptor agonist (127), GABA (44, 54), and orexins (69) could all stimulate I cell CCK secretion. Both radioligand binding studies and Northern blot analyses suggested the presence of bombesin-like receptors in CCK cells (131). Bombesin-stimulated CCK secretion from STC-1 cells was PKC dependent and involved the MAPK pathway (101) and calmodulin (136). Liddle and colleagues (127) demonstrated the presence of β-adrenergic receptors in STC-1 cells, the stimulation of which led to cAMP production and CCK release (127). Addition of GABA depolarizes STC-1 cells, activating voltage-gated Ca\(^{2+}\) channels and subsequent CCK release (44). Further work found that, in addition to GABA\(_A\) receptors (44), functional GABA\(_C\) receptors were also present on STC-1 cells (54), and both played important roles in CCK secretion. Neuropeptide orexin stimulates CCK release in STC-1 cells, via orexin1 and orexin2 receptors (69). The distribution of CCK-immunoreactive cells is similar in birds and mammals (111). In ostrich, CCK is mainly produced in duodenum, whereas, in chicken, CCK production is concentrated at the transit from jejunum to ileum (61). Chicken CCK cell secretory granules are \(\sim 230\) nm in diameter (111). In chicken, both dietary proteins and amino acids are potent stimulators of CCK release (37, 150). In addition, two DBIs have been purified from chicken intestine (19).

### PLASMA CCK CONCENTRATION

Hormones classically exert their effect through the circulation (115). Therefore, measurement of plasma CCK is an important parameter in CCK physiology. Plasma CCK concentration can be measured by bioassay or radioimmunoassay (114) with the use of antibodies. Plasma CCK concentrations are usually in the picomolar range in each of the animal species examined (see Table 1). In humans, premeal plasma CCK concentration measured by bioassays or by radioimmunoassays in different studies varied from 1.1 \(\pm\) 0.1 to 2.8 \(\pm\) 0.5 pM; after a meal, plasma CCK concentration varied from 4.6 \(\pm\) 0.6 to 8.2 \(\pm\) 1.3 pM (see Table 1). An exception is a report that human basal CCK level was 8.3 \(\pm\) 2.5 pM and after a meal was 24.4 \(\pm\) 6.5 pM (14). The variation of plasma CCK concentration is partly because of different techniques and different antibodies used and the ingested food used to elevate CCK concentration. Before meals, rat plasma CCK concentrations were found to be in the range of 0.5 \(\pm\) 0.2 to 2.5 \(\pm\) 0.3 pM; after feeding, concentrations increased to 4.4 \(\pm\) 0.8 to \(\sim 13.4 \pm 3.8\) pM (29, 30, 81, 128). Basal CCK plasma concentration in chicken was found to be 5–10 pM; after feeding, this increased to 15–40 pM (37, 86).

### Table 1. Plasma CCK concentration in different animal species

<table>
<thead>
<tr>
<th>Species</th>
<th>Before meal</th>
<th>After meal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>2.0 (\pm) 0.2</td>
<td>7.4 (\pm) 0.7</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>2.0 (\pm) 0.2</td>
<td>4.6 (\pm) 0.7</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>1.0 (\pm) 0.2</td>
<td>6.0 (\pm) 1.6</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>8.3 (\pm) 2.5</td>
<td>24.4 (\pm) 6.5</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2.8 (\pm) 0.5</td>
<td>6.5 (\pm) 0.7</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>1.5 (\pm) 0.5</td>
<td>6.3 (\pm) 1.7</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>1.7 (\pm) 0.7</td>
<td>29.8 (\pm) 2.9</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>1.1 (\pm) 0.1</td>
<td>8.2 (\pm) 1.3</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>1.13 (\pm) 0.10</td>
<td>4.92 (\pm) 0.34</td>
<td>114</td>
</tr>
<tr>
<td>Rat</td>
<td>1.9 (\pm) 0.3</td>
<td>13.4 (\pm) 3.8</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>9.7 (\pm) 1.6</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>0.5 (\pm) 0.2</td>
<td>7.9 (\pm) 1.9</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>0.85 (\pm) 0.1</td>
<td>8.2 (\pm) 1.1</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>2.5 (\pm) 0.3</td>
<td>8.9 (\pm) 0.6</td>
<td>30</td>
</tr>
<tr>
<td>Dog</td>
<td>16</td>
<td>45</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>1.8 (\pm) 0.9</td>
<td>4.6 (\pm) 1.1</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>5.3 (\pm) 0.6</td>
<td>9.4 (\pm) 1.6</td>
<td>135</td>
</tr>
<tr>
<td>Cat</td>
<td>10.6 (\pm) 1.4</td>
<td>27.6 (\pm) 4.8</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>10–30</td>
<td>80–100</td>
<td>6</td>
</tr>
<tr>
<td>Pig</td>
<td>0.5 (\pm) 0.3</td>
<td>12.3 (\pm) 1.5</td>
<td>17</td>
</tr>
<tr>
<td>Chicken</td>
<td>5–10</td>
<td>15–40</td>
<td>37–86</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SE. *Medium-chain triglyceride meal; †long-chain triglyceride meal.
DIFFERENT MOLECULAR FORMS OF PLASMA CCK

CCK is a heterogeneous hormone and present in different molecular forms in mammals: CCK-83, CCK-58, CCK-39, CCK-33, CCK-22, and CCK-8 (Fig. 1). The different forms are all carboxyamidated and O-sulfated and are all ligands for the CCK1 receptor (11, 93, 114, 115). The endopeptidolysis of proCCK occurs mainly at monoarginyl sites, but the presence of CCK-22 shows that the Lys-61 site is also cleaved (11, 115).

Processing of proCCK is cell specific (11, 115). The plasma CCK originates almost entirely from I cells in small intestinal mucosa (78), which contain a mixture of medium-sized CCK molecules (CCK-58, CCK-33, CCK-22, and CCK-8) that are released into the blood circulation (11, 115). CCK is also a widespread neurotransmitter in the nervous system (114), and neurons mainly release CCK-8 (117). Thus brain and gut contain drastically different molecular forms of CCK. This is because proCCK is processed by different isoforms of the prohormone convertase. Prohormone convertase 1 is present in the intestines, whereas prohormone convertase 2 processes proCCK in the brain (116). Different animal species also have their plasma CCK in diverse forms (Table 2). CCK-33 was found to be the predominant form in human plasma, CCK-22 being the second most abundant, with CCK-58 less abundant (118). Moderate amounts of CCK-8 in human plasma have also been reported (114). CCK-58 is the major circulating form of CCK in canine blood (32, 135). The most abundant forms of CCK in cat plasma were found to be CCK-8, CCK-33, and CCK-58 (7). In pig plasma, the bioactive species comprised CCK-58, CCK-33, CCK-22, and CCK-8, with CCK-22 being predominant (17). Substantial amounts of CCK-22- and CCK-8-like peptides were reported in rabbit plasma, with a small amount of CCK-33-like peptide (113). The plasma forms of CCK in ostrich and chicken are not certain, but analysis of intestinal extracts indicated that some CCK-70 was present, and the dominant forms were CCK-8 and CCK-7 (61). Human proproCCK shares 55% identity with chicken amino acid sequence, further suggesting that CCK is highly conserved among different vertebrate species (60). It may be worthwhile to note here that strict blood collection procedures may be needed to prevent possible degradation of CCK during sample processing. With this new method, Reeve et al. (112) found that CCK-58 was the only major endocrine form of CCK in the rat.

CCK RECEPTOR IN DIFFERENT ANIMAL SPECIES

CCK binds to CCK1 receptors (CCK1R) on vagal fibers (75) or pancreatic acinar cells (93, 103, 142, 152) to evoke pancreatic enzyme secretion and to CCK receptors in the gastrointestinal tract to regulate gastrointestinal motility (46, 70, 87, 99). Receptors for CCK and gastrin are members of the G-protein-coupled receptor superfamily. Two receptor subtypes for CCK and gastrin have been classified (93, 103, 142). CCK1R, found in gall bladder, exocrine pancreas, and limited areas of the central nervous system, is highly selective for sulfated CCK analogs, whereas the CCK2 receptor (CCK2R), present in widespread areas in the brain and stomach, has high affinity for both sulfated and nonsulfated CCK and gastrin analogs (142).

Table 2. Plasma CCK forms in different animal species

<table>
<thead>
<tr>
<th>Plasma CCK Molecular Forms</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
</tr>
<tr>
<td>CCK-58*</td>
<td>112</td>
</tr>
<tr>
<td>Human</td>
<td></td>
</tr>
<tr>
<td>CCK-58, CCK-33,* CCK-22, CCK-8</td>
<td>114, 118</td>
</tr>
<tr>
<td>Canine</td>
<td></td>
</tr>
<tr>
<td>CCK-58*</td>
<td>32, 135</td>
</tr>
<tr>
<td>Cat</td>
<td></td>
</tr>
<tr>
<td>CCK-58, CCK-33, CCK-8</td>
<td>7</td>
</tr>
<tr>
<td>Pig</td>
<td></td>
</tr>
<tr>
<td>CCK-58, CCK-33, CCK-22,* CCK-8*</td>
<td>17</td>
</tr>
<tr>
<td>Rabbit</td>
<td></td>
</tr>
<tr>
<td>CCK-33, CCK-22,* CCK-8*</td>
<td>113</td>
</tr>
<tr>
<td>Chicken, intestine</td>
<td></td>
</tr>
<tr>
<td>CCK-70 CCK-8* CCK-7*</td>
<td>61</td>
</tr>
<tr>
<td>Ostrich, intestine</td>
<td></td>
</tr>
<tr>
<td>CCK-70 CCK-8* CCK-7*</td>
<td>61</td>
</tr>
</tbody>
</table>

*Indicates the most abundant form of CCK in blood.
Human CCK1R has >90% homology to the rat and guinea pig CCK1R (25). CCK1R of cynomolgus monkey is 98% identical to the human CCK1R (49). Rabbit CCK1R is 92% homologous (87% identity) to the human and 85% identical to the rat CCK1R (97).

Different animal species share ~90% identity in their CCK1R amino acid sequence (102). It is noteworthy that there was a seven-amino acid insertion (GGGGGGS) in the predicted third intracellular loop of the mouse receptor that has not been seen in CCK receptors from any other species (40) (see Figs. 2 and 3). The major differences in the sequences are in predicted intracellular domains, with the third intracellular loop being predominant and with the COOH-terminal tail also harboring several differences (40, 49).

Like other members of the G-protein-coupled receptor superfamily, CCK1R has a heptahelical transmembrane (TM) structure (4, 24, 91, 143) (Fig. 3). Mierke and colleagues (43) built a model of the human CCK1R. The TM motifs each form an α-helix that embeds into the lipid bilayer (42, 107). There is a disulfide bond between C18 and C29 in the NH2 terminus [CCK1R(1–47)] (107). The first and second extracellular loops are connected by a disulfide bond (C114–C196), which plays an important structural role. The extracellular loops and the NH2 terminus are vital for both recognition and binding of CCK (91). The COOH-terminal portion of the third cytoplasmic loop of CCK1R contains a stretch of charged residues that are thought to form an amphipathic α-helical extension of the sixth transmembrane domain in a critical orientation for G protein activation (143). Theoretical models of CCK1R have also been built by others (3, 28).

CCK receptors are present in chicken brain, pancreas, cecum, hypothalamus, and gallbladder, all with a dissociation constant (Kd) of 1 nM (121). In chicken, two CCK receptor subtypes exist: a central subtype in brain and hypothalamus that resembles the mammalian CCK2R in agonist binding and a peripheral subtype in pancreas, gallbladder, and cecum that resembles the mammalian CCK1R in agonist binding (102, 121, 140). These similarities notwithstanding, however, rodent CCK1 antagonist L-364,718 behaves as a chicken CCK2R antagonist, whereas rodent CCK2 antagonist L-365,260 has very low affinity for both receptor subtypes in chicken (121). At the amino acid level, chicken brain CCK receptor (CCK-Ck) shared ~50% homology with mammalian and Xenopus laevis CCK receptors (102) (see Fig. 2). CCK-Ck resembled rodent CCK2Rs regarding agonist binding (CCK-8, CCK-4, gastrin-17). However, CCK-Ck showed higher affinity for the rodent CCK1R antagonist devazepide than for the rodent CCK2R antagonist L-365,260 (102, 121, 140).

Human and canine CCK2/gastrin receptors share 90% amino acid sequence identity and have similar agonist affinities (10, 66, 71). However, human and canine receptor binding affinities for antagonists L-365,260 and L-364,718 differ by 20-fold. Beinborn et al. (10) found that a single amino acid in the sixth transmembrane domain of the CCK2/gastrin receptor corresponding to Val-319 in the human homologue was critical in determining binding affinity for antagonists L-365,260 and L-364,718. Substitution of Val-319 by leucine decreases affinity for L-365,260 20-fold and increases affinity for L-364,718; an isoleucine in the same position of human receptor selectively increases affinity for L-364,718 (10). Kopin et al. (67) identified eight amino acid residues in membrane domains adjacent to the cell surface; these residues project into a putative ligand-binding pocket of the human CCK2R, and this pocket is very important for ligand-receptor interaction. These data suggest that ligand-binding amino acid residues, particularly antagonist-binding residues in avian CCK receptors, may be different than those in mammals. Theoretical model building for the avian CCK receptor may help to understand this difference.

Early work indicated that a common ancestor CCK receptor diverged into CCK1R and CCK2R at or before the level of the divergence of birds and mammals from reptiles (104, 140). The evolution of CCK probably followed the evolution of CCK receptors. Johnsen (60) reviewed the sequence variations of CCK molecules in lower animals. More detailed studies on CCK receptors in lower animals are obviously needed.

**CCK STIMULATION OF EXOCRINE PANCREATIC SECRETION: DIRECT AND INDIRECT PATHWAYS**

Physiologists have used different models to investigate exocrine pancreatic secretion, including the in situ pancreas perfusion model and isolated pancreatic acini. The latter model makes it possible to study pancreatic acinar cell responses without influence from neural or hormonal factors, and single cell responses can be easily investigated. Comparison of data from these two general models revealed significant variations in the in-born mechanisms in different animal species.

**Rodents.** CCK-stimulated amylase release in isolated rodent pancreatic acinar cells is typically “bell-shaped.” At lower CCK concentrations, amylase release increased progressively with increasing concentrations, whereas, at higher CCK concentrations, amylase release gradually diminished with increasing CCK concentrations. This results in a maximal stimulating CCK concentration of ~100 pM (57, 122, 123). This bell shape may be due to simultaneous occupation in different proportions of high-affinity (Kd = 26 pM), low-capacity and low-affinity (Kd = 2.2 nM), high-capacity CCK1R states (123).

Further examination of the dose-response curve revealed that rodent CCK1Rs are exceptionally sensitive to CCK, with a threshold concentration at 1 pM (23, 57, 115, 149). Picomolar CCK concentrations induced significant amylase secretion in all isolated rodent (rat, mouse, and guinea pig) pancreatic acini, and maximal stimulating CCK concentration was 100 pM (149). CCK concentration of 10 pM typically induces regular Ca2+ oscillations in individual rat pancreatic acinar cells in intact acini (2). This also applies to mouse and rabbit pancreatic cells (5, 146). Atropine does not alter secretory responses to CCK in isolated rodent pancreatic acini (2, 56, 147), indicating that CCK’s effect on rat pancreatic enzyme secretion in isolated pancreatic acini is not dependent on cholinergic stimulation. Hence, in rodents, physiological concentrations of CCK could stimulate pancreatic enzyme secretion by direct stimulation of CCK1Rs on pancreatic acinar cells (51, 124).

CCK, however, could also influence exocrine pancreatic secretion in vivo by a neural pathway in rodents. Bilateral vagotomy, pretreatment with atropine or hexamethonium, or perivagal treatment with capsaicin in anesthetized rats completely abolished pancreatic protein secretion in response to low doses of CCK, suggesting that CCK at physiological levels stimulates pancreatic enzyme secretion via a capsaicin-sensitive afferent vagal pathway originating from the gastroduode-
nal mucosa (74). In both rats and guinea pigs, atropine decreased CCK-evoked pancreatic secretory response in vivo (110). In vivo infusion of CCK-JMV-180 (a CCK analog), the high-affinity agonist of CCK1R, causes dose-dependent increases in pancreatic protein secretion in rats blockable by CCK1R antagonist L-364,718, and acute vagotomy in anesthetized rats and perivagal application of capsaicin in conscious rats will abolish pancreatic secretory responses to CCK-JMV-180, demonstrating that CCK acts through high-affinity CCK1Rs on nerves to mediate pancreatic protein secretion (73, 105).

Fig. 3. Structure of human CCK1 receptor (CCK1R), CCK-8, and CCK-8-CCK1R interaction. Middle: serpentine representation of the CCK1R. The NH2-terminal CCK1R(1–47), third extracellular loop CCK1R(329–357), and residues (W39, Q40, A334, Y338) involved in interaction with CCK-8 are denoted (91). Top left: complex of CCK1R(1–47) with CCK-8. Receptor fragment is shown as blue and CCK-8 as red ribbons. W39, Q40 of CCK1R(1–47), Tyr-27, and Met-28 of CCK-8 are displayed as ball-and-stick representation (107). Top right: structure of CCK1R(329–357). Transmembrane domain 7 (TM7) and TM6 are depicted on the left and right, respectively. Residues of receptor fragment are color coded according to hydrophobicity (blue = polar, red = hydrophobic) (42). Bottom left: structure of CCK-8. Helix-like loop in center of CCK-8 is highlighted by ribbon diagram. Protons involved in intermolecular interaction with NH2 terminus of CCK1R are denoted as spheres (91). Bottom right: interactions of CCK-8 (gray ribbon) with NH2 terminus and third extracellular loop (EC3) of CCK1R (black ribbon). The side chains of the amino acids of CCK-8 and CCK1R stabilizing the CCK-8-CCK1R complex are illustrated as ball-and-sticks and sticks, respectively. The residues of CCK-8 are denoted by three-letter code and those of CCK1R by one-letter code (42).

CCK receptors have been detected in rat vagus nerves with the use of in vitro receptor autoradiography, and these receptors are transported toward peripheral nerve endings from the nodose ganglia (153). CCK1Rs and axonal transport are found in vagal trunks and all abdominal vagal branches (95). Both CCK1R and CCK2R have been found to be present in the cervical vagus and the nucleus of the solitary tract (20). Furthermore, the existence of functional CCK1Rs in the nodose ganglion has been confirmed (12, 145). Both CCK1R and CCK2R have been found to be synthesized in nodose ganglion cells, and these receptors can be transported to the periphery along afferent fibers in both rats and humans (96).

CCK may also act on low-affinity CCK1Rs to trigger Ca2+ influx into vagal afferent neurons, which in turn may result in acute activation of vagal afferent neurons (130). Electrophysiological evidence showed that both high- and low-affinity CCK1Rs exist on rat vagal afferent fibers, and the vagal CCK-receptor field includes the regions innervated by the gastric, celiac, and hepatic vagal branches (76). Recent work...
indicates that CCK1Rs exist in both high- and low-affinity states in rat nodose ganglia cells. Activation of high-affinity CCK1Rs in isolated rat nodose ganglia cells elicits regular Ca\(^{2+}\) oscillations, whereas stimulation of low-affinity CCK1Rs evokes a Ca\(^{2+}\) transient followed by a small and sustained Ca\(^{2+}\) plateau. Such Ca\(^{2+}\) signaling involves both \(G_q\) and L-type Ca\(^{2+}\) channels (68). CCK acts through high-affinity CCK1Rs on rat vagal afferent fibers to mediate pancreatic secretion (73). The above observations provide evidence for a role for CCK receptors in vagal afferent fibers in pancreatic digestive enzyme secretion.

CCK receptors are also present on rabbit vagus nerve, and vagal CCK receptors include both CCK1 and CCK2/gastrin subtypes (83). In rabbit vagal afferent (nodose) ganglion, high concentrations of CCK- and neuropeptide Y-binding sites have been found in 10.6% and 9.2% of the nodose ganglion neurons, respectively; both CCK (CCK1/2) and neuropeptide Y (Y1/2) receptor binding sites are expressed by discrete populations of neurons in the nodose ganglia (41). These data suggest that CCK released from peripheral tissues (mainly from I cells in the small intestinal mucosa) may interact with CCK receptors in vagal afferent fibers to modulate a neural circuit.

In addition, it has been found that CCK (10 nM to 10 \(\mu\)M) stimulates \[^{3H}\]acetylcholine release from rat pancreatic lobules, which was blocked by TTX, by Ca\(^{2+}\)-free medium, and by CCK antagonist L-364,718, suggesting that CCK may act by stimulation of neural acetylcholine release (132). This provides an additional site for CCK action through the neural pathway, but apparently with much lower affinity, because at least nanomolar CCK concentrations were needed to stimulate acetylcholine release from the rat pancreatic lobules (132).

Together, these findings suggest that in both rodents and other animals (rabbit) CCK can act both directly on acinar cells and through neural pathways to stimulate exocrine pancreatic secretion (Fig. 4A).

**Humans.** Quantitative RT-PCR indicated that, in human pancreas, CCK1R mRNA was \(~30\) times lower than CCK2R mRNA, which was \(~10\) times further lower than M3 muscarinic acetylcholine receptor mRNA (58). In situ hybridization completely failed to detect either CCK1R or CCK2R mRNA in adult human pancreas (58, 59). In isolated human pancreatic acini, CCK or secretin stimulation did not produce any functional responses, and no significant specific binding for CCK...
receptors was found (58, 59, 94). This is in contrast to the high density of CCK1Rs revealed by microautoradiography in the mucosa and in smooth muscles in the human duodenum (94). However, after adenoviral-mediated transfection of either CCK1R or CCK2R gene to human pancreatic acinar cells, these cells became responsive to CCK stimulation (58). These data indicate that human pancreatic acinar cells do not respond to CCK stimulation due to insufficient levels of CCK1R gene expression. With advanced quantitative RT-PCR technology, CCK1R transcription in human pancreas has now become detectable (38), although the cell type expressing it was not determined.

In vivo human studies found that the highly specific CCK1R antagonist MK-329 (also known as L-364,718 and devazepide) only slightly affected pancreatic enzyme secretion and gastric emptying but significantly inhibited bile secretion, indicating that CCK is not an essential mediator of postprandial pancreatic enzyme secretion in humans (16). Others found that CCK in vivo stimulated pancreatic secretion by interacting with neural cholinergic system. Atropine in human subjects completely suppressed low-dose CCK-induced digestive enzyme secretion, indicating that CCK-mediated exocrine pancreatic secretion required a cholinergic tone in vivo (108). Atropine blocked significantly meal-stimulated pancreatic digestive enzyme secretion, and the response to graded doses of exogenous CCK was significantly inhibited by both atropine and loxiglumide, a CCK1R antagonist, suggesting that pancreatic enzyme secretion is predominantly dependent on a cholinergic tone and that CCK modulates the enzyme-secretory response in human (1). Atropine (5 μg·kg⁻¹·h⁻¹) reduced the CCK-stimulated increase in pancreatic enzyme secretion and essentially blocked postprandial enzyme secretion (9). In another study, trypsin output stimulated by physiological doses of CCK was inhibited by atropine by 84.0%, whereas lipase output was inhibited by 78.6% in humans (132). Thus the human exocrine pancreas is crucially dependent on a cholinergic background, with CCK only modulating this secretory response (9).

The above data suggest that CCK can act on vagal afferent fibers, which explains how physiological plasma CCK levels act via vagal cholinergic pathways to stimulate pancreatic secretion. Although knowledge of vagal CCK1Rs has come from rodents, other work suggests that this information is also applicable to humans (105). The fact that CCK1Rs exist in human duodenum seems to support such a notion (possibly mediating both vagal afferent input and duodenal-pancreas reflex) (34). Therefore, physiological levels of CCK appear to act entirely via vagal cholinergic pathways to mediate pancreatic secretion in humans (Fig. 4B).

**Birds.** The case for avian CCK stimulation of exocrine pancreatic secretion is different from that for both rats and humans, although the plasma CCK levels seem to be similar in all animal species. Proteins and fatty acids are the major luminal dietary stimuli for CCK release in rat (29, 30, 72, 81, 84). Duodenal infusion of casein, for example, resulted in elevation of plasma CCK from fasting levels of 0.5 ± 0.1 to 3.8 ± 0.4 pM (72). This picomolar postprandial plasma CCK concentration is sufficient to stimulate enzyme secretion and trigger Ca²⁺ oscillation in rat pancreatic acini (2, 21–23, 149).

Soya proteins and amino acid mixtures mimicking soya protein composition could increase gut CCK release in chicks (150). In chickens, plasma CCK increases from a basal level (control diet) of 9.6 ± 0.6 to 13.4 ± 0.6 and to 18.1 ± 0.8 pM 90 min after ingestion of a diet supplemented with 100 and 1,000 mg soybean trypsin inhibitor, respectively (37). Plasma CCK concentration is also significantly enhanced in chicks fed medium-chain triglycerides but not in chicks fed long-chain triglycerides (86). However, in chickens, such picomolar CCK...
concentrations do not stimulate pancreatic acini secretion; CCK concentrations 100 times higher are required to elicit secretory response from isolated chicken pancreatic acini. This has led to the belief that endogenous CCK does not have any important role in digestive enzyme secretion from exocrine pancreas in birds (35, 36, 98, 125).

In the isolated duck pancreatic acini, picomolar concentrations of CCK do not stimulate either an increase in \([\text{Ca}^{2+}]_i\), or digestive enzyme secretion. CCK at 1 nM was required for stimulation of amylsase secretion, with a maximal effect being achieved at 10 nM (149). Vasoactive intestinal peptide (VIP)/pituitary adenylate cyclase-activating peptide (PACAP) receptor (VPAC) agonists such as PACAP38 and PACAP27 and VIP alone had little effect on amylsase secretion in duck pancreatic acini but could make picomolar concentrations of CCK effective. Furthermore, PACAP27 and VIP shifted the maximal stimulating CCK concentrations from 10 to 1 nM (149). Subthreshold CCK (10 pM) in the presence of VPAC agonists induced Ca\(^{2+}\) spikes. CCK (10 nM)-induced secretion was inhibited by CCK1R antagonist FK-480 (1 \(\mu\)M). Gastrin did not stimulate amylsase secretion and did not induce \([\text{Ca}^{2+}]_i\) increase (149). These data suggest that duck pancreatic acini possess both CCK1 and VPAC receptors and that simultaneous activation of both is required for each to play a physiological role (149) (Fig. 5A). As such, VIP/PACAP sensitization of CCK stimulation could probably be broken down into two components. At higher CCK concentrations, VIP/PACAP may primarily serve the purpose of priming zymogen granules for exocytosis; at lower CCK concentrations, VIP/PACAP may also sensitize Ca\(^{2+}\) signal generation (Fig. 5B).

The presence of VIP/PACAP innervation in avian exocrine pancreas provides a direct line of evidence that VIP or VIP-like receptors are likely to have a physiological role in exocrine pancreatic secretion. In situ studies show that chicken VIP increased the flow of pancreatic juice in turkey (27), Vaillant et al. (139) identified VIP in the gut and pancreas of turkey. Mensah-Brown and Pallott (90) found that Houbara Bustard (Chlamydotis undulata) exocrine pancreas was innervated by VIP, galanin, neuropeptide Y, and some other neurotransmitters. Mirabella et al. (92) found that, in duck, both PACAP38 and PACAP27 are present in neurons and fibers of the enteric nervous system, and PACAP is colocalized with VIP. Peeters et al. (106) also suggested that PACAP receptor exists in chicken pancreas. The well-established innervation of VIP/PACAP and related receptors in avian pancreas lend support to a role for VIP/PACAP receptors in avian exocrine pancreatic secretion (149).

Presently, there is scant information about vagal reflexes and their potential role in exocrine pancreatic secretion in birds. However, there is evidence to indicate that chicken exocrine pancreatic secretion is controlled by the vagus nerve (48). In addition, turkey pancreatic secretion may be controlled by a cholinergic pathway (126). Therefore, neural or vagal reflexes may also exist in avian species. Figure 4C depicts the presently known situation for exocrine pancreatic secretion in birds.

CONCLUSIONS AND PERSPECTIVES

The hormone CCK is the most important mediator of postprandial pancreatic secretion, particularly concerning digestive enzyme output. Pancreatic secretion can be influenced by CCK either directly via actions on pancreatic acinar cells or indirectly via actions on afferent vagus nerves. The mechanisms of action are species specific. In rodents, CCK acts both directly through the blood and neurally to mediate pancreatic secretion. In humans, CCK appears to act entirely via vagal cholinergic pathways to mediate pancreatic secretion. In birds, VIP/PACAP and CCK are mutually dependent to directly stimulate exocrine pancreatic secretion at physiological concentrations. This latter conclusion implies mutual dependence of the endocrine and nervous systems.

In birds, whether VIP/PACAP and CCK are also needed together to excite the sensory nerves, triggering vagovagal and enteropancreatic reflexes to stimulate pancreatic enzyme secretion, remains unclear. This will require future studies.

Avian CCK receptors have not received much attention before, largely because of the uncertainty about its physiological role in exocrine pancreatic secretion. Now that its role is established and significant differences in CCK receptors of bird and mammals are quite obvious both structurally and functionally, more attention should be directed to that respect in the future.

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