Published in final edited form as:

Pharmacol Ther. 2008 July ; 119(1): 83–95. doi:10.1016/j.pharmthera.2008.05.001.

Structural basis of cholecystokinin receptor binding and regulation

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Abstract

Two structurally-related guanine nucleotide-binding protein-coupled receptors for two related peptides, cholecystokinin (CCK) and gastrin, have evolved to exhibit substantial diversity in specificity of ligand recognition, in their molecular basis of binding these ligands, and in their mechanisms of biochemical and cellular regulation. Consistent with this, the CCK₁ and CCK₂ receptors also play unique and distinct roles in physiology and pathophysiology. The paradigms for ligand recognition and receptor regulation and function are reviewed in this article, and should be broadly applicable to many members of this remarkable receptor superfamily. This degree of specialization is instructive and provides an encouraging basis for the diversity of potential drugs targeting these receptors and their actions that can be developed.

Keywords

cholecystokinin; gastrin; photoaffinity labeling; GPCR; molecular modeling

1. Introduction

Guanine nucleotide-binding protein (G protein)-coupled receptors comprise the largest and most diverse group of receptors in the genome, representing the predominant site of action of existing approved drugs on the market. This superfamily includes three distinct families of receptors that include targets for regulatory molecules as different as photons, odorants, biogenic amines, peptides, proteins, glycoproteins, lipids, and even viral particles. This list includes molecules of markedly diverse structures, with sizes, charges, solubilities, and biological behaviors vastly different. Remarkably, these have been postulated to have evolved from a single evolutionary precursor (Kolakowski, 1994).

The family of G protein-coupled receptors that has been most extensively studied and that is best understood is Family A (or Class I), the rhodopsin- β adrenergic receptor family. Included in Family A are structurally highly homologous receptors for the gastrointestinal endocrine and neural polypeptide, cholecystokinin (CCK). These are classified as CCK₁ and CCK₂ based on their highly distinctive ligand selectivities (previously identified as types A and B CCK receptors, related to their prominent presence in “alimentary tract” and “brain”) (Dufresne et

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al., 2006). While there had been the prediction of a distinct receptor for gastrin, another structurally-related polypeptide hormone produced in the gastric antrum, this turned out to be the CCK₂ receptor (Kopin et al., 1992). Despite being highly homologous and approximately 50 percent identical, particularly in the predicted transmembrane segments where these two receptors are 70 percent identical, and having clearly evolved from a common precursor, the CCK₁ and CCK₂ receptors exhibit remarkable functional differences. These differences are the focus of the current report. We believe that the ability for variation in mechanisms of ligand binding and activation of receptors that are even closely related and in mechanisms of the regulation of these receptors provide extraordinary opportunities for the introduction of highly selective therapeutic agents.

2. Naturally-occurring peptide ligands – structure-activity relationships

CCK was discovered in porcine duodenal extracts, based on its ability to stimulate gallbladder contraction and pancreatic exocrine secretion (Ivy & Oldberg, 1928). It was subsequently identified in the brain where it was found to represent one of the most abundant neuropeptides present (Miller et al., 1984). We now recognize that CCK is present as a variety of different length peptides that are produced from a single 115-residue preprohormone precursor, all sharing their carboxyl-terminal-amide sequence. These range in length from 58, 39, 33 and 8 residues, with each containing a sulfated tyrosine residue seven residues from the carboxyl terminus (Eysselein et al., 1990; Rehfeld et al., 2001). Shorter, non-sulfated CCK peptides have also been isolated from the brain (Rehfeld, 1980). The carboxyl-terminal heptapeptide-amide is also shared with gastrin polypeptides. Those peptides are produced from a distinct 101-residue preprohormone precursor, yielding dominant mature products 34 and 17 amino acid residues in length, with a carboxyl-terminal amide and a tyrosine that is sulfated approximately half of the time located six residues from their carboxyl terminus (Dockray et al., 2001). Figure 1 illustrates the sequences of major molecular forms of CCK and gastrin peptides, highlighting the region shared between the two hormones. The CCK₁ receptor requires the carboxyl-terminal heptapeptide-amide that includes the sulfated tyrosine found in CCK peptides for high affinity binding and biological activity (Miller, 1991). For this reason, gastrin is a low affinity ligand and weakly potent agonist at this receptor. The CCK₂ receptor requires the carboxyl-terminal tetrapeptide-amide that is shared between all CCK and gastrin peptides (Miller, 1991). Table 1 illustrates the structure-activity relationships for the action of CCK and gastrin peptides at both types of CCK receptors.

3. Receptor distribution and actions

CCK₁ receptors are responsible for a number of biological activities important for nutrient assimilation. These include stimulation of post-cibal gallbladder contraction, pancreatic exocrine secretion, gastrointestinal motility, and satiety (Liddle, 1994). These are mediated by receptors on gallbladder muscularis, pancreatic neurons (in human, and also directly on pancreatic acinar cells in rodents), pyloric smooth muscle, and enteric neurons and central nervous system nuclei. CCK₂ receptors are present in the gastric oxyntic mucosa and widely distributed in the brain, with their highest concentrations in the striatum, cerebral cortex, and limbic system (Noble & Roques, 1999). In addition to stimulating gastric acid secretion, these receptors play roles in anxiety and nociception (Noble, 2007). It is important to note that a variety of neoplasms have also been reported to express CCK₁ and CCK₂ receptors (Reubi et al., 1997), with both having the potential to stimulate cell growth, possibly contributing to carcinogenesis and aggressive tumor behavior (Dabrowski et al., 1997). These trophic effects are clearly important considerations in the development of agonists acting at these receptors.

4. Receptor structure

Sequences and predicted topologies and potential post-translational modifications of human CCK₁ and CCK₂ receptors are illustrated in Figure 2. These receptors are typical of the G protein-coupled receptor superfamily in having seven hydrophobic segments predicted to represent transmembrane helices that form a helical bundle domain, and are typical of Family A in sharing the signature sequences of this family within these regions (Kolakowski, 1994). These sequences likely provide the appropriate architectural motifs to achieve the preferred structure for proper activity and regulation, and are, therefore, conserved in both CCK₁ and CCK₂ receptors. Included here are the motifs representing E/DRY at the intracellular side of transmembrane segment three and NPxxY at the intracellular side of transmembrane segment seven. Other notable features of these CCK receptors are sites of glycosylation on external loop and tail regions, a conserved disulfide bond linking the predicted first and second extracellular loop regions, a site of palmitoylation on vicinal cysteines on the intracellular side of the predicted seventh transmembrane segment, and multiple potential sites for serine and threonine phosphorylation in internal loop and tail regions. The critical functional roles of the sites of glycosylation of the CCK₁ receptor for proper folding and trafficking to the cell surface have been reported (Hadac et al., 1996). In addition to the conserved disulfide bond linking the first and second extracellular loops that is shared by CCK₁ and CCK₂ receptors, the CCK₁ receptor also has been shown to possess another intradomain disulfide bond within its amino terminus (Pellegrini & Mierke, 1999; Ding et al., 2003). Palmitoylation has also been directly demonstrated for the CCK₁ receptor (Miller, unpublished observation). Both types of CCK receptors have been shown to be phosphorylated, with the distinct sites of phosphorylation carefully mapped for the CCK₁ receptor (Klueppelberg et al., 1991; Ozcelebi & Miller, 1995; Ozcelebi et al., 1996).

Residues that are identical (filled with white lettering) or homologous (gray filled) between the two types of CCK receptors have been highlighted in Figure 2. These begin to provide insight into the levels of similarity in different regions of these receptors. Clearly, the highest degree of identity and similarity resides within the predicted transmembrane segments (70 percent identity in these regions). The residues that are distinct in these two types of CCK receptors are distributed throughout their structures, in extracellular regions, intracellular regions, and all faces of their helical bundle regions. This provides the molecular basis for generating the observed differences between these two receptors in their ligand binding, signaling, and regulation.

5. Molecular basis of natural peptide ligand binding

A broad variety of experimental approaches have been utilized to examine the molecular basis of ligand binding to CCK receptors. This includes structure-activity relationship studies of ligands and receptors, modifying the ligands during synthesis and typically modifying the receptors by mutagenesis (Miller & Lybrand, 2002). Types of mutagenesis include segmental deletions and site-specific modifications. Table 2 and Table 3 describe a number of such mutations that have been studied for the CCK₁ and CCK₂ receptors, respectively. Deletion of a residue or group of residues in such studies without incurring loss of function may suggest that the residue or region of interest is not important for that function. Loss of function in such studies is much harder to interpret. This can occur as the result of a direct or an indirect interference with the function of interest. Mutations of G protein-coupled receptors can also result in trapping of the receptor intracellularly in the endoplasmic reticulum or in some other compartment of the biosynthetic pathway short of delivery to the plasma membrane where normal receptor function occurs (Conn et al., 2006; Janovick et al., 2007). Quality control for the biosynthesis of this group of receptors seems to be quite stringent. Clearly, with the natural ligand being a soluble peptide that cannot cross the plasma membrane, abnormalities of

delivery to the cell surface in form that is normally folded is a critical requirement for normal function.

Another specialized form of mutagenesis includes the construction of chimeric forms of these receptors, exchanging large areas, smaller segments, or distinct residues between CCK₁ and CCK₂ receptors (Kopin et al., 1995). Chimeric receptors may be particularly informative when two receptors are structurally closely related with distinctive functional features, such as the unique structural specificity of the two types of CCK receptors. These characteristics are clearly in place for the CCK₁ and CCK₂ receptors. The other feature that is critical for effective interpretation of such studies is that the ligand should bind to both with similar or analogous mechanisms – this is an area where chimeric studies between these two receptors have likely been complicated and compromised. Table 4 lists a series of chimeric constructs between CCK₁ and CCK₂ receptors.

A number of additional lines of evidence suggest that despite binding with similar levels of high affinity, CCK peptides bind to these two subtypes of receptors in quite distinct manner (Silvente-Poirot et al., 1998; Harikumar et al., 2005a; Harikumar et al., 2006). These include differences in sites of receptor labeling in photoaffinity studies utilizing probes with sites of covalent attachment intrinsic to the pharmacophoric region, and differences in microenvironments of fluorescence indicators situated in distinct regions of CCK.

Photoaffinity labeling is an experimental approach that provides direct data for the spatial approximation between residues within a ligand and its receptor (Pearson & Miller, 1987; Dawson et al., 2002). For this to be most informative, the photolabile ligand should be structurally related to the natural hormone whose binding is being investigated. For CCK, high affinity, biologically active photolabile analogues have been developed with sites of covalent attachment in six of seven positions within the CCK pharmacophore (Miller & Lybrand, 2002). To date, four of these have been used to establish predominant sites of labeling the CCK₁ receptor. A smaller number of constraints have been experimentally established for analogous spatial approximation of residues at the CCK₂ receptor (Gimpl et al., 1996; Anders et al., 1999). It is notable that the same photolabile probe that represents a high affinity, full agonist ligand for both CCK₁ and CCK₂ receptors covalently labels distinct residues in these receptors (Dong et al., 2005). A summary of the studies identifying sites of photolabeling of these receptors is shown in Table 5.

Another method of exploring the microenvironment of a docked receptor ligand utilizes fluorescent analogues that retain key features of that ligand (Harikumar et al., 2002; Harikumar et al., 2005a). A number of fluorescent analogues of CCK and gastrin have been developed (Harikumar et al., 2002; Harikumar et al., 2005a). Some of these have been shown to retain high affinity binding and biological activity. Description of the fluorescence properties of such probes while bound to a receptor can be quite informative. Some of these probes have now been characterized while bound to the CCK₁ and CCK₂ receptors (Harikumar et al., 2002; Harikumar & Miller, 2005; Harikumar et al., 2005a; Harikumar et al., 2006). Here, too, the same probes that bind with similar affinity, potency, and efficacy to both receptors have been shown to yield distinct fluorescence properties, reflecting their occupation of distinct microenvironments when bound to these receptors (Harikumar et al., 2002; Harikumar & Miller, 2005; Harikumar et al., 2005a).

The most informative of these studies have utilized fluorescent analogues of CCK having an Aladan indicator that is highly sensitive to the polarity of its microenvironment situated in positions 24, 29, and 33 of CCK (numbering based on 33 amino acid form originally isolated), at the amino terminus, mid-region, and carboxyl terminus of the pharmacophoric domain. The mid-region probe was least accessible to the aqueous milieu as determined by fluorescence

emission spectra and iodide quenching, which was not altered by changes in conformation from active to inactive. Accessibilities of the amino- and carboxyl-terminal probes were both affected by receptor conformation. The position 24 probe was more easily quenched in the active than in the G protein-uncoupled conformation for both the CCK₁ and CCK₂ receptors. In contrast, the position 33 probe docked at the CCK₁ receptor was more easily quenched in the active conformation, whereas the same probe docked at the CCK₂ receptor was more easily quenched in the inactive conformation. Fluorescence anisotropy and red edge excitation shift determinations confirmed these observations and supported the proposed movements. It is possible that the carboxyl terminus of CCK resides closer to the extracellular milieu in the CCK₁ receptor and in the intramembranous helical bundle in the CCK₂ receptor.

Application of these insights have resulted in the proposal of molecular models of natural ligand-occupied CCK receptors (Harikumar et al., 2004; Henin et al., 2006). It is remarkable that, even after the collection of so many experimentally-derived constraints of different types, two quite distinct three-dimensional models continue to be actively considered in the current literature. The general structural features of the receptor conformation in these two models are similar, with the greatest degree of similarity residing in the proposed helical bundle area. This obviously reflects the crystal structure reported for rhodopsin (Palczewski et al., 2000), another member of Family A G protein-coupled receptors. The loop regions are much less well resolved and reflect the very small number of reported experimental constraints. It is notable that the amino-terminal region of CCK resides at the surface of the lipid bilayer nested within extracellular loops in both models. Studies ranging from mutagenesis, complementary mutagenesis, and photoaffinity labeling have all placed the acidic tyrosine-sulfate moiety of CCK in spatial approximation with arginine¹⁹⁷ in the second extracellular loop (Gigoux et al., 1999b; Ding et al., 2002; Arlander et al., 2004). The major difference between these two molecular models is the placement of the carboxyl-terminal region of CCK (Harikumar et al., 2004; Henin et al., 2006). In the model based primarily on photoaffinity-derived constraints, this portion of the peptide is also at the surface of the lipid bilayer approximated with the amino-terminal tail, just above the first transmembrane segment (Harikumar et al., 2004). In contrast, in the model relying more heavily on receptor mutagenesis, this part of CCK is shown to dip into the bilayer within the helical bundle (Henin et al., 2006). Close spatial approximation in this model has been postulated between CCK residue Asp³² (based on the numbering of the 33 amino acid peptide originally isolated) and receptor residue Arg³³⁶ at the extracellular side of transmembrane segment six, and CCK residue Phe³³ with receptor residues Phe²¹⁸ in transmembrane segment five and Trp³²⁶, Ile³²⁹, and Phe³³⁰ in transmembrane segment six. Figure 3 illustrates the model based largely on photoaffinity labeling, with covalently labeled residues highlighted. Additionally, the residues identified in the mutagenesis approach of the contrasting model as being adjacent to the carboxyl-terminal end of CCK in that model are highlighted to illustrate how far removed these are from the peptide docking site in the contrasting model.

6. Molecular basis of non-natural ligand binding

An extensive variety of non-natural ligands for CCK receptors have been developed (see critical reviews (Herranz, 2003; Blakeney et al., 2007; Kalindjian & McDonald, 2007)). These fall into the categories of (1) amino acid derivatives, typified by proglumide and benzotript, (2) benzodiazepines developed after the natural product asperlicin was identified, (3) ureidoacetamides representing acyclic analogues of the most active and selective benzodiazepines, (4) quinazolinones also based on the structure of asperlicin, and (5) dipeptoids rationally developed based on the pharmacophore of CCK and gastrin. Other chemical classes of non-natural ligands have also been developed.

The first highly selective and potent non-peptidyl ligands developed and reported to work at CCK receptors were benzodiazepines (Freidinger, 1989). These have also been the most extensively studied in regard to mechanism of binding to CCK receptors. It is notable that minor variations in the structures of these ligands were able to change their relative selectivities from CCK₁ to CCK₂ (Freidinger, 1989) and from antagonist to agonist at the CCK₁ receptor (Aquino et al., 1996). These data support the presence of binding pockets in these receptors that are related to each other, yet that have adequate differences to allow distinctive patterns of action. Indeed, extensive receptor mutagenesis has been performed exploring the basis of binding of these compounds (Kopin et al., 1994). Of note, in both subtypes of CCK receptors, mutagenesis studies point to the importance of a pocket in the upper third of the lipid bilayer within the helical confluence as a potential site of docking these compounds (Martin-Martinez et al., 2005). This location has been further supported with a recent study in which a benzodiazepine ligand for the CCK₁ receptor was directly identified in a photoaffinity labeling experiment (Hadac et al., 2006).

It is interesting that the differences in the two distinctive major models of CCK binding to the CCK₁ receptor provide substantial differences in their implications for the benzodiazepine ligands. In the one based predominantly on photoaffinity labeling data (Harikumar et al., 2004; Hadac et al., 2006), the proposed benzodiazepine binding site would be predicted to represent an allosteric site, while the other model based predominantly on receptor mutagenesis data (Henin et al., 2006) illustrates the binding sites for peptide and non-peptidyl ligands as overlapping. Once this important question is resolved, there may be leads for the development of allosteric modulators of these important receptors.

Some receptor mutagenesis has been performed in attempt to determine the molecular basis of the action of other classes of non-natural ligands of CCK receptors, but these studies have not yet been extensive enough to be definitive. It is certainly conceivable that the dipeptoid compounds that were developed with the pharmacophore of the natural peptide ligand in mind could bind to a region more closely related to the binding of CCK.

7. Regulated post-translational modifications of CCK receptors – biochemical regulation

Biochemical modifications of receptors provide rapid and often reversible mechanisms for their regulation that are used by most cells. The most common regulated biochemical modification of G protein-coupled receptors is phosphorylation. This has been studied for both types of CCK receptors, although the level of detail of our understanding for the CCK₁ receptor is substantially greater than that of the CCK₂ receptor. Phosphorylation of serine and threonine residues has been described, with no tyrosine phosphorylation detected (Ozcelebi et al., 1995; Ozcelebi & Miller, 1995; Ozcelebi et al., 1996). The distribution of potential sites of serine and threonine phosphorylation in cytosolic domains can be seen in the snake diagrams depicting the amino acid sequences of the CCK receptors in Figure 2. The CCK₁ receptor is phosphorylated on residues within the third intracellular loop and carboxyl-terminal tail. This occurs in response to agonist stimulation of this receptor (homologous activation) or in response to heterologous activation of other receptors that activate protein kinase C or activation of that enzyme directly (Rao et al., 1997). Homologous activation of the CCK receptor by CCK results in activation of both signaling kinases, representing protein kinase C isoforms, and G protein-coupled receptor kinases (Gates et al., 1993). Heterologous activation of the CCK receptor occurs only through protein kinase C. Agonist-stimulated phosphorylation of the CCK₁ receptor has been shown to achieve a stoichiometry of five moles of phosphate per mole of receptor (Ozcelebi & Miller, 1995). The CCK₂ receptor seems to have its predominant region of phosphorylation within its carboxyl-terminal tail (Pohl et al., 1997). The details here are less clearly defined.

The functional impact of CCK₁ receptor phosphorylation is to desensitize this receptor, interfering with its coupling to its G protein (Rao et al., 1997). This may be mediated via arrestin binding. Of particular interest, this biochemical event has been shown to be independent of cellular modes of desensitization in the CCK₁ receptor (Rao et al., 1997), while it seems to effect internalization of the CCK₂ receptor (Pohl et al., 1997). Another interesting feature of the agonist-stimulated phosphorylation of the CCK₁ receptor is its hierarchical nature, whereby early and particularly accessible sites of phosphorylation by protein kinase C within the third loop result in a conformational change that facilitates the further phosphorylation within the carboxyl-terminal tail and other sites within the third loop (Ding et al., 2000; Rao et al., 2000). If the two key sites of early phosphorylation (serines 260 and 264 in the rat CCK₁ receptor) are mutated to alanine residues that cannot be phosphorylated, the other sites do not get phosphorylated. Similarly, if these serine residues are mutated to aspartic acid residues to mimic the phosphorylated serines, all subsequent events occur (Rao et al., 2000). A particularly interesting event that is dependent on the conformational change in the third loop induced by these phosphorylation events is the exposure of an epitope within the second intracellular loop that regulates intracellular trafficking after receptor internalization. This was identified in a series of peptide competition experiments in which each of the intracellular loop regions of the CCK₁ receptor was coexpressed with intact receptor and agonist-stimulated receptor trafficking was studied (Ding et al., 2000). This emphasizes the complexity of the program of conformational changes that occur in receptors after agonist activation, affecting both signaling and regulatory domains.

Another biochemical modification of receptors that can be regulated is its palmitoylation (Bouvier et al., 1995). It resides in a critical location to possibly affect what has been called the eighth helical segment that runs parallel to the lipid bilayer just inside the seventh transmembrane segment. There are no available data to support this modification of the CCK receptor as being a regulated event.

8. Cellular mechanisms of regulation of CCK receptors

Cellular mechanisms of receptor regulation tend to be slower than the biochemical mechanisms. They frequently rely on solubilities and access to various cellular compartments. With a signaling system having a soluble ligand that cannot cross the lipid bilayer and that is delivered from the extracellular milieu, any movement of the receptor off the cell surface functionally desensitizes it. Two such internalization pathways have been described for the CCK₁ receptor, clathrin-mediated endocytosis and potocytosis via caveolae (Roettger et al., 1995b). Similarly, coupling between receptor and G protein requires lateral mobility in the plasma membrane and access for the two molecules to meet each other (Roettger et al., 2001). A novel mechanism of CCK₁ receptor desensitization was described in which the rat pancreatic acinar cell receptor becomes immobilized in a specialized plasma membrane domain that is depleted in G proteins (Roettger et al., 1995a). As such, this “insulated” domain yields functional desensitization. Different types of cells may therefore utilize different components of this desensitization repertoire. Adding the biochemical mechanisms as additional mechanisms that can be utilized independently from cellular mechanisms, the ability to optimize the level of sensitivity of the signaling system and its need for protection from over-stimulation for a particular cell in a specific physiological setting is truly remarkable. The possibility of inappropriate pancreatic exocrine secretion into an interstitial compartment resulting in autodigestion is an example of this (Steer & Meldolesi, 1987).

9. Physiology and pathophysiology of CCK receptor function

The physiologic roles of CCK and gastrin and their receptors, as reviewed above and in extensive review articles (Liddle, 1994; Dockray, 2004), are broad and significant. Essentially

all of the physiologic actions of CCK relate to nutrient homeostasis that is clearly very important to the survival of the organism. This spans activities related to nutrient emulsification with bile coming from its site of sequestration in the gallbladder, nutrient digestion by pancreatic proteolytic, lipolytic, and amylolytic enzymes, nutrient transit along the gastrointestinal tract and its impact on exposure to absorptive surface and its rate of absorption, and resultant satiety. Gastrin plays a direct role in stimulating gastric acid secretion to establish an optimal luminal environment for peptic activity and for vitamin B12 absorption. Despite the apparent complexity of the coordination of all of these secretory, absorptive, contractile, and motility events along the gastrointestinal tract and the need to ensure their appropriateness for the nutrient composition of a particular meal, digestion proceeds seamlessly and in a transparent manner for the vast majority of the population who have no gastrointestinal complaints.

It is interesting that dysfunction of this signaling system has been implicated in irritable bowel syndrome, where there may be a hyper-reactive motility response to CCK (Harvey & Read, 1973; Snape et al., 1977; Kellow et al., 1987; Kamath et al., 1991). This syndrome is extremely common and the source of substantial disability and negative economic impact for our society. Unfortunately, use of CCK antagonists has not had adequate positive impact in this syndrome to become one of the standard therapeutic approaches (Cremonini et al., 2005). Dysfunction of gastrin signaling is not a syndrome that has a significant clinical presentation. More often than not, patients are afflicted by excessive acid secretion and its sequelae, rather than inadequate acid secretion that could result from an inadequate gastrin response to a meal or from an unresponsive receptor for this hormone. Hypochlorhydria can play a role in bacterial overgrowth syndromes, but they often occur in a broader context, such as in patients with pernicious anemia or dysmotility states.

An area of substantial potential interest that has not yet been fully developed is the sensitivity of CCK receptors to their lipid microenvironment. This has been systematically investigated *in vitro* for the CCK₁ receptor (Harikumar et al., 2005b). In these studies, the cholesterol and sphingolipid composition of the cell were manipulated to examine their effects on CCK receptor function. Of note, CCK₁ receptor affinity and signaling were affected by the cholesterol composition, with both too much and too little of this lipid resulting in reduced potency of CCK action on this receptor. This may be clinically important, since CCK function in animals (and humans) with lipid abnormalities has been reported to follow a similar pattern (Behar et al., 1993; Yu et al., 1995; Xiao et al., 1999; Xiao et al., 2000). The site of defect in these models has been localized to the interface between a normal CCK₁ receptor and its normal G protein. Modification of sphingolipid composition of the cell was found to affect CCK₁ receptor trafficking within the cell, resulting in abnormal receptor recycling (Harikumar et al., 2005b). The sensitivity of the CCK₂ receptor to its lipid microenvironment has not yet been examined, but acid secretory abnormalities and their sequelae have not been associated with lipid abnormalities. This suggests that there may be differential sensitivity to the lipid environment of these two closely-related receptors. If present, this likely results from some structural difference in the lipid-embedded residues in the CCK₁ and CCK₂ receptors, with such differences known to be quite limited.

10. Conclusions

Two structurally-similar G protein-coupled receptors for two highly related peptides, CCK and gastrin, have evolved to exhibit remarkable diversity in specificity of ligand recognition and mechanisms of biochemical and cellular regulation, as well as playing unique and distinct roles in physiology and pathophysiology. The paradigms for ligand recognition and receptor regulation and function should be broadly applicable to many members of this remarkable receptor superfamily.

Acknowledgements

This work was supported by National Institutes of Health Grant DK32878, the Fiterman Foundation, and Mayo Clinic.

Abbreviations

Bpa, *p*-benzoyl-L-phenylalanine; CCK, cholecystokinin; ECL, extracellular loop; G protein, guanine nucleotide-binding protein; GPCR, G protein-coupled receptor; *p*NO₂-Phe, *p*-nitro-phenylalanine.

REFERENCES

- Anders J, Bluggel M, Meyer HE, Kuhne R, Ter Laak AM, Kojro E, et al. Direct identification of the agonist binding site in the human brain cholecystokininB receptor. *Biochemistry* 1999;38:6043–6055. [PubMed: 10320330]
- Aquino CJ, Armour DR, Berman JM, Birkemo LS, Carr RAE, Croom DK, et al. Discovery of 1,5-benzodiazepines with peripheral cholecystokinin (CCK-A) receptor agonist activity. 1. Optimization of the agonist "Trigger". *J Med Chem* 1996;39:562–569. [PubMed: 8558528]
- Arlander SJ, Dong M, Ding XQ, Pinon DI, Miller LJ. Key differences in molecular complexes of the cholecystokinin receptor with structurally related peptide agonist, partial agonist, and antagonist. *Mol Pharmacol* 2004;66:545–552. [PubMed: 15322246]
- Behar J, Rhim BY, Thompson W, Biancani P. Inositol trisphosphate restores impaired human gallbladder motility associated with cholesterol stones. *Gastroenterology* 1993;104:563–568. [PubMed: 8425698]
- Beinborn M, Quinn SM, Kopin AS. Minor modifications of a cholecystokinin-B/gastrin receptor non-peptide antagonist confer a broad spectrum of functional properties. *J Biol Chem* 1998;273:14146–14151. [PubMed: 9603914]
- Beinborn M, Ren Y, Blaker M, Chen C, Kopin AS. Ligand function at constitutively active receptor mutants is affected by two distinct yet interacting mechanisms. *Mol Pharmacol* 2004;65:753–760. [PubMed: 14978254]
- Blakeney JS, Reid RC, Le GT, Fairlie DP. Nonpeptidic ligands for peptide-activated G protein-coupled receptors. *Chem Rev* 2007;107:2960–3041. [PubMed: 17622179]
- Bläker M, Ren Y, Seshadri L, McBride EW, Beinborn M, Kopin AS. CCK-B/gastrin receptor transmembrane domain mutations selectively alter synthetic agonist efficacy without affecting the activity of endogenous peptides. *Mol Pharmacol* 2000;58:399–406. [PubMed: 10908308]
- Bouvier M, Moffett S, Loisel TP, Mouillac B, Hebert T, Chidiac P. Palmitoylation of G-protein-coupled receptors: A dynamic modification with functional consequences. *Biochem Soc Trans* 1995;23:116–120. [PubMed: 7758677]
- Conn PM, Knollman PE, Brothers SP, Janovick JA. Protein folding as posttranslational regulation: evolution of a mechanism for controlled plasma membrane expression of a G protein-coupled receptor. *Mol Endocrinol* 2006;20:3035–3041. [PubMed: 16556733]
- Cremonini F, Camilleri M, McKinzie S, Carlson P, Camilleri CE, Burton D, et al. Effect of CCK-1 antagonist, dexloxiglumide, in female patients with irritable bowel syndrome: a pharmacodynamic and pharmacogenomic study. *Am J Gastroenterol* 2005;100:652–663. [PubMed: 15743365]
- Dabrowski A, Detjen KM, Logsdon CD, Williams JA. Stimulation of both CCK-A and CCK-B receptors activates MAP kinases in AR42J and receptor-transfected CHO cells. *Digestion* 1997;58:361–367. [PubMed: 9324163]
- Dawson ES, Henne RM, Miller LJ, Lybrand TP. Molecular models for cholecystokinin-A receptor. *Pharmacol Toxicol* 2002;91:290–296. [PubMed: 12688371]
- Ding XQ, Dolu V, Hadac EM, Schuetz M, Miller LJ. Disulfide bond structure and accessibility of cysteines in the ectodomain of the cholecystokinin receptor: Specific mono-reactive receptor constructs examine charge-sensitivity of loop regions. *Recept Chan* 2003;9:83–91.
- Ding XQ, Pinon DI, Furse KE, Lybrand TP, Miller LJ. Refinement of the conformation of a critical region of charge-charge interaction between cholecystokinin and its receptor. *Mol Pharmacol* 2002;61:1041–1052. [PubMed: 11961122]

- Ding XQ, Rao RV, Kuntz SM, Holicky EL, Miller LJ. Impaired resensitization and recycling of the cholecystokinin receptor by Co-expression of its second intracellular loop. *Mol Pharmacol* 2000;58:1424–1433. [PubMed: 11093782]
- Dockray GJ. Clinical endocrinology and metabolism. Gastrin. *Clin Endocrinol Metab* 2004;18:555–568.
- Dockray GJ, Varro A, Dimaline R, Wang T. The gastrins: their production and biological activities. *Annu Rev Physiol* 2001;63:119–139. [PubMed: 11181951]
- Dong M, Ding XQ, Thomas SE, Gao F, Lam PC, Abagyan R, et al. Role of lysine187 within the second extracellular loop of the type A cholecystokinin receptor in agonist-induced activation. Use of complementary charge-reversal mutagenesis to define a functionally important interdomain interaction. *Biochemistry* 2007;46:4522–4531. [PubMed: 17381074]
- Dong M, Liu G, Pinon DI, Miller LJ. Differential docking of high-affinity peptide ligands to type A and B cholecystokinin receptors demonstrated by photoaffinity labeling. *Biochemistry* 2005;44:6693–6700. [PubMed: 15850403]
- Dufresne M, Seva C, Fourmy D. Cholecystokinin and gastrin receptors. *Physiol Rev* 2006;86:805–847. [PubMed: 16816139]
- Escriveau C, Gigoux V, Archer E, Verrier S, Maigret B, Behrendt R, et al. The biologically crucial C terminus of cholecystokinin and the non-peptide agonist SR-146,131 share a common binding site in the human CCK1 receptor - Evidence for a crucial role of Met-121 in the activation process. *J Biol Chem* 2002;277:7546–7555. [PubMed: 11724786]
- Eysselein VE, Eberlein GA, Hesse WH, Schaeffer M, Grandt D, Williams R, et al. Molecular variants of cholecystokinin after endogenous stimulation in humans: a time study. *Am J Physiol* 1990;258:G951–G957. [PubMed: 2360639]
- Freidinger RM. Cholecystokinin and gastrin antagonists. *Med Res Rev* 1989;9:271–290. [PubMed: 2666802]
- Galés C, Kowalski-Chauvel A, Dufour MN, Seva C, Moroder L, Pradayrol L, et al. Mutation of Asn-391 within the conserved NPXXY motif of the cholecystokinin B receptor abolishes Gq protein activation without affecting its association with the receptor. *J Biol Chem* 2000;275:17321–17327. [PubMed: 10748160]
- Galés C, Poirot M, Taillefer J, Maigret B, Martinez J, Moroder L, et al. Identification of tyrosine 189 and asparagine 358 of the cholecystokinin 2 receptor in direct interaction with the crucial C-terminal amide of cholecystokinin by molecular modeling, site-directed mutagenesis, and structure/affinity studies. *Mol Pharmacol* 2003;63:973–982. [PubMed: 12695525]
- Gates LK, Ulrich CD, Miller LJ. Multiple kinases phosphorylate the pancreatic cholecystokinin receptor in an agonist-dependent manner. *Am J Physiol* 1993;264:G840–G847. [PubMed: 8498511]
- Gigoux V, Escriveau C, Fehrentz JA, Poirot S, Maigret B, Moroder L, et al. Arginine 336 and asparagine 333 of the human cholecystokinin-A receptor binding site interact with the penultimate aspartic acid and the C-terminal amide of cholecystokinin. *J Biol Chem* 1999a;274:20457–20464. [PubMed: 10400673]
- Gigoux V, Escriveau C, Silvente-Poirot S, Maigret B, Gouilleux L, Fehrentz JA, et al. Met-195 of the cholecystokinin-A receptor interacts with the sulfated tyrosine of cholecystokinin and is crucial for receptor transition to high affinity state. *J Biol Chem* 1998;273:14380–14386. [PubMed: 9603948]
- Gigoux V, Maigret B, Escriveau C, Silvente-Poirot S, Bouisson M, Fehrentz JA, et al. Arginine 197 of the cholecystokinin-A receptor binding site interacts with the sulfate of the peptide agonist cholecystokinin. *Protein Sci* 1999b;8:2347–2354. [PubMed: 10595537]
- Gimpl G, Anders J, Thiele C, Fahrenholz F. Photoaffinity labeling of the human brain cholecystokinin receptor overexpressed in insect cells - Solubilization, deglycosylation and purification. *Eur J Biochem* 1996;237:768–777. [PubMed: 8647124]
- Go WY, Holicky EL, Hadac EM, Rao RV, Miller LJ. Identification of a domain in the carboxy terminus of CCK receptor that affects its intracellular trafficking. *Am J Physiol* 1998;275:G56–G62.
- Gouldson P, Legoux P, Carillon C, Delpech B, Le Fur G, Ferrara P, et al. The antagonist SR 146131 and the antagonist SR 27897 occupy different sites on the human CCK(1) receptor. *Eur J Pharmacol* 2000;400:185–194. [PubMed: 10988332]

- Hadac EM, Dawson ES, Darrow JW, Sugg EE, Lybrand TP, Miller LJ. Novel benzodiazepine photoaffinity probe stereoselectively labels a site deep within the membrane-spanning domain of the cholecystokinin receptor. *J Med Chem* 2006;49:850–863. [PubMed: 16451051]
- Hadac EM, Ghanekar DV, Holicky EL, Pinon DI, Dougherty RW, Miller LJ. Relationship between native and recombinant cholecystokinin receptors - role of differential glycosylation. *Pancreas* 1996;13:130–139. [PubMed: 8829180]
- Hadac EM, Pinon DI, Ji Z, Holicky EL, Henne RM, Lybrand TP, et al. Direct identification of a second distinct site of contact between cholecystokinin and its receptor. *J Biol Chem* 1998;273:12988–12993. [PubMed: 9582333]
- Harikumar KG, Clain J, Pinon DI, Dong M, Miller LJ. Distinct molecular mechanisms for agonist peptide binding to types A and B cholecystokinin receptors demonstrated using fluorescence spectroscopy. *J Biol Chem* 2005a;280:1044–1050. [PubMed: 15520004]
- Harikumar KG, Miller LJ. Fluorescence resonance energy transfer analysis of the antagonist- and partial agonist-occupied states of the cholecystokinin receptor. *J Biol Chem* 2005;280:18631–18635. [PubMed: 15757907]
- Harikumar KG, Pinon DI, Miller LJ. Fluorescent indicators distributed throughout the pharmacophore of cholecystokinin provide insights into distinct modes of binding and activation of type A and B cholecystokinin receptors. *J Biol Chem* 2006;281:27072–27080. [PubMed: 16857665]
- Harikumar KG, Pinon DI, Wessels WS, Dawson ES, Lybrand TP, Prendergast FG, et al. Measurement of intermolecular distances for the natural agonist peptide docked at the cholecystokinin receptor expressed in situ using fluorescence resonance energy transfer. *Mol Pharmacol* 2004;65:28–35. [PubMed: 14722234]
- Harikumar KG, Pinon DL, Wessels WS, Prendergast FG, Miller LJ. Environment and mobility of a series of fluorescent reporters at the amino terminus of structurally related peptide agonists and antagonists bound to the cholecystokinin receptor. *J Biol Chem* 2002;277:18552–18560. [PubMed: 11893747]
- Harikumar KG, Puri V, Singh RD, Hanada K, Pagano RE, Miller LJ. Differential effects of modification of membrane cholesterol and sphingolipids on the conformation, function, and trafficking of the G protein-coupled cholecystokinin receptor. *J Biol Chem* 2005b;280:2176–2185. [PubMed: 15537636]
- Harvey RF, Read AE. Effect of cholecystokinin on colonic motility and symptoms in patients with irritable bowel syndrome. *Lancet* 1973;i:1. [PubMed: 4118538]
- Henin J, Maigret B, Tarek M, Escrieut C, Fourmy D, Chipot C. Probing a model of a GPCR/ligand complex in an explicit membrane environment: the human cholecystokinin-1 receptor. *Biophys J* 2006;90:1232–1240. [PubMed: 16326901]
- Herranz R. Cholecystokinin antagonists: pharmacological and therapeutic potential. *Med Res Rev* 2003;23:559–605. [PubMed: 12789687]
- Ivy AC, Oldberg E. A hormone mechanism for gallbladder contraction and evacuation. *Am J Physiol* 1928;86:599–613.
- Jagerschmidt A, Guillaume N, Goudreau N, Maigret B, Roques BP. Mutation of Asp¹⁰⁰ in the second transmembrane domain of the cholecystokinin B receptor increases antagonist binding and reduces signal transduction. *Mol Pharmacol* 1995;48:783–789. [PubMed: 7476907]
- Jagerschmidt A, Guillaume N, Roques BP, Noble F. Binding sites and transduction process of the cholecystokinin receptor: Involvement of highly conserved aromatic residues of the transmembrane domains evidenced by site-directed mutagenesis. *Mol Pharmacol* 1998;53:878–885. [PubMed: 9584214]
- Jagerschmidt A, Guillaume-Rousselet N, Vikland ML, Goudreau N, Maigret B, Roques BP. His³⁸¹ of the rat CCK_B receptor is essential for CCK_B versus CCK_A receptor antagonist selectivity. *Eur J Pharmacol* 1996;296:97–106. [PubMed: 8720482]
- Janovick JA, Brothers SP, Knollman PE, Conn PM. Specializations of a G-protein-coupled receptor that appear to aid with detection of frequency-modulated signals from its ligand. *FASEB J* 2007;21:384–392. [PubMed: 17172315]
- Kalindjian SB, McDonald IM. Strategies for the design of non-peptide CCK2 receptor agonist and antagonist ligand. *Cur Top Med Chem* 2007;7:1195–1204.

- Kamath PS, Gaisano HY, Phillips SF, Miller LJ, Charboneau JW, Brown ML, et al. Abnormal gallbladder motility in irritable bowel syndrome: evidence for target-organ defect. *Am J Physiol* 1991;260:G815–G819. [PubMed: 2058671]
- Kellow JE, Miller LJ, Phillips SF, Zinsmeister AR, Charboneau JW. Altered sensitivity of the gallbladder to cholecystokinin octapeptide in irritable bowel syndrome. *Am J Physiol* 1987;253:G650–G655.
- Kennedy K, Escrieu C, Dufresne M, Clerc P, Vaysse N, Fourmy D. Identification of a region of the N-terminal of the human CCKA receptor essential for the high affinity interaction with agonist CCK. *Biochem Biophys Res Commun* 1995;213:845–852. [PubMed: 7654246]
- Kennedy K, Gigoux V, Escrieu C, Martinez J, Moroder L, Frehel D, et al. Identification of two amino acids of the human cholecystokinin-A receptor that interact with the N-terminal moiety of cholecystokinin. *J Biol Chem* 1997;272:2920–2926. [PubMed: 9006937]
- Klueppelberg UG, Gates LK, Gorelick FS, Miller LJ. Agonist-regulated phosphorylation of the pancreatic cholecystokinin receptor. *J Biol Chem* 1991;266:2403–2408. [PubMed: 1989991]
- Kolakowski LF. GCRDb: a G-protein-coupled receptor database. *Recept Chans* 1994;2:1–7.
- Kopin AS, Beinborn M, Lee Y-M, McBride EW, Quinn SM. The CCK-B/gastrin receptor. Identification of amino acids that determine nonpeptide antagonist affinity. *Ann NY Acad Sci* 1994;713:67–78. [PubMed: 8185216]
- Kopin AS, Lee YM, McBride EW, Miller LJ, Lu M, Lin HY, et al. Expression cloning and characterization of the canine parietal cell gastrin receptor. *Proc Natl Acad Sci U S A* 1992;89:3605–3609. [PubMed: 1373504]
- Kopin AS, McBride EW, Quinn SM, Kolakowski LF Jr, Beinborn M. The role of the cholecystokinin-B/gastrin receptor transmembrane domains in determining affinity for subtype-selective ligands. *J Biol Chem* 1995;270:5019–5023. [PubMed: 7890609]
- Langer I, Tikhonova IG, Travers MA, rcher-Lahlou E, Escrieu C, Maigret B, et al. Evidence that interspecies polymorphism in the human and rat cholecystokinin receptor-2 affects structure of the binding site for the endogenous agonist cholecystokinin. *J Biol Chem* 2005;280:22198–22204. [PubMed: 15817487]
- Liddle, RA. Cholecystokinin. In: Walsh, JH.; Dockray, GJ., editors. *Gut peptides: biochemistry and physiology*. New York: Raven Press; 1994. p. 175-216.
- Marco E, Foucaud M, Langer I, Escrieu C, Tikhonova IG, Fourmy D. Mechanism of activation of a G protein-coupled receptor, the human cholecystokinin-2 receptor. *J Biol Chem* 2007;282:28779–28790. [PubMed: 17599907]
- Martin-Martinez M, Marty A, Jourdan M, Escrieu C, Archer E, Gonzalez-Muniz R, et al. Combination of molecular modeling, site-directed mutagenesis, and SAR studies to delineate the binding site of pyridopyrimidine antagonists on the human CCK1 receptor. *J Med Chem* 2005;48:4842–4850. [PubMed: 16033264]
- Miller, LJ. Heterogeneity of CCK receptors: Classification and characterization. In: Adler, G.; Beglinger, C., editors. *CCK antagonists in gastroenterology. Basic and clinical status*. 1 ed. Berlin: Springer-Verlag; 1991. p. 27-34.
- Miller LJ, Jardine I, Weissman E, Go VL, Speicher D. Characterization of cholecystokinin from the human brain. *J Neurochem* 1984;43:835–840. [PubMed: 6086839]
- Miller LJ, Lybrand TP. Molecular basis of agonist binding to the type A cholecystokinin receptor. *Pharmacol Toxicol* 2002;91:282–285. [PubMed: 12688369]
- Noble F. Pharmacology of CCKRs and SAR studies of peptidic analog ligands. *Cur Top Med Chem* 2007;7:1173–1179.
- Noble F, Roques BP. CCK-B receptor: chemistry, molecular biology, biochemistry and pharmacology. *Prog Neurobiol* 1999;58:349–379. [PubMed: 10368033]
- Ozcelebi F, Holtmann MH, Rentsch RU, Rao RV, Miller LJ. Agonist-stimulated phosphorylation of the carboxyl-terminal tail of the secretin receptor. *Mol Pharmacol* 1995;48:818–824. [PubMed: 7476911]
- Ozcelebi F, Miller LJ. Phosphopeptide mapping of cholecystokinin receptors on agonist-stimulated native pancreatic acinar cells. *J Biol Chem* 1995;270:3435–3441. [PubMed: 7531708]

- Ozcelebi F, Rao RV, Holicky E, Madden BJ, McCormick DJ, Miller LJ. Phosphorylation of cholecystokinin receptors expressed on Chinese hamster ovary cells: similarities and differences relative to native pancreatic acinar cells. *J Biol Chem* 1996;271:3750–3755. [PubMed: 8631990]
- Paillasse MR, Deraeve C, de MP, Mhamdi L, Favre G, Poirot M, et al. Insights into the cholecystokinin 2 receptor binding site and processes of activation. *Mol Pharmacol* 2006;70:1935–1945. [PubMed: 16998007]
- Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, et al. Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* 2000;289:739–745. [PubMed: 10926528]
- Pearson RK, Miller LJ. Affinity labeling of a novel cholecystokinin-binding protein in rat pancreatic plasmalemma using new short probes for the receptor. *J Biol Chem* 1987;262:869–876. [PubMed: 3805011]
- Pellegrini M, Mierke DF. Molecular complex of cholecystokinin-8 and N-terminus of the cholecystokinin A receptor by NMR spectroscopy. *Biochemistry* 1999;38:14775–14783. [PubMed: 10555959]
- Pohl M, Silvente-Poirot S, Pisegna JR, Tarasova NI, Wank SA. Ligand-induced internalization of cholecystokinin receptors - Demonstration of the importance of the carboxyl terminus for ligand-induced internalization of the rat cholecystokinin type B receptor but not the type A receptor. *J Biol Chem* 1997;272:18179–18184. [PubMed: 9218453]
- Rao RV, Holicky EL, Kuntz SM, Miller LJ. CCK receptor phosphorylation exposes regulatory domains affecting phosphorylation and receptor trafficking. *Am J Physiol* 2000;279:C1986–C1992.
- Rao RV, Roettger BF, Hadac EM, Miller LJ. Roles of cholecystokinin receptor phosphorylation in agonist-stimulated desensitization of pancreatic acinar cells and receptor-bearing Chinese hamster ovary cholecystokinin receptor cells. *Mol Pharmacol* 1997;51:185–192. [PubMed: 9203622]
- Rehfeld JF. Cholecystokinin. *Clin Gastroenterol* 1980;9:593–607. [PubMed: 6107189]
- Rehfeld JF, Sun G, Christensen T, Hillingso JG. The predominant cholecystokinin in human plasma and intestine is cholecystokinin-33. *J Clin Endocrinol Metab* 2001;86:251–258. [PubMed: 11232009]
- Reubi JC, Schaer JC, Waser B. Cholecystokinin (CCK)-A and CCK-B gastrin receptors in human tumors. *Cancer Res* 1997;57:1377–1386. [PubMed: 9102227]
- Roettger BF, Hellen EH, Burghardt TP, Miller LJ. Multiple ligand and cell-dependent states of lateral mobility of plasmalemmal G protein-coupled cholecystokinin receptors. *J Fluor* 2001;11:237–246.
- Roettger BF, Rentsch RU, Hadac EM, Hellen EH, Burghardt TP, Miller LJ. Insulation of a G protein-coupled receptor on the plasmalemmal surface of the pancreatic acinar cell. *J Cell Biol* 1995a;130:579–590. [PubMed: 7622559]
- Roettger BF, Rentsch RU, Pinon D, Holicky E, Hadac EM, Larkin JM, et al. Dual pathways of internalization of the cholecystokinin receptor. *J Cell Biol* 1995b;128:1029–1042. [PubMed: 7896869]
- Silvente-Poirot S, Escricuot C, Galès C, Fehrentz JA, Escherich A, Wank SA, et al. Evidence for a direct interaction between the penultimate aspartic acid of cholecystokinin and histidine 207, located in the second extracellular loop of the cholecystokinin B receptor. *J Biol Chem* 1999;274:23191–23197. [PubMed: 10438490]
- Silvente-Poirot S, Escricuot C, Wank SA. Role of the extracellular domains of the cholecystokinin receptor in agonist binding. *Mol Pharmacol* 1998;54:364–371. [PubMed: 9687578]
- Silvente-Poirot S, Wank SA. A segment of five amino acids in the second extracellular loop of the cholecystokinin-B receptor is essential for selectivity of the peptide agonist gastrin. *J Biol Chem* 1996;271:14698–14706. [PubMed: 8663021]
- Snape WJ, Carlson GM, Matarazzo SA, Cohen S. Evidence that abnormal myoelectrical activity produces colonic motor dysfunction in the irritable bowel syndrome. *Gastroenterology* 1977;72:383–387. [PubMed: 832784]
- Steer ML, Meldolesi J. The cell biology of experimental pancreatitis. *N Engl J Med* 1987;316:144–150. [PubMed: 3540666]
- Wang HL. A site-directed mutagenesis study on the conserved alanine residue in the distal third intracellular loops of cholecystokinin and neurotensin receptors. *Br J Pharmacol* 1997a;121:310–316. [PubMed: 9154342]

- Wang HL. Basic amino acids at the C-terminus of the third intracellular loop are required for the activation of phospholipase C by cholecystokinin-B receptors. *J Neurochem* 1997b;68:1728–1735. [PubMed: 9084447]
- Wu SV, Yang M, Avedian D, Birnbaumer M, Walsh JH. Single amino acid substitution of serine-82 to asparagine in first intracellular loop of human cholecystokinin (CCK)-B receptor confers full cyclic AMP responses to CCK and gastrin. *Mol Pharmacol* 1999;55:795–803. [PubMed: 10220557]
- Wu V, Yang M, McRoberts JA, Ren J, Seensalu R, Zeng N, et al. First intracellular loop of the human cholecystokinin-A receptor is essential for cyclic AMP signaling in transfected HEK-293 cells. *J Biol Chem* 1997;272:9037–9042. [PubMed: 9083028]
- Xiao ZL, Chen Q, Amaral J, Biancani P, Behar J. Defect of receptor-G protein coupling in human gallbladder with cholesterol stones. *Am J Physiol* 2000;278:G251–G258.
- Xiao ZL, Chen Q, Amaral J, Biancani P, Jensen RT, Behar J. CCK receptor dysfunction in muscle membranes from human gallbladders with cholesterol stones. *Am J Physiol* 1999;276:G1401–G1407. [PubMed: 10362643]
- Yu P, Chen Q, Harnett KM, Amaral J, Biancani P, Behar J. Direct G protein activation reverses impaired CCK signaling in human gallbladders with cholesterol stones. *Am J Physiol* 1995;269:G659–G665. [PubMed: 7491956]

Fig. 1.

Shown are the amino acid sequences of major molecular forms of human CCK and gastrin peptides. The pharmacophoric regions recognized by the CCK₁ and CCK₂ receptors are highlighted.

Fig. 2.

Shown are the amino acid sequences of the human CCK₁ (panel A) and CCK₂ (panel B) receptors. These illustrate the proposed topology of these receptors. The filled black circles with white lettering represent conserved identical residues, while the gray circles represent homologous residues. Dotted lines represent disulfide bonds that have been demonstrated to exist.

Fig. 3.

Three-dimensional molecular model of the CCK-occupied CCK₁ receptor. Panel A illustrates this model from above and panel B illustrates the same model from the side view. The human CCK₁ receptor structure is shown in gray. The position of the docked CCK ligand (Tyr-Gly-Asp-Tyr(SO₃)-Nle-Gly-Trp-Nle-Asp-Phe(NH₂), representing amino-terminally-extended CCK-26-33 with the natural Met residues replaced by Nle residues) (shown as space-filling in white with the peptide backbone shown from amino terminus to carboxyl terminus blue-to-red) reflects the model based on photoaffinity labeling data (Harikumar et al., 2004). Key affinity labeled residues within the receptor are illustrated in tan. Also shown in blue are

receptor residues postulated as interacting with the carboxyl terminus of CCK in the contrasting model based on mutagenesis data (Henin et al., 2006).

Table 1
Structure-activity relationships for natural CCK and gastrin peptide action at CCK receptors

CCK₁ receptor: relative potencies and binding affinities -

CCK-58 ≥ CCK-8 >>> CCK-8 desulfate > gastrin-17, CCK-4

CCK-58 and CCK-8 bind with approximate K_i values of 0.6-1 nM, with desulfation of CCK-8 resulting in a 500-fold reduction in affinity, and gastrin and CCK-4 having a 1,000–10,000-fold reduction in affinity.

CCK₂ receptor: relative potencies and binding affinities -

CCK-8, CCK-58 ≥ gastrin-17, CCK-8 desulfate > CCK-4

CCK-8, CCK-58, gastrin, and CCK-8 desulfate all bind with approximate K_i values of 0.3–1 nM, with CCK-4 losing approximately 10-fold affinity.

Table 2
Mutagenesis of CCK₁ receptors. CCK₁ receptor modifications

Domain(s)	Species	Residue(s)	Functional characterization *			References
			Binding	Biological activity		
				Potenc	Efficacy %	
N-terminus	rat	Δ 1–30	1.0	3.0	100	(Ding et al., 2003)
N-terminus	human	Δ 1–37	1.0 (high), 1.1 (low)	-	-	(Kennedy et al., 1995)
N-terminus	human	Δ 1–42	n.d.	-	-	(Kennedy et al., 1995)
N-terminus	rat	C18S & C29S	1.0	1.5	100	(Ding et al., 2003)
N-terminus	human	K37A	-	1.3	100	(Gouldson et al., 2000)
N-terminus	human	E38D	1.1	-	-	(Kennedy et al., 1997)
N-terminus	human	E38A	0.9 (high), 0.4 (low)	0.8	100	(Gouldson et al., 2000)
N-terminus	human	W39F	12.9	15.0	100	(Kennedy et al., 1997)
N-terminus	human	W39I	8.7	-	-	(Kennedy et al., 1997)
N-terminus	human	W39A	18.9 (high), 1.1 (low)	1.5	100	(Gouldson et al., 2000)
N-terminus	human	Q40N	20.9	22.6	100	(Kennedy et al., 1997)
N-terminus	human	Q40E	2.7	-	-	(Kennedy et al., 1997)
N-terminus	human	Q40A	0.7 (high [*]), 1.9 (low [*])	1.1	100	(Gouldson et al., 2000)
N-terminus	human	A42V	1.0	-	-	(Kennedy et al., 1997)
TM1	human	Q44A	10.0 (high), 1.3 (low)	2.0	100	(Gouldson et al., 2000)
TM1	human	Y48A	2200 (high), n.d. (low)	450	70	(Gouldson et al., 2000)
TM1	human	L50A	1.0 (high), 0.7 (low)	0.8	93	(Escrieut et al., 2002)
TM1	human	I51A	1.7 (high), 0.7 (low)	0.9	63	(Escrieut et al., 2002)
TM1	human	L53A	1.2 (high), 0.7 (low)	0.8	93	(Escrieut et al., 2002)
ICL1	human	R68L	0.3	-	-	(Wu et al., 1999)
ICL1	human	N69S	1.1	-	-	(Wu et al., 1999)
ICL1	human	M72L	0.1	-	-	(Wu et al., 1999)
ICL1	human	R68L & N69S	0.2	-	-	(Wu et al., 1999)
TM2	human	L90A	1.4 (high), 1.2 (low)	2.0	100	(Gouldson et al., 2000)
TM2	human	C91A	0.9 (high), 1.3 (low)	4.8	100	(Gouldson et al., 2000)
TM2	human	C94L	>10,000 (high), n.d. (low)	n.d.	n.d.	(Gouldson et al., 2000)
TM2	rat	C94S	1.0	-	-	(Ding et al., 2003)
TM2	human	C94L	62 (high), n.d. (low)	28	45	(Escrieut et al., 2002)
TM2	human	F97A	9,100 (high), n.d. (low)	30	100	(Gouldson et al., 2000)
TM2	human	N98A	970 (high), n.d. (low)	595	30	(Gouldson et al., 2000)
ECL1	human	N102A	2.5	-	-	(Silvente-Poirot et al., 1998)
ECL1	human	L103A	n.d.	68	61	(Silvente-Poirot et al., 1998)
ECL1	human	L104M	1.0	-	-	(Arlander et al., 2004)
ECL1	rat	K105A	0.8 (high), 2.0 (low)	2.5	100	(Gouldson et al., 2000)
ECL1	human	F107A	n.d.	2885	77	(Silvente-Poirot et al., 1998)
ECL1	human	F109A	0.7	-	-	(Silvente-Poirot et al., 1998)
TM3	rat	C114S	n.d.	1000	100	(Ding et al., 2003)
TM3	human	K115A	n.d.	35	55	(Gouldson et al., 2000)
TM3	human	T118A	0.9 (high), 1.4 (low)	0.9	100	(Gouldson et al., 2000)
TM3	human	T118S	1.4 (high), 1.4 (low)	0.7	100	(Gouldson et al., 2000)
TM3	human	M121A	13.2 (high), n.d. (low)	16.5	100	(Gouldson et al., 2000)
TM3	human	M121V	16.0 (high), n.d. (low)	n.d.	n.d.	(Escrieut et al., 2002)
TM3	human	M121A	1.8 (high), n.d. (low)	0.8	48	(Escrieut et al., 2002)
TM3	human	G122L	2,700 (high), n.d. (low)	>2,500	50	(Gouldson et al., 2000)
TM3	human	V125A	0.4 (high), 0.4 (low)	2.3	72	(Escrieut et al., 2002)
ECL2	human	K187A	2.0 (high), 6.4 (low)	2.5	100	(Gouldson et al., 2000)
ECL2	rat	K187D	0.8	233	100	(Dong et al., 2007)
N-terminus;	rat	D5K	2.6	9.6	100	(Dong et al., 2007)
N-terminus;	rat	D5K & K187D	3.2	1.0	100	(Dong et al., 2007)

Domain(s)	Species	Residue(s)	Functional characterization *			References
			Binding	Biological activity		
				Potenc	Efficacy %	
ECL2	human	M195L	2.8 (high), 29.8 (low)	54	100	(Gigoux et al., 1998)
ECL2	rat	C196S	n.d.	n.d.	n.d.	(Ding et al., 2003)
ECL2	human	R197A	n.d.	890	70	(Gouldson et al., 2000)
ECL2	human	R197M	n.d.	1470	70	(Gigoux et al., 1999b)
ECL2	rat	R197K	17	400	100	(Ding et al., 2002)
ECL2	rat	R197A	n.d.	n.d.	n.d.	(Ding et al., 2002)
ECL2	rat	R197D	n.d.	n.d.	n.d.	(Ding et al., 2002)
ECL2	rat	R197E	n.d.	>10,000	20	(Ding et al., 2002)
ECL2	human	F198A	n.d.	50	60	(Gouldson et al., 2000)
ECL2	human	F198A	2.9	-	-	(Silvente-Poirot et al., 1998)
ECL2	human	L199A	0.8 (high), 0.1 (low)	1.5	100	(Gouldson et al., 2000)
ECL2	human	L200A	970 (high), n.d. (low)	45	100	(Gouldson et al., 2000)
TM5	human	W209A	n.d.	720	100	(Gouldson et al., 2000)
TM5	human	H210A	0.4 (high), 1.5 (low)	1.0	100	(Gouldson et al., 2000)
TM5	human	L214A	0.2 (high), 0.1 (low)	0.9	100	(Gouldson et al., 2000)
TM5	human	L217A	5 (high), 2.1 (low)	0.6	60	(Gouldson et al., 2000)
TM5	human	F218A	2.3 (high), n.d. (low)	0.4	35	(Gouldson et al., 2000)
ICL3	rat	S260A, S264A	Eliminates PKC-mediated receptor phosphorylation			(Escrieut et al., 2002)
TM6	human	W326A	0.2 (high), 1.1 (low)	4.3	80	(Ozelebi et al., 1996; Rao et al., 1997)
TM6	human	I329A	0.5 (high), 8.5 (low)	38	74	(Escrieut et al., 2002)
TM6	human	I329F	n.d.	468	80	(Escrieut et al., 2002)
TM6	human	I329A	n.d.	60	100	(Gouldson et al., 2000)
TM6	human	F330A	10.5 (high), n.d. (low)	2.6	57	(Escrieut et al., 2002)
TM6	human	F330A	0.07 (high), 0.06 (low)	1.5	60	(Gouldson et al., 2000)
TM6	human	N333A	n.d.	1933	62	(Gouldson et al., 2000)
TM6	human	N333A	n.d.	1351	60	(Gigoux et al., 1999a)
TM6	human	R336A	n.d.	1425	100	(Gouldson et al., 2000)
TM6	human	R336D	n.d.	303	20	(Gouldson et al., 2000)
TM6	human	R336M	n.d.	9300	81	(Gigoux et al., 1999a)
ECL3	rat	V342M	1.0	-	-	(Hadac, et al., 1998)
ECL3	human	R345A	0.6 (high), 0.4 (low)	2.5	100	(Gouldson et al., 2000)
ECL3	human	R346A	0.9 (high), 0.9 (low)	0.6	100	(Gouldson et al., 2000)
TM7	human	I352A	n.d.	213	86	(Escrieut et al., 2002)
TM7	human	L356A	805 (high), n.d. (low)	11.3	100	(Gouldson et al., 2000)
TM7	human	L356A	0.8 (high), 8 (low)	29	90	(Escrieut et al., 2002)
TM7	human	S359A	0.5 (high), 0.5 (low)	0.7	100	(Gouldson et al., 2000)
TM7	human	Y360F	2.2 (high), 5.5 (low)	32	96	(Escrieut et al., 2002)
C-terminus	rat	Δ 385–429	No change in receptor trafficking			(Pohl et al., 1997)
C-terminus	rat	Δ 409–429	1.7	5.1	100	(Go et al., 1998)
C-terminus	rat	Δ 394–429	No change in receptor trafficking	1.5	100	(Go et al., 1998)
C-terminus	rat	Y386A	0.5	Inhibits receptor trafficking 4.6	100	(Go et al., 1998)
C-terminus	rat	C403A & C404A	0.9	No change in receptor trafficking 3.7	100	(Go et al., 1998)
C-terminus	rat	C403A & C404A	0.5	No change in receptor trafficking	100	(Go et al., 1998)

* Functional characterization is expressed as multiples of binding affinity and potency and as percentages of efficacy of CCK-8 or CCK-9 action at the wild type receptor, unless specified. Some authors differentiate effects on high and low affinity binding sites.

(Δ) deletion.
(n.d.) not detectable.
(-) not determined.

Table 3
Mutagenesis of CCK₂ receptors. CCK₂ receptor modifications

Domain(s)	Species	Residue(s)	Functional characterization *			References
			Binding	Biological activity		
				Potency	Efficacy %	
N-terminus	rat	Δ 1–53	2.8	-	-	(Jagerschmidt et al., 1998)
N-terminus	rat	E51A	1.2	-	-	(Silvenne-Poirot et al., 1998)
N-terminus	rat	L52A	1.0	-	-	(Silvenne-Poirot et al., 1998)
N-terminus	rat	E53A	2.0	-	-	(Silvenne-Poirot et al., 1998)
N-terminus	rat	M54A	1.5	-	-	(Silvenne-Poirot et al., 1998)
TM1	rat	A55L	0.6	-	-	(Silvenne-Poirot et al., 1998)
TM1	rat	I56A	2.1	-	-	(Silvenne-Poirot et al., 1998)
TM1	rat	R57A	21.3	-	-	(Silvenne-Poirot et al., 1998)
TM1	human	R57A	4.0 (high), 8.0 (low)	6.0	100	(Marco et al., 2007)
TM1	rat	I58A	1.2	-	-	(Silvenne-Poirot et al., 1998)
TM1	human	Y61A	11.6	-	100	(Bläker, et al., 2000)
TM1	human	Y61A	0.3 (high), 1.7 (low)	1.0	100	(Marco et al., 2007)
ICL1	human	G80I	0.2 (CCK), 0.7 (gastrin)	7	170	(Wu et al., 1999)
ICL1	human	L81R	4.0 (CCK), 32.4 (gastrin)	0.8	120	(Wu et al., 1999)
ICL1	human	S82N	2.9 (CCK), 20 (gastrin)	19.6	150	(Wu et al., 1999)
ICL1	human	S82D	0.8 (CCK), 4.5 (gastrin)	-	-	(Wu et al., 1999)
ICL1	human	S82T	0.8 (CCK), 7.9 (gastrin)	-	-	(Wu et al., 1999)
ICL1	human	S82A	1.0 (CCK), 5.9 (gastrin)	-	-	(Wu et al., 1999)
ICL1	human	L85M	1.3 (CCK), 12.8 (gastrin)	20.6	50	(Wu et al., 1999)
ICL1	human	L81R & S82N	0.2 (CCK), 1.4 (gastrin)	-	-	(Wu et al., 1999)
TM2	rat	D100N	1.2	1.0	50	(Jagerschmidt et al., 1995)
TM2	human	F110A	1.3	-	100	(Bläker, et al., 2000)
TM2	human	T111A	1.9	-	100	(Bläker, et al., 2000)
ECL1	rat	L113A	0.9	-	-	(Silvenne-Poirot et al., 1998)
ECL1	rat	N115A	15.6	-	-	(Silvenne-Poirot et al., 1998)
ECL1	rat	L116A	6.2	-	-	(Silvenne-Poirot et al., 1998)
ECL1	rat	M117A	2.3	-	-	(Silvenne-Poirot et al., 1998)
ECL1	rat	G118K	2.0	-	-	(Silvenne-Poirot et al., 1998)
ECL1	rat	T119A	1.6	-	-	(Silvenne-Poirot et al., 1998)
ECL1	rat	F120A	n.d.	440	100	(Marco et al., 2007)
ECL1	human	F120A	3.0 (high), 6.0 (low)	5.0	100	(Paillasse et al., 2006)
ECL1	rat	F120W	0.2	0.15	100	(Paillasse et al., 2006)
ECL1	rat	F120Y	19	16	100	(Paillasse et al., 2006)
ECL1	rat	F120H	139	86	100	(Paillasse et al., 2006)
ECL1	rat	F120M	215	142	100	(Paillasse et al., 2006)
ECL1	rat	F120L	275	201	90	(Paillasse et al., 2006)
ECL1	rat	I121A	1.3	-	-	(Silvenne-Poirot et al., 1998)
ECL1	rat	F122A	8.0	-	-	(Silvenne-Poirot et al., 1998)
ECL1	rat	T124A	0.7	-	-	(Silvenne-Poirot et al., 1998)
ECL1	rat	V125A	0.8	-	-	(Silvenne-Poirot et al., 1998)
ECL1	rat	I126A	1.0	-	-	(Silvenne-Poirot et al., 1998)
TM3	rat	C127A	n.d.	n.d.	n.d.	(Silvenne-Poirot et al., 1998)

Domain(s)	Species	Residue(s)	Functional characterization *			References
			Binding	Biological activity		
				Potency	Efficacy %	
TM3	human	S131A	1.0	-	100	(Bläker, et al., 2000)
TM3	human	M134A	0.8 (CCK), 0.4 (gastrin)	-	4 (constitutive)	(Beinborn et al., 2004)
TM3	human	M134A	0.6 (high), 12.0 (low)	1.0	120	(Marco et al., 2007)
TM3	human	M134L	1.7	2.0	100	(Dong et al., 2005)
TM3	human	G135A	0.9	-	100	(Bläker, et al., 2000)
TM3	human	V138A	0.3 (high), 1.8 (low)	1.3	90	(Marco et al., 2007)
TM4	human	M186A	10.2	-	100	(Bläker, et al., 2000)
TM4	human	M186A	1.3 (high), 20.8 (low)	2.2	120	(Marco et al., 2007)
TM4	rat	Y189F	30	60.7	90	(Galés et al., 2003)
TM4	human	Y189F	9 (high), 8 (low)	11.0	100	(Marco et al., 2007)
TM4	human	Y189A	136 (high), 156 (low)	109	220	(Marco et al., 2007)
TM4	human	T193A	44.4	-	100	(Bläker, et al., 2000)
ECL2	rat	C205A	n.d.	n.d.	n.d.	(Bläker, et al., 2000)
ECL2	human	H207A	260	302	100	(Silvente-Poirot et al., 1998)
ECL2	human	H207A	27 (high), 18 (low)	35	90	(Silvente-Poirot et al., 1999)
ECL2	human	H207D	n.d.	33824	70	(Marco et al., 2007)
TM5, TM7	human	S219H & S379H	0.1 (CCK), 0.1 (gastrin)	-	9 (constitutive)	(Silvente-Poirot et al., 1999)
TM5	human	S219A	0.7	-	110	(Beinborn et al., 2004)
TM5	human	L222A	0.6 (high), 1.1 (low)	-	110	(Bläker, et al., 2000)
TM5	human	L223A	0.8 (high), 9.0 (low)	1.2	190	(Marco et al., 2007)
TM5	human	L226A	1.4	0.9	120	(Marco et al., 2007)
TM5	human	F227A	1.1	-	100	(Bläker, et al., 2000)
TM5	human	F227A	0.5	-	90	(Bläker, et al., 2000)
TM5	rat	F227A	0.9 (high), n.d. (low)	1.1	100	(Jagerschmidt et al., 1998)
TM5	human	F227A	2.1 (CCK), 2.8 (gastrin)	0.7	150	(Marco et al., 2007)
ICL3	human	E288K	0.6 (CCK), 0.6 (gastrin)	-	3 (constitutive)	(Beinborn et al., 2004)
ICL3	human	L325E	3.4 (CCK), 5.5 (gastrin)	-	20 (constitutive)	(Beinborn et al., 1998)
ICL3	human	V332E	0.8	-	17 (constitutive)	(Beinborn et al., 2004)
ICL3	rat	A332E	1.1	0.8	110	(Wang, 1997a&b)
ICL3	rat	K333M & K334T & R335L	1.3	n.d.	n.d.	(Wang, 1997b)
ICL3	rat	K333M	0.8	1.7	70	(Wang, 1997b)
ICL3	rat	K334T	1.1	2.3	30	(Wang, 1997b)
ICL3	rat	R335L	1.3	n.d.	10	(Wang, 1997b)
ICL3	rat	K333R & K334R & R335K	0.8	1.2	90	(Wang, 1997b)
TM6	human	W346A	1.8	-	100	(Bläker, et al., 2000)
TM6	human	W346A	0.9 (high), 5.5 (low)	1.8	170	(Marco et al., 2007)
TM6	rat	F347A	1.2	-	20	(Jagerschmidt et al., 1998)
TM6	human	V349A	3.7	-	120	(Bläker, et al., 2000)
TM6	human	V349A	0.8 (high), 4.2 (low)	1.2	100	(Marco et al., 2007)
TM6	human	Y350A	1.3	-	110	(Bläker, et al., 2000)
TM6	human	Y350A	0.7 (high), 1.0 (low)	0.8	130	(Bläker, et al., 2000)
TM6	human	Y350A	0.7 (high), 1.0 (low)	0.8	130	(Marco et al., 2007)
TM6	rat	W351A	n.d.	6.7	70	(Jagerschmidt et al., 1998)
TM6	human	N353L	1.7	-	110	(Bläker, et al., 2000)
TM6	human	N353L	0.5 (CCK), 0.2 (gastrin)	-	-	(Beinborn et al., 2004)
TM6	human	N353A	32 (high), 41 (low)	32	90	(Marco et al., 2007)

Domain(s)	Species	Residue(s)	Functional characterization *			References	
			Binding	Biological activity			
				Potency	Efficacy %		
TM6	human	T354A	2.7	-	120	(Bläker, et al., 2000)	
TM6	rat	N358A	19	30	90	(Galés et al., 2003)	
TM6	human	R356A	76 (high), n.d. (low)	65	100	(Marco et al., 2007)	
TM6	human	R356K	1.0 (high), 1.0 (low)	1.6	100	(Langer et al., 2005)	
TM6	human	R356H	10 (high), 18 (low)	12	100	(Langer et al., 2005)	
TM6	human	R356D	932 (high), n.d. (low)	224	100	(Langer et al., 2005)	
TM7	human	S379A	1.0	-	100	(Bläker, et al., 2000)	
TM7	rat	H381L	2.5	-	-	(Jagerschmidt et al., 1996)	
TM7	rat	H381F	2.5	-	-	(Jagerschmidt et al., 1996)	
TM7	human	H381A	1.5 (high), 5.2 (low)	6.8	90	(Marco et al., 2007)	
TM7	human	Y385A	2.6 (high), 14.9 (low)	1.2	100	(Marco et al., 2007)	
TM7	human	Y385F	0.8 (high), 8.1 (low)	1.2	140	(Marco et al., 2007)	
TM7	human	S387A	2.0 (high), 1.8 (low)	0.7	80	(Marco et al., 2007)	
TM7	human	N391A	1.6	n.d.	n.d.	(Galés et al., 2000)	
TM7	rat	N391D	0.5	1.0	100	(Jagerschmidt et al., 1995)	
C-terminus	rat	Δ 409–452	Inhibits receptor internalization; no change in binding or biological activity.				(Pohl et al., 1997)

* Functional characterization is expressed as multiples of binding affinity and potency and as percentages of efficacy of CCK-8 or CCK-9 action at the wild type receptor, unless specified. Some authors differentiate effects on high and low affinity binding sites.

(Δ) deletion.

(n.d.) not detectable.

(-) not determined.

(constitutive) agonist-independent signaling

Table 4
Chimeric CCK₁-CCK₂ receptor constructs. Chimeric CCK₁-CCK₂ receptor constructs

Domain(s)	Species	Residue(s)	Functional characterization *			References
			Binding	Biological activity		
				Potency	Efficacy %	
TM1 to C-terminus TM1, ICL1, TM2, ECL1 TM1	human	Replace 50–447 CCK ₂ R→CCK ₁ R	6.9 (CCK), n.d. (gastrin)	0.8	100	(Wu et al., 1997)
	human	Replace 50–134 CCK ₂ R→CCK ₁ R	6.5 (CCK), 74 (gastrin)	4.1	45	(Wu et al., 1997)
	human	R57Q CCK ₂ R→CCK ₁ R	6.1 (CCK), 8.0 (gastrin)	-	-	(Kopin et al., 1995)
	human	Replace 80–85 CCK ₂ R→CCK ₁ R	7.5 (CCK), 5.5 (gastrin)	1.2	50	(Wu et al., 1997)
ICL1	human	T111N CCK ₂ R→CCK ₁ R	1.4 (CCK), 1.2 (gastrin)	-	-	(Kopin et al., 1995)
TM2	human	CCK ₂ R→CCK ₁ R	5.9 (CCK), 17 (gastrin)	-	-	(Kopin et al., 1995)
TM3	human	A129T & V130T & S131T CCK ₂ R→CCK ₁ R	1.6 (CCK), 1.2 (gastrin)	-	-	(Kopin et al., 1995)
TM3	human	A129T CCK ₂ R→CCK ₁ R	2.1 (CCK), 1.1 (gastrin)	-	-	(Kopin et al., 1995)
TM3	human	V130T CCK ₂ R→CCK ₁ R	1.4 (CCK), 8.5 (gastrin)	-	-	(Kopin et al., 1995)
TM3	human	S131T CCK ₂ R→CCK ₁ R	0.1 (CCK), 0.1 (gastrin)	29	50	(Wu et al., 1997)
TM3 to C-terminus ICL2, TM4, ECL2, TM5	human	Replace 134–447 CCK ₂ R→CCK ₁ R	1.6 (CCK), 97 (gastrin)	-	-	(Silvente-Poirot, & Wank, 1996)
ICL2, TM4, ECL2, TM5	rat	Replace 146–246 CCK ₂ R→CCK ₁ R	0.9 (CCK), 0.5 (gastrin)	-	-	(Silvente-Poirot, & Wank, 1996)
ICL2, TM4, ECL2, TM5	rat	Replace 146–203, 217–235 CCK ₂ R→CCK ₁ R	n.d. (CCK), n.d. (gastrin)	n.d.	n.d.	(Silvente-Poirot, & Wank, 1996)
ICL2, TM4, ECL2, TM5	rat	Replace 180–235 CCK ₁ R→CCK ₂ R	1.4 (CCK), 85 (gastrin)	-	-	(Silvente-Poirot, & Wank, 1996)
TM5	rat	CCK ₂ R→CCK ₁ R	n.d. (CCK), n.d. (gastrin)	n.d.	n.d.	(Silvente-Poirot et al., 1998)
ECL2	rat	Replace 195–200 CCK ₂ R→CCK ₁ R	2.2	-	-	(Silvente-Poirot et al., 1998)
ECL2	rat	Q204M CCK ₂ R→CCK ₁ R	n.d.	3044	100	(Silvente-Poirot et al., 1998)
ECL2	rat	H207F CCK ₂ R→CCK ₁ R	3.6 (CCK), 166 (gastrin)	-	-	(Silvente-Poirot et al., 1998)
ECL2	rat	Replace 204–209 CCK ₂ R→CCK ₁ R	1.6 (CCK), 158 (gastrin)	-	-	(Silvente-Poirot, & Wank, 1996)
ECL2, TM5	rat	Replace 204–209, 217–235 CCK ₂ R→CCK ₁ R				

Domain(s)	Species	Residue(s)	Functional characterization *			References
			Binding	Biological activity		
				Potency	Efficacy %	
TM5 to C-terminus TM5	human	Replace 218–447 CCK ₂ R→CCK ₁ R	0.3 (CCK), 0.2 (gastrin)	24	50	(Wu et al., 1997)
	rat	T217S & S219Q & V220T & L221F CCK ₂ R→CCK ₁ R	1.0 (CCK), 0.8 (gastrin)	-	-	(Kopin et al., 1995)
TM5	rat	S219Q CCK ₂ R→CCK ₁ R	1.1 (CCK), 0.8(gastrin)	-	-	(Kopin et al., 1995)
TM5	rat	S219H CCK ₂ R→CCK ₁ R	5.7 (CCK), 4.1 (gastrin)	-	-	(Kopin et al., 1995)
TM5	rat	Replace 217–235 CCK ₂ R→CCK ₁ R	1.4 (CCK), 0.9 (gastrin)	-	-	(Silvente-Poirot, & Wank, 1996)
ICL3 to C-terminus TM6	human	CCK ₂ R→CCK ₁ R Replace 270–447 CCK ₂ R→CCK ₁ R	2.3 (CCK), 2.2 (gastrin)	5.0	130	(Wu et al., 1997)
	human	V349I & Y350F CCK ₂ R→CCK ₁ R	1.0 (CCK), 1.0 (gastrin)	-	-	(Kopin et al., 1995)
TM6	human	T354A CCK ₂ R→CCK ₁ R	3.5 (CCK), 1.9 (gastrin)	-	-	(Kopin et al., 1995)
TM7	human	H376L CCK ₂ R→CCK ₁ R	29 (CCK), 17.1 (gastrin)	-	-	(Kopin et al., 1995)

* Functional characterization is expressed as multiples of binding affinity and potency and as percentages of efficacy of CCK-8 or CCK-9 action at the wild type receptor, unless specified.

(n.d.) not detectable.

(-) not determined.

Table 5

Site-selective photoaffinity labeling data for CCK receptors.

CCK₁ receptor			
Photolabile probe	Domain	Residue	Reference
Bpa ²⁴ CCK	ECL 3	Glu ^{345*}	(Ding et al., 2001)
pNO ₂ -Phe ²⁷ CCK	ECL 2	Arg ¹⁹⁷	(Arlander et al., 2004)
Bpa ²⁹ CCK	ECL 3	His ^{347*}	(Hadac et al., 1998)
pNO ₂ -Phe ³³ and BPA ³³ CCK	N terminus	Trp ³⁹	(Ji et al., 1997; Hadac et al., 1999)
CCK₂ receptor			
Photolabile probe	Domain	Residue	Reference
Bpa ²⁴ CCK	ECL 1	Phe ¹²²	(Dong et al., 2005)
Bpa ³³ CCK	ECL 1	Thr ¹¹⁹	(Dong et al., 2005)

Numbering of CCK probes based on CCK-33; Bpa, p-benzoyl-L-phenylalanine; pNO₂-Phe, p-nitro-phenylalanine; ECL, extracellular loop.

* Glu³⁴⁵ and His³⁴⁷ identified in rat CCK₁ receptor correspond to Glu³⁴⁴ and Arg³⁴⁶, respectively, in the human CCK₁ receptor sequence.