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Metabolic actions of the type 1 cholecystokinin receptor: its potential as a therapeutic target

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Abstract

Cholecystokinin regulates appetite and reduces food intake by activating the type 1 CCK receptor (CCK1R). Attempts to develop CCK1R agonists for obesity have yielded active agents that have not reached clinical practice. In this review we discuss why, along with new strategies to target CCK1R more effectively. We examine signaling events and the possibility of developing agents that exhibit ligand-directed bias, to dissociate satiety activity from undesirable side effects. Potential allosteric sites of modulation are also reviewed, along with desired properties of a positive allosteric modulator without intrinsic agonist action as another strategy to treat obesity. These new types of CCK1R-active drugs could be useful as stand-alone agents or as part of a rational drug combination for management of obesity.

Keywords

cholecystokinin; type 1 cholecystokinin receptor; positive allosteric modulators; biased agonists; satiety

Cholecystokinin regulation of appetite

Obesity is a global public health problem, also responsible for the epidemic increase in type 2 diabetes mellitus and related co-morbidities and mortality [1, 2]. Nutritional homeostasis and energy expenditure are perturbed in obesity, and understanding the mechanisms that control food intake is critical in efforts to develop therapies to manipulate appetite and **satiety**. The gastrointestinal tract plays a central role in nutritional homeostasis, as a site of caloric assimilation, but also as a key regulator of appetite and energy balance, with these functions contributing to establishment and maintenance of body weight. The gut accomplishes its regulatory roles through an intrinsic neuroendocrine system comprised of cells that secrete regulatory peptides in response to luminal contents, which are distributed

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along the gastrointestinal tract and provide feedback to control key digestive events to optimize digestion and nutrient absorption [3].

A role for a parenterally-administered intestinal extract to reduce food intake was first demonstrated in 1937 [4], and cholecystokinin (CCK), an intestinal peptide, was the first purified factor shown to elicit this effect in 1973 [5]. Subsequently, CCK-like peptides, peptoids, and non-peptidyl agonists have confirmed their ability to reduce food intake in multiple species, including man [6]. This effect was further corroborated by the observation that a **type 1 CCK receptor** (CCK1R) antagonist to increase food intake in rodents [7, 8], and by a genetic defect in CCK1R processing resulting in obesity [9].

CCK exists as a family of different length peptides that share their carboxyl-terminal octapeptide-amide, known to contain the **pharmacophoric domains** critical for recognition by type 1 (CCK1Rs) and **type 2 CCK receptors** (CCK2Rs) (Fig. 1). CCK and gastrin share their carboxyl-terminal pentapeptide-amide, with both peptides recognizing CCK2R (gastrin receptor) with similar high affinities and potencies. The CCK1R, however, requires the unique amino-terminal extension present only in CCK, including a sulfated tyrosine, for high affinity binding and full biological activity. CCK peptides are synthesized in neuroendocrine I cells scattered throughout the mucosa of the proximal intestine, and are secreted in response to fat and protein in the lumen. Classical targets for this hormone are CCK1Rs present in gallbladder, pancreas, and pylorus, where they facilitate optimal food absorption by contributing to micelle formation, stimulating delivery of lipolytic and proteolytic enzymes, and titrating rate of delivery of nutrients to the absorptive surface. In addition to these classical digestive events, this hormone also acts on CCK1Rs present on vagal afferent neurons within the wall of the gastrointestinal tract, with these ultimately activating central nervous system neurons, including the nucleus of the solitary tract, important for appetite regulation [10].

The well-established activity of CCK to control appetite through peripheral receptors was the basis for active agonist drug development programs for the management of obesity by major pharmaceutical companies. Indeed, multiple candidates have been identified, with some entering clinical trials [11, 12]. However, none of these agents have reached approval for clinical use, and all such programs seem to have been terminated, because the therapeutic endpoint, defined as having greater effect on body weight than acute dieting and lifestyle modifications, was not met. There has also been concern that highly potent CCK1R agonists with long duration of action might exhibit side effects of abdominal cramping, nausea, and diarrhea, as well as the possibility of trophic effects that could contribute to progression of pancreatic cancer [13]. The regulatory bar for safety and efficacy is quite high for such drugs, since they will likely be administered to relatively healthy people for long periods of time.

In recent years, there have been a series of insights that could support this pendulum swinging back to warrant further efforts to develop CCK1R-active drugs, although the characteristics of those drugs may be different from those that had been sought earlier. Strategies to develop safer, more effective drugs include considerations that will be the

major focus of this review, (i) ligand-directed **bias** in signaling, (ii) **allosteric** modulation of endogenous agonist action, and (iii) benefits of combination therapies.

Ligand-directed bias in signaling

It has become clear that **G protein-coupled receptors (GPCRs)** can activate multiple signaling cascades via independent mechanisms, beyond receptor coupling to a single dominant heterotrimeric G protein. This might involve coupling with more than one G protein and association with arrestin, as well as through interactions with other molecules [14–16]. While the dominant natural **full agonist** can stimulate a spectrum of events, it is not uncommon for other natural and synthetic agonists to result in differences in this spectrum. Particularly when one set of signaling events might be associated with an undesirable side effect or toxic effect, it becomes advantageous to identify a ligand that reduces or does not activate the unwanted pathway, while maintaining the beneficial biological action.

CCK receptors are closely related members of the class A GPCR family that couple predominantly with G_q (and related family members, G_{11} and G_{14}), resulting in phospholipase C-β activation, with release of diacylglycerol and intracellular calcium, and activation of protein kinase C. This enzyme is responsible for many intracellular regulatory events, having substantial impact on numerous signaling pathways. In response to high agonist concentration, these receptors additionally couple with G_s to yield cAMP and activate protein kinase A, also having many cross-regulatory effects. CCK1R has been described to couple with another distinct G protein, G_{13}, to result in activation of RhoA, with impact on the cytoskeleton, as well as activating phospholipase D [17]. Additionally, both CCKR subtypes are known to become associated with β-arrestin, which not only plays a role in desensitization, but also acts as a scaffold initiating multiple signaling events [18, 19]. They are also known to be associated with more distal signaling events [20, 21]. The interconnections between all of these pathways are too numerous to delineate here, and outside the scope of this article. The most extensive characterization of these events has utilized rodent pancreatic acinar cells (see [20, 22]). It is not yet clear whether similar events are stimulated in other cells naturally expressing this receptor that might be more relevant to metabolic effects of this hormone, such as vagal afferent neurons.

Differences in the biological effects of various natural forms of CCK are potentially important. While the earliest studies utilized natural porcine CCK-33, all more recent studies used synthetic CCK, specifically CCK-8, due to ease of synthesis and inclusion of the minimal fragment with full activity and potency at CCK1R, the carboxyl-terminal heptapeptide-amide. In feeding studies, this peptide has been shown to reproducibly reduce meal size [6], however, its impact on inter-meal interval has been variable, with some studies demonstrating more frequent meals (shortening of inter-meal interval), capable of offsetting the beneficial impact of reduced meal size [23, 24]. It is now appreciated that the dominant form of CCK released into the circulation is a longer form of this hormone with an amino-terminal extension, CCK-58 [25]. CCK-58 has been consistently shown to not only reduce meal size, but also extend the inter-meal interval [23, 24]. This provides important reassurance that the "**satiation**" effect of CCK (reduced meal size) is not offset by reduced "satiety" (increased meal frequency). While *in vitro* analysis has demonstrated that these

two species of CCK behave in a similar manner for CCK1R binding and ability to stimulate calcium responses [26], other signaling events have not been systematically studied. This suggests that there could be agonist-dependent differences in signaling events stimulated by these forms of CCK. This could also reflect the quantitative hepatic extraction of CCK-8 from the portal circulation, with longer forms of CCK less efficiently extracted [27]. This might also result in differential exposure of molecular forms of CCK to CCK1Rs present on different cells, when it is delivered via the bloodstream to the systemic circulation (endocrine route).

There is also substantial evidence for local delivery of CCK (paracrine route) [28]. CCK binding to CCK1Rs present on vagal afferent neurons within the wall of the intestine could be an early event, not influenced by differential extraction of different forms of hormone from the portal circulation. This is supported by studies in which very low doses of CCK-58 were administered locally into small vessels within the gut wall and shown to elicit reduced food intake, while similar systemic infusions had no effect [29–32]. This was reproduced with luminal infusion of the non-nutrient trypsin inhibitor, camostat, to stimulate release of endogenous CCK. Reduction in meal size and prolongation of inter-meal interval were blocked by duodenal myotomy and by locally-infused CCK1R antagonist.

This powerful approach was also applied to selective infusions into the celiac artery (supplying splenic, left gastric and common hepatic arteries) and the more distal cranial mesenteric artery (supplying pancreatico-duodenal, jejunal, and ileocolic arteries), with the former responsible for regulating meal size, while the latter was responsible for regulating inter-meal interval [31, 32]. CCK1R-bearing vagal afferent neurons are present in both vascular distributions, but it is fascinating that regulation of meal size is an event that appears to be regulated higher in the gut than regulation of the inter-meal interval. There are not yet any data to track specific neurons originating in these two distinct locations along the gut or to characterize signaling events that might be unique to specific neurons.

While there are data to suggest that the impact of CCK on feeding reflects only a subset of the signaling events stimulated by a full agonist, we do not understand what particular signaling event might be responsible for this. The best studied **partial agonist** of the CCK1R is CCK-JMV-180 [33], a phenyl ethyl-ester analogue of CCK [34]. Unfortunately, this ligand does not possess the feeding effects of CCK when studied in rats [35, 36]. Since it also does not exhibit the supra-maximal inhibition of pancreatic secretion correlated with low affinity receptor occupation that is typical of CCK, it has been operationally described as a “high affinity agonist and low affinity antagonist of CCK1R” [33]. Instead, it likely represents a partial agonist, stimulating only a subset of events activated by natural full agonist, while possibly missing one or more of those events activated by high concentrations of CCK. Evidence for this comes from stimulation of cells with low dose phorbol ester to transform the CCK-JMV-180 dose-response curve to a CCK-like curve, reintroducing supra-maximal inhibition [34]. Given the interdependency of signaling events, it is not yet clear what the critical event for feeding control might be. Unfortunately, there have not yet been any partial agonists of CCK1R described that reduce food intake, which could be used to refine our understanding of responsible pathways.

Lack of insights into signaling events that might best correlate with feeding effects of CCK makes development of a biased agonist that might be useful in this clinical setting quite challenging. It will be helpful to more fully characterize signaling events stimulated by CCK-8, CCK-58, and CCK-JMV-180, as well as other promising candidate drugs that might be identified in the future. This might provide insights into activities that could be used in high throughput screening to discover candidates with profiles of interest.

Allosteric modulation of endogenous agonist action

A potentially powerful new strategy is the use of a **positive allosteric modulator** (PAM) of CCK action at CCK1R, which would possess no intrinsic agonist activity [16]. There is precedent for this type of drug in Cinacalcet, a PAM that acts as a calcimimetic by augmenting calcium-induced responses at the calcium-sensing receptor [16]. Such a drug would occupy a site on the CCK1R that is spatially distinct from where natural agonist docks, and would not elicit any biological responses that might contribute to trophic activity. Only when there would be physiologically-relevant release of CCK to occupy the orthosteric site of action within the CCK1R, would there be a biological response. This would occur during a finite period after starting to eat, thereby providing the opportunity to reduce meal size. PAMs would increase responses to CCK by increasing hormonal potency or efficacy or both, thereby amplifying the biological response to a given concentration of CCK. Presumably, since CCK-58 is the dominant circulating form of CCK that also possesses satiation and satiety effects, a PAM might enhance these effects. Another advantage of this type of PAM without intrinsic agonist activity would be the likelihood that, unlike full agonists, it would not be expected to result in receptor down-regulation or internalization, which could blunt or eliminate the beneficial impact of CCK on eating behavior.

Since all existing small molecule CCK1R agonists were identified in screening for full agonists, PAMs have not been identified. Recently, several small molecule agonists were tested to determine if they also possessed PAM activity (**ago-PAMs**), however no significant PAM activity was identified. Nevertheless, one of these, a benzodiazepine ligand in which the portion of the molecule most responsible for agonist activity (“agonist trigger”) had been defined [37], was studied in form modifying that region [38]. The hope was that the conformation of the pocket accommodating such a drug would be closer to that in the activated receptor than to that in its inactive state, thereby lowering the energy barrier for receptor activation [37]. Unfortunately, this modification also resulted in docking the compound differently from the agonist, and this strategy was not successful in producing a PAM without agonist activity [37]. Such a strategy continues to be potentially viable should an ago-PAM be identified in the future.

There have been substantial advances in understanding the molecular basis of docking CCK to CCK1R (Fig. 2). This is challenging, since there is not yet a crystal structure for this receptor, and, while the helical bundle of this class A GPCR would be expected to be similar to structures of other family members [39], conformations of extracellular loop and tail regions are difficult to predict. Additionally, the natural peptide is very flexible, which adds uncertainty to this process. There have been extensive structure-activity and fluorescence studies involving both CCK and its receptor. There has also been more direct analysis of

spatial approximations between positions along the CCK pharmacophore with residues in the receptor [40]. Building these data into working models of the CCK-occupied CCK1R, there have been two predominant proposals [40–42]. In both, the peptide resides primarily at the external surface of the bilayer, with interactions with loop residues, whereas one model (identified as alternative model in Fig. 2) illustrates the peptide carboxyl terminus diving into the helical bundle [41, 42], while the proposed model retains it at the cell surface, above the first transmembrane segment [43]. The distinction between these models has important implications for allosteric modulator development. The alternative model suggests that the orthosteric ligand partially occupies the small molecule binding pocket within the helical bundle [41, 42], thereby precluding simultaneous CCK1R occupation by both CCK and a small molecule ligand, and making it impossible for the small molecule to modulate CCK function. In contrast, the proposed model shows the orthosteric site to be spatially distinct from the intrahelical pocket [40], supporting this pocket as a valid target for allosteric drug development. A recent report provided pharmacologic evidence that the small molecule-docking pocket and sites for docking natural CCK are indeed distinct and not overlapping, since occupation with a benzodiazepine slowed the rate of dissociation of radiolabeled CCK, only possible with non-overlapping sites of docking [44].

Understanding the molecular basis for docking small molecules to CCK1R has also advanced considerably (Fig. 2) [45–48]. These studies have supported the predicted allosteric nature of the pocket within the helical bundle (spatially distinct from the site of docking the natural **orthosteric** ligand). They have also established the distinct nature of the conformation of this pocket in inactive and active states. These molecular models have been shown to possess substantial predictive value in screening potential ligands *in silico* [45, 46]. Since no PAMs have yet been described for this receptor, we cannot be sure what that conformation might represent. Hopefully, such insights could also be useful in the rational selection and/or development and refinement of drugs having the desired characteristics.

Another possible target for allosteric modulators of this receptor is the outside (lipid face) of the helical bundle (Fig. 2). **Lateral allosterism** through lipid interactions is now appreciated as a way to modulate GPCR function [49]. The CCK1R has been shown to be particularly sensitive to the cholesterol composition of the lipid bilayer [50, 51]. Membrane cholesterol elevated to levels reported in metabolic syndrome [52, 53], result in functional uncoupling of CCK binding from CCK-stimulated biological activity [50, 54–56]. In contrast, the closely-related CCK2R is not sensitive to this lipid [55, 56]. This functional difference for structurally-related GPCRs that bind and are activated by CCK with similar high affinity and potency is an ideal setting to utilize chimeric CCK1R/CCK2R constructs to explore the molecular basis for this difference. This approach was utilized to demonstrate that the impact of cholesterol is mediated directly through interaction with a cholesterol recognition motif on transmembrane segment 3 [55–57]. Of interest, while CCK2R possesses the same major determinants of this motif, the functional impact of cholesterol is distinct. This could be explained by different residues around the key determinants resulting in differential siting of the lipid, or it could be explained by more distal events initiated by cholesterol interaction with the receptor, such as differential impact on G protein interaction or on polar networks involved in stabilizing conformations of these receptors.

Other structurally-related sterols, such as bile acids, seem to be capable of interacting with the same site on CCK1R, with ursodeoxycholic acid able to compete for cholesterol interaction and to reverse the negative effects of that lipid [58]. This may provide proof-of-principle for ultimate development of a drug that might act similarly. However, the biological distribution of bile acids is practically limited to the enterohepatic circulation, since these molecules are efficiently absorbed in the ileum and extracted by the liver, and result in minimal exposure to the metabolically relevant CCK1Rs. If this mechanism is utilized therapeutically, there would be advantage for an agent that more efficiently reaches the systemic circulation. β-sitosterol, a plant sterol, was recently shown to act much like ursodeoxycholic acid to correct CCK1R dysfunction when in a high cholesterol membrane environment [59].

Benefits of combination therapies

While CCK is clearly involved in short term regulation of eating, mediated largely through CCK1Rs on vagal afferent neurons, there is also evidence for a role of this hormone in longer-term regulatory events. This is more complex, and likely involves both peripheral and central sites of action and possible interactions among various hormones and receptors, as well as their signaling events. Table 1 lists a number of peripheral mediators of appetite and energy expenditure [60], and their reported interactions with CCK. This includes products of gastrointestinal neuroendocrine cells (ghrelin, leptin, GIP, orexin, GLP-1, oxyntomodulin, and PYY), products of the pancreatic islet (insulin, glucagon, somatostatin, PP, and amylin), and products coming from adipocytes (leptin). However, the additive and/or synergistic effect between most of these factors has not been systematically explored.

There has been substantial interest in synergistic roles for CCK and leptin, a peptide secreted by adipocytes as an indication of fat stores and nutritional status, both in short-term inhibition of food intake and in long-term impact on body weight [61–63]. Leptin enhances the sensitivity to CCK, while absence of leptin and its receptor markedly reduce the effects of CCK on feeding. Leptin resistance due to hyperphagia is believed to contribute toward maintenance of obesity, while being an unlikely primary cause of this clinical state. While CCK does not stimulate leptin release from adipocytes [64], it does stimulate leptin release from gastric endocrine cells, further emphasizing the interdependence of these factors [62].

We have come to think of the proximal and distal gut as playing distinct regulatory roles in nutrient homeostasis. The proximal gut, where most CCK is secreted, is a key location for titrating delivery of food to the intestine, where most digestive events and absorption occur. Indeed, CCK affects all these events, with impact on pylorus to slow gastric emptying if too much food is entering the small bowel, with a stimulatory impact on gallbladder emptying to contribute to micelle formation critical for fat emulsification, and with stimulatory effects on secretion of pancreatic zymogens for digestion of complex nutrients. Of particular interest, recent studies reviewed above [31, 32] suggest that even within the “proximal gut,” CCK may include spatial differences in action, with the most proximal events stimulated by CCK acting at CCK1Rs contributing to limiting meal size, and the more distal events stimulated by this same hormone and receptor contributing to extending the inter-meal interval. In evolution, it has been more of a challenge to ensure adequate nutrition, than to prevent

excessive feeding. CCK seems to be designed to optimize nutrient assimilation, rather than having a dominant role in preventing over-eating. The most distal part of the small intestine (ileum), where GLP-1 and PYY are secreted, is the site of the intestinal “brake”, helping to prevent over-eating and wasting nutrients that get dumped into the colon.

The complementary nature of the distribution and function of many of these mediators and their receptors may justify combination therapy in an effort to mimic what physiology has taught us, while limiting side effects and toxicity. Many such combinations have been attempted, with encouraging effects observed [65–67].

Concluding remarks and future perspectives

CCK has long been recognized as a key physiologic regulator of appetite, and CCK1R as a potential target for obesity drugs. However, as discussed here, full agonists acting at this receptor have not yet advanced to clinical practice. This is due to the presence of substantial challenges to identify agents with optimal characteristics, including adequate potency and duration of action to be useful, yet minimizing side effects and potential toxicity, as well as receptor down-regulation and desensitization. We now make a case for developing CCK1R-active drugs that might exhibit ligand-directed bias and/or positive allosteric modulation in the absence of intrinsic agonist activity that might be useful as stand-alone agents or as part of new combination therapy approaches for this major public health problem. Challenges to the rational development of such drugs relate to insufficient insights into specific signaling events responsible for inducing satiation and satiety, versus those that might contribute to trophic effects, as well as lack of understanding of the conformation of the intramembranous allosteric pocket conferring PAM activity, independent of stabilization of G protein association and agonist action (see Outstanding Questions Box).

OUTSTANDING QUESTIONS BOX

What are the specific post-CCK1R signaling events most critical for mediating CCK-stimulated satiety? Are these limited to a single signaling pathway that can be targeted with a biased agonist of this receptor? Similarly, what events and/or pathways contribute most to unwanted side effects limiting use of potent, long-duration agonists that might be minimized by a biased agonist?

What are the post-CCK1R signaling events and ultimate destination of vagal afferent neurons that are responsible for satiation (meal size) and those (believed to originate more distally along the gut) that are responsible for satiety (inter-meal interval)? Localized infusions of CCK-58 suggest that these complementary effects are mediated by distinct neural fibers.

What are the spatial characteristics (conformation) of the allosteric intrahelical small molecule-binding pocket compatible with PAM activity, independent of stabilizing G protein association? What are the key molecular determinants for the action of such a drug? Absence of agonist activity is key for safety, limiting duration of action to a finite

physiologically-relevant period after a meal, and for efficacy, ensuring that such a drug is retained in place on the receptor without stimulating down-regulation or internalization. These insights could be useful for rational development and/or refinement of drug candidates.

How can negative effects of elevated membrane cholesterol on CCK stimulus-activity coupling at CCK1R be reversed? Strategies of a "corrective PAM" and of displacing cholesterol from its site of action outside the helical bundle are discussed. The concept of different types of drugs being necessary and useful along the clinical continuum from being overweight to being morbidly obese with metabolic syndrome is novel and potentially important for therapeutic strategies.

What are the benefits of utilizing these new types of CCK1R-active drugs alone versus in combination with other satiety agents? Would specific combinations be particularly relevant to particular clinical states?

GLOSSARY BOX

Ago-PAM
ligand that possesses both agonist activity (capable of stimulating a biological response) and the ability to enhance the biological response to another agonist, most often the natural hormonal stimulant of the receptor

Allosteric site
a site within a receptor where an allosteric ligand can dock that is topographically distinct from where the natural hormonal ligand docks, providing the opportunity for both allosteric and orthosteric ligands to bind to the receptor simultaneously. This provides an opportunity to modulate the binding and/or action of the orthosteric agonist

Biased agonist
a receptor ligand that stimulates a set of signaling events and biological responses that is distinct (often a subset) of the spectrum of such events stimulated by the natural agonist

Full agonist
a receptor ligand capable of stimulating the full spectrum (and intensity) of signaling events and biological responses stimulated by the natural hormonal agonist

G protein-coupled receptor (GPCR)
a G protein-coupled receptor, representing the most frequent membrane receptor, which possesses seven transmembrane helical segments that come together to form a bundle, and which transduces signaling events in the cell through interaction at its cytosolic face with a heterotrimeric G protein

Lateral allosterism
impact on a membrane receptor coming from within the lipid bilayer, likely mediated through the outside of the helical bundle of a GPCR

Orthosteric site
the site of docking an endogenous natural hormonal ligand to a receptor

Partial agonist
a receptor ligand capable of stimulating only a submaximal signaling or biological response relative to the natural hormonal agonist

Pharmacophoric domain
the portion of a ligand or receptor that is responsible (adequate and sufficient) for its binding and/or biological activity

Positive allosteric modulator (PAM)
a ligand that interacts with a receptor at a site that is spatially distinct from that where the natural hormone binds, and that enhances the action of the natural hormonal agonist

Satiation
the tendency to have your appetite satisfied and to feel full during a meal, thus limiting the amount of food consumed

Satiety
the feeling or state of being sated and not being hungry after a meal, with the duration of this state determining the frequency of eating and the inter-meal interval

Type 1 cholecystokinin receptor (CCK1R)
a CCK peptide-binding class A GPCR that is the most common CCK receptor in the periphery, where it mediates gallbladder emptying, pancreatic exocrine secretion, gastric emptying, and appetite (vagal afferent neurons), and is present in select brain nuclei where it influences appetite

Type 2 cholecystokinin receptor (CCK2R)
a gastrin and CCK peptide-binding class A GPCR that mediates gastric parietal cell acid secretion in the periphery, and that is the dominant receptor for CCK in the brain, with a role in panic

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TRENDS BOX

Cholecystokinin, acting though CCK1R on vagal afferent neurons, is an important physiologic regulator of appetite. While potent receptor agonists have been developed, they have not reached clinical use, due to limited efficacy and potential side effects and toxicity.

Activation of CCK1R stimulates multiple interacting signaling cascades, initiated through the G_q family, G_{13}, $G_{s,}$ and arrestin. Biased agonists can potentially stimulate a subset of events responsible for satiety, independent of events contributing to trophic and other side effects.

Positive allosteric modulators without intrinsic agonist activity can potentially enhance the satiety effect of endogenous CCK-58 released during a meal, reducing meal size, and extending inter-meal intervals.

New types of CCK1R-active drugs may be useful as stand-alone agents or part of rational drug combinations

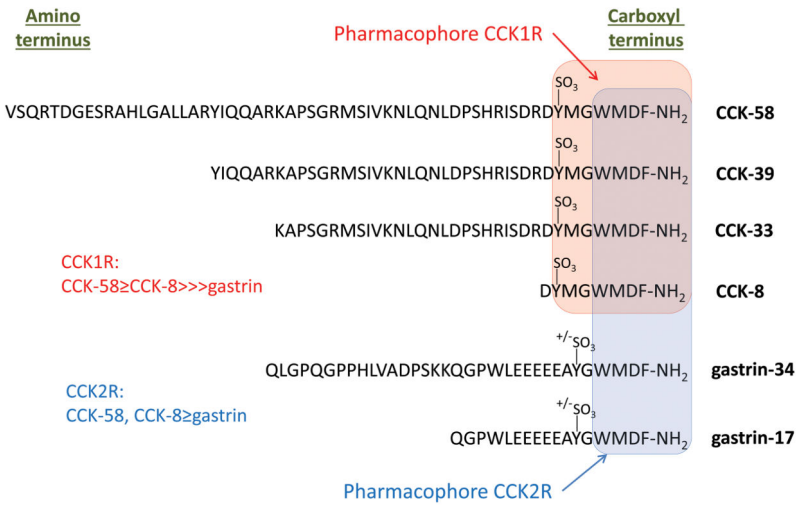

Fig. 1. Structural relationship between CCK and gastrin ligands, and CCK1R and CCK2R pharmacophores

Shown are naturally-occurring forms of CCK and gastrin peptides, sharing their carboxyl-terminal amide sequences. Highlighted in orange are the minimal fragments necessary and sufficient for high affinity binding and potent biological responses at CCK2R and CCK1R (pharmacophoric regions).

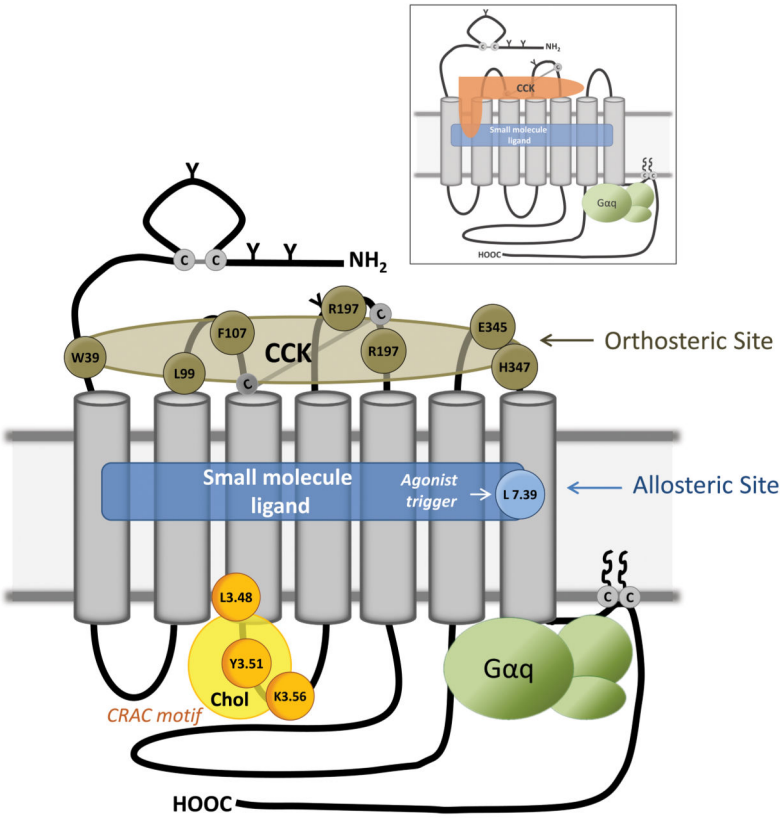

Fig. 2. Proposed sites of action of natural CCK and small molecule ligands acting at the CCK1R

Shown are proposed general modes of binding natural peptide agonist and allosteric modulators of CCK1R [40]. Consistent with the shape-changing character of GPCRs, the distinct sites of interaction with various ligands, mediators, and regulators can all affect each other. This includes the site of orthosteric peptide ligand interaction at the external face of the receptor (rat CCK1R residues that have been photoaffinity labeled are identified in tan), the intrahelical intramembranous site of allosteric small molecule ligand binding (blue, with human CCK1R residue believed to interact with the agonist trigger highlighted), the extrahelical intramembranous site of sterol interaction (CRAC motif residues in human CCK1R highlighted in yellow/orange), and the cytosolic site of interaction with the heterotrimeric G protein (highlighted in green). Shown in the inset panel is an alternative model [41, 42] in which the carboxyl-terminal end of the orthosteric peptide ligand dips into the helical bundle and occupies the same space as the small molecule ligands, making it impossible for simultaneous occupation with both CCK and ligands targeting this site. The intramembranous pocket in this model would no longer be considered “allosteric”.

Table 1

Peripheral mediators of appetite and energy expenditure, and reported interactions with CCK.

Peptide	Site of synthesis	Action on vagal afferent neurons	Effect on food intake	Interaction with CCK	Reference
Ghrelin	X/A cells in stomach, with less in intestine and colon	+	increase	Feeding studies report that the anorexic effect of CCK was blocked by pre-administration of ghrelin. Conversely, pretreatment with CCK inhibits the orexigenic effect of ghrelin.	[68]
Leptin	Adipocytes; Stomach	+	decrease	Leptin enhances the sensitivity to CCK; CCK stimulates leptin release from gastric endocrine cells.	[61], [62]
GIP	K cells in proximal small intestine	+	decrease	GIP potentiates CCK-stimulated pancreatic enzyme secretion.	[69]
Endocannabinoids	Autocrine, paracrine sources	+	increase	CCK downregulates expression of CB1 receptors in vagal afferent neurons.	[70]
Orexin (gut)	CNS; Neurons and endocrine cells in the gut	+	increase	Orexin A inhibits the excitatory effect of CCK on vagal afferent nerve discharge.	[71]
GLP-1	L cells in distal small intestine	+	decrease	GLP-1 stimulates islet β cell CCK production and secretion	[72]
Oxyntomodulin	L cells in distal small intestine	ND	decrease	ND	
PYY	L cells in distal small intestine	+	decrease	CCK increases plasma PYY levels. CCK1R antagonist devazepide attenuates PYY 3–36- induced activation of the vagal afferent pathway and inhibition of gastric emptying.	[73], [74]
Insulin	Islet β cells	+	decrease	Physiological levels of CCK stimulates insulin secretion.	[75], [76]
Amylin	Islet β cells	-	decrease	Amylin and CCK interact synergistically to reduce meal size; Amylin antagonists attenuate CCK's anorectic action, but the converse is not true; anorectic effect of CCK is severely affected in mice lacking amylin.	[65], [77], [78]
Glucagon	Islet α cells	+	decrease	CCK reinstates glucagon's satiety effect in sham feeding in rats with open gastric cannulas.	[79]
Somatostatin	Islet δ cells	ND	decrease	Somatostatin inhibits release of CCK; CCK increases plasma levels of somatostatin.	[80], [81]
PP	Islet γ cells	+	decrease	CCK causes an increase in the plasma levels of PP.	[82], [83]

GLP-1, Glucagon-Like Peptide-1; PYY, Peptide YY; GIP, Gastric Inhibitory Polypeptide; PP, Pancreatic Polypeptide; + signifies reported action on vagal afferent neurons; ND, not determined