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REVIEW

Involvement of endogenous CCK and CCK_1 receptors in colonic motor function

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Cholecystokinin (CCK) is a brain-gut peptide; it functions both as a neuropeptide and as a gut hormone. Although the pancreas and the gallbladder were long thought to be the principal peripheral targets of CCK, CCK receptors are found throughout the gut. It is likely that CCK has a physiological role not only in the stimulation of pancreatic and biliary secretions but also in the regulation of gastrointestinal motility. The motor effects of CCK include postprandial inhibition of gastric emptying and inhibition of colonic transit. It is now evident that at least two different receptors, CCK_1 and CCK_2 (formerly CCK-A and CCK-B, respectively), mediate the actions of CCK. Both localization and functional studies suggest that the motor effects of CCK are mediated by CCK_1 receptors in humans. Since CCK is involved in sensory and motor responses to distension in the intestinal tract, it may contribute to the symptoms of constipation, bloating and abdominal pain that are often characteristic of functional gastrointestinal disorders in general and irritable bowel syndrome (IBS), in particular. CCK_1 receptor antagonists are therefore currently under development for the treatment of constipation-predominant IBS. Clinical studies suggest that CCK_1 receptor antagonists are effective facilitators of gastric emptying and inhibitors of gallbladder contraction and can accelerate colonic transit time in healthy volunteers and patients with IBS. These drugs are therefore potentially of great value in the treatment of motility disorders such as constipation and constipation-predominant IBS.
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Keywords: Cholecystokinin; CCK_1 receptor antagonist; colon; irritable bowel syndrome

Abbreviations: CCK, cholecystokinin; C-IBS, constipation-predominant IBS; CTT, colonic transit time; IBS, irritable bowel syndrome

Introduction

Irritable bowel syndrome (IBS) is the most commonly identified functional gastrointestinal disorder. It is characterized by recurrent and often disabling abdominal pain associated with altered defecation (Thompson *et al*., 1999). Cholecystokinin (CCK) is a peptide known to be a potent regulator of gastrointestinal motility. Its effects include stimulation of postprandial gallbladder contraction, inhibition of gastric emptying, and inhibition of colonic transit (Crawley & Corwin, 1994). Alterations in CCK release and in tissue responses to the peptide have been implicated in the pathogenesis of IBS (Kellow *et al*., 1988). These studies indicate that either exaggerated release or increased sensitivity to CCK could contribute to the symptoms (Simren *et al*., 2001). This paper reviews our present knowledge of CCK and CCK receptors and summarizes the available data on the involvement of CCK_1 receptors in the regulation of colonic motility. We also report promising clinical investigations that indicate the beneficial effects of CCK_1 receptor blockade in IBS patients.

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CCK

The actions of CCK include stimulation of exocrine and endocrine secretion, motility and growth in the gastrointestinal tract, and regulation of satiety, anxiety, pain and behavior in the central and peripheral nervous systems. It is therefore a prototype of a class of agents known as brain-gut peptides, functioning both as a neuropeptide and as a gut hormone (Crawley & Corwin, 1994; Noble *et al*., 1999). Although the pancreas and gallbladder were long thought to be the principal targets of CCK in the gastrointestinal tract, CCK receptors are actually found throughout the gut (Crawley & Corwin, 1994; Noble *et al*., 1999). It is therefore likely that CCK also has a physiological role in the regulation of gastrointestinal motility.

There are two principal sources of CCK: endocrine I cells in the duodenal wall that are in contact with the lumen of the intestine, and peptidergic nerves both in the enteric nervous system (ENS) and in the central nervous system (CNS). In the periphery, CCK-containing neurons are found in the myenteric plexus, submucosal plexus and muscle layers of the small intestine and colon, and in the celiac plexus and the vagus nerve (Liddle, 1997).

CCK, initially characterized as a 33-amino-acid peptide, is present in a variety of biologically active molecular forms, all

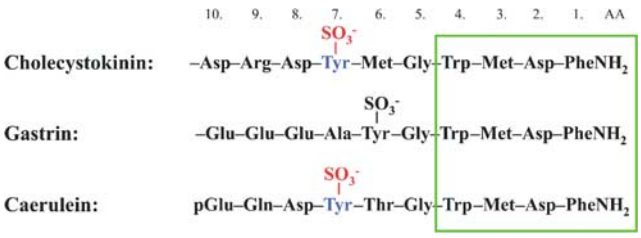

Figure 1 Structural similarities of members of the CCK/gastrin peptide family: CCK, gastrin and the amphibian skin peptide caerulein. All of these peptides share a common feature, the same amidated tetrapeptide Trp-Met-Asp-Phe-NH_2 at the C-terminal. The characteristic CCK-like activity depends on the sulfated Tyr residue at the seventh position.

derived from a 115-amino-acid precursor prepro-CCK (Deschenes *et al*., 1984). They include CCK-58, CCK-39, CCK-33, CCK-22, sulfated CCK-8 and CCK-7, unsulfated CCK-8 and CCK-7, CCK-5, and CCK-4 (Rehfeld & Hansen, 1986). All of these, as well as the closely related peptide gastrin and the amphibian skin peptide caerulein, share a common amidated tetrapeptide Trp-Met-Asp-Phe-NH_2 at the C-terminal. Within the CCK/gastrin family of peptides, the characteristic CCK-like activity depends on the sulfated Tyr residue at the seventh position. If the Tyr residue is not sulfated, or if another amino-acid residue is present at this location, the peptide behaves as a gastrin analogue and loses its CCK-like potency by a factor of about 1000 (Wank, 1998) (Figure 1).

CCK receptors

The biological actions of CCK are mediated by two distinct receptors originally denoted CCK-A (where 'A' indicated alimentary type) and CCK-B ('B' for brain type), based on their anatomical location. They are now termed CCK_1 and CCK_2, respectively, because of evidence indicating overlapping areas of localization (Noble *et al*., 1999). The existence and anatomical distribution of these CCK receptors was subsequently confirmed by molecular cloning. It had long been known that the gastrin and CCK_2 receptors were similar, so it was no surprise when molecular biological investigations showed that a single gene encodes both the brain and stomach CCK_2/gastrin receptors (Lee *et al*., 1993).

The CCK_1 receptor has an approximately 1000-fold greater affinity for CCK than for gastrin, while the CCK_2 receptor has the same high affinity for both CCK and gastrin. In addition, while the CCK_1 receptor responds to sulfated CCK with a 1000-fold greater potency than non-sulfated CCK, the CCK_2 receptor does not discriminate between the two (Noble *et al*., 1999). The CCK_1 receptor, like the CCK_2 receptor, belongs to the class A, rhodopsin-like family of G-protein-coupled receptors (Archer *et al*., 2003) (Figure 2). The receptor activates a $G_{q/11}$-mediated pathway leading to activation of phospholipase C. High agonist concentrations can also activate adenylyl cyclase *via* a G_s-mediated pathway (Wu *et al*., 1997).

The physiological and pathophysiological significance of CCK receptors can be investigated in either CCK-deficient or CCK-receptor-deficient animal models. The drawback of such studies is the tendency for adaptation to obscure the changes in function. For example, in CCK-deficient mice, pancreatic function does not change greatly because the lack of CCK is

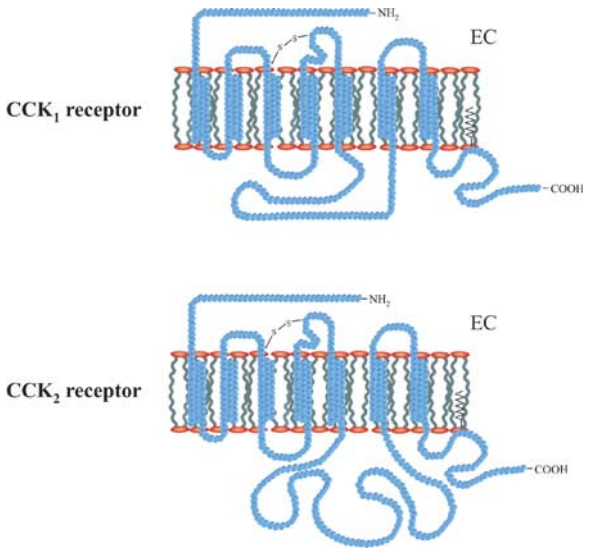

Figure 2 Predicted membrane topology of CCK_1 and CCK_2 receptors. Both belong to the family of G-protein-coupled receptors with seven transmembrane domains. A large portion of the amino-acid sequence is conserved. EC = extracellular side of the membrane.

compensated by other mechanisms (Lacourse *et al*., 1999). The formation of gallstones in CCK_1-receptor knockout mice suggests, however, that gallbladder control is either monofactorial or that CCK_1 receptors are involved in a single pathway controlling gallbladder motility (Sato *et al*., 2003). Limited amounts of data are also available in CCK_2-receptor-deficient mice obtained through gene targeting (Nagata *et al*., 1996) and in Otsuka Long-Evans Fatty Tokushima (OLEFT) rats which have no functional CCK_1 receptors (Kobayashi *et al*., 1996). However, no information is available to indicate whether gastrointestinal motility is affected in either CCK_1- or CCK_2-receptor null rats, mice or humans. On the other hand, a substantial modification in the central dopaminergic system has been reported in CCK_1-receptor-deficient rats (Feifel *et al*., 2003). These data are extremely important since they reveal the interaction of CCK with other major transmitter systems that may also affect colonic motility and sensation.

Species variability in the localization of CCK_1 and CCK_2 receptors also needs special attention. For example, in rodents, pain perception in the spinal cord is primarily mediated by CCK_2 receptors (Wiesenfeld-Hallin *et al*., 2002). In contrast, the majority of the receptors involved in this process in primates are CCK_1 receptors (Ghilardi *et al*., 1992). Furthermore, a recent study suggests that CCK_1 receptor blockade potentiates opiate analgesia (Simpson *et al*., 2002). These observations carry the message that data from animal studies can be extrapolated to humans only at a rather limited level. Nonetheless, the nociceptive, antianalgesic effects of CCK mediated by CCK_1 receptors provide a further and potentially important pharmacological target for the development of drugs to treat gastrointestinal motor disorders related to pain sensation.

CCK_1 receptor antagonists

A specific approach for evaluating the importance of CCK in the regulation of gastrointestinal function is to establish

whether blockade of the CCK receptor lessens or abolishes the response to endogenous stimulants thought to act through CCK release. Immunoneutralization, the administration of specific, high-affinity, anti-CCK antibodies, is one approach to this (Reidelberger *et al*., 1994), but in most cases the use of a specific and competitive receptor blocker is certainly better. A clear definition of the role of CCK in the physiology of gastric motor activity was hampered for a long time by the lack of specific and potent nonpeptide antagonists of CCK receptors. The development of such compounds has stimulated a broad investigation into the physiological actions of CCK and its role in certain diseases (Scarpignato, 1992; D'Amato & Rovati, 1997). At least 10 classes of CCK receptor antagonists are now available (D'Amato & Rovati, 1997). In this review, we refer only to those for which advanced clinical data are available. (Figure 3).

Among the amino-acid derivatives, proglumide was the first to be discovered, more than 35 years ago in Rotta Research Laboratorium SpA. However, its low potency and specificity (it also effectively binds CCK_2 receptors) stimulated the synthesis of glutaramic acid derivatives, the most promising of which were the compounds CR-1409 (Rotta Research Laboratorium SpA) and CR-1505 (Rotta Research Laboratorium SpA), lorglumide, and loxiglumide, respectively. These are potent, specific, and competitive antagonists of CCK_1 receptors. They are active after oral administration and are able to antagonize the effects of both endogenous and exogenous CCK. Since loxiglumide is a racemic mixture, both isomeric forms could be obtained. The dextro isomer dexloxiglumide is about twice as potent as the parent compound because the anti-CCK activity is specific to the R form whereas the S form is almost ineffective (D'Amato & Rovati, 1997).

The first selective nonpeptide CCK_1 receptor antagonist asperlicin was discovered during screening of microbial fermentation media in 1985 by scientists at Merck. Chemical modification of asperlicin, retaining its benzodiazepine skeleton, has led to the discovery of a line of potent and selective CCK_1 receptor antagonists, devazepide (also known as L364,718 or MK-329) being the most potent and widely studied among these (Chang & Lotti, 1986). The CCK_1 receptor antagonist activity of lintitript (also known as SR-27,897) was discovered through random screening of a large chemical library at Sanofi. The selectivity and potency of this compound for the CCK_1 receptor has also been well characterized (Gully *et al*., 1993).

Among the CCK_1 receptor antagonists, dexloxiglumide is of particular interest in the present context since this compound is under clinical development for IBS treatment. Functional studies, both *in vitro* and *in vivo*, confirm that dexloxiglumide is a highly potent CCK_1 receptor antagonist. In rat pancreatic acinar cells, it displaced the concentration–response curve for CCK-8 to the right without affecting the maximum response, suggesting a competitive antagonism. Schild analysis gave a straight line with a slope (0.90 ± 0.36) that was not significantly different from unity. The calculated pA_2 for dexloxiglumide was 6.41 ± 0.38 (Revel *et al*., 1999). On isolated human gallbladder, the compound had a similar affinity for CCK_1 receptors when the effects of three CCK_1 antagonists (dexloxiglumide, lorglumide, and amiglumide) were compared. All the three antagonists showed competitive inhibition of CCK-8-induced gallbladder contractions with pA_2 values of 7.00, 6.95, and 6.71 for lorglumide, dexloxiglumide, and amiglumide, respectively (Maselli *et al*., 2001) (Figure 4). These results are in line with those obtained using other CCK_1 receptor antagonists (Herranz, 2003).

In vivo experiments have confirmed the results from the *in vitro* studies: intravenous dexloxiglumide, like other CCK_1 receptor antagonists, reduced rat pancreatic exocrine secretion induced by submaximal CCK-8 stimulation ($0.5\,nmol\,kg^{-1}\,h^{-1}$) in a dose-dependent manner with an ID_{50} of $0.64\,mg\,kg^{-1}$ (Revel *et al*., 1999). In chronic studies, both exogenous CCK and endogenous CCK, released by intraduodenal trypsin inhibitor camostate (Ono Pharmaceutical Co Ltd), increased the weight of the pancreas, the total pancreatic protein and DNA, and the trypsin and amylase content. Dexloxiglumide ($25\,mg\,kg^{-1}$ i.p.) administered together with the CCK agonist caerulein ($1\,\mu g\,kg^{-1}$) reduced the peptide-induced increase in pancreatic weight, protein, and enzyme content in rats. Similarly, when dexloxiglumide was given together with camostate ($200\,mg\,kg^{-1}$), all the observed changes were reduced by the antagonist (Varga *et al*., 1998). When CCK receptor subtype selectivity was tested *in vivo* in rats, dexloxiglumide, at doses sufficient to completely block CCK_1 receptor-mediated inhibition of gastric emptying (ID_{50} $1.14\,mg\,kg^{-1}$), was ineffective against the pentagastrin-induced gastric acid secretion mediated by CCK_2 receptors (Scarpignato *et al*., 1996) (Figure 5). A range of selective and potent CCK_1 receptor antagonists similar to dexloxiglumide have now been characterized and are available for further studies (Herranz, 2003).

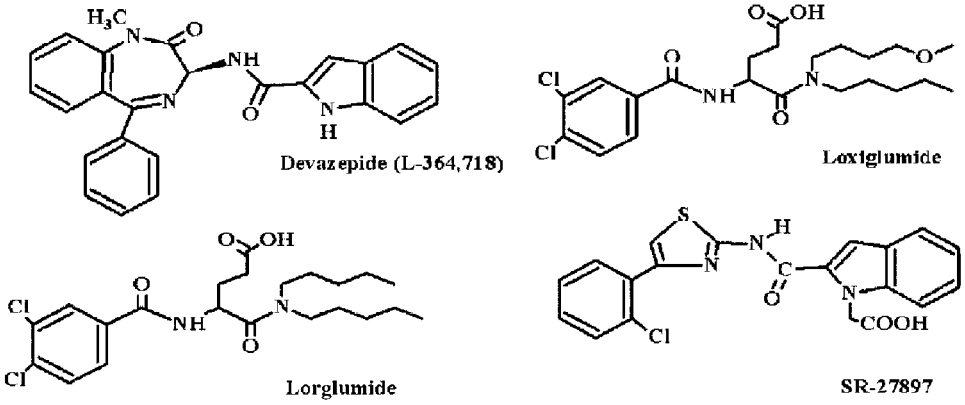

Figure 3 Chemical structures of CCK_1 receptor antagonists: devazepide, lorglumide, loxiglumide and lintitript.

CCK_1 receptor heterogeneity

Although the nucleotide sequences of cloned cDNAs of CCK_1 receptors from pancreas and from gastric and gallbladder smooth muscle are identical (De Weerth *et al*., 1993), their affinity states are different (Maubach *et al*., 1991; Moran *et al*., 1994; Taniguchi *et al*., 1995; Varga *et al*., 1988; 1998; Kisfalvi *et al*., 2001) (Figure 6). The available evidence suggests that the CCK_1 receptor exists in both high- and low-affinity states (Bianchi *et al*., 1994; Rivard *et al*., 1994; Tsunoda & Owyang, 1995) and that CCK occupancy of these results in the initiation of different intracellular events and consequent biological responses. The CCK analogue JMV-180, which is an agonist at the high-affinity CCK_1 receptors and an antagonist towards the low-affinity ones, is a useful tool for functionally distinguishing the two receptor states (Rivard *et al*., 1994; Tsunoda & Owyang, 1995; Tsunoda *et al*., 1996).

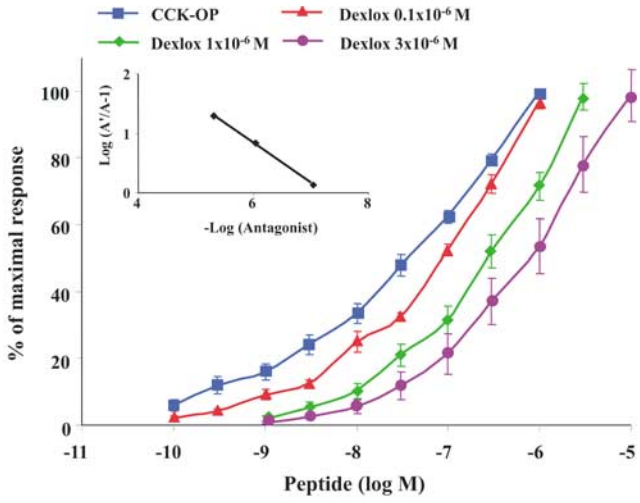

Figure 4 Inhibition of CCK-induced contractions of human gallbladder by CCK_1 receptor blockade. Effects of dexloxiglumide at 0.1 μM (red), 1 μM (green), 3 μM (purple) on the contractile responses produced by CCK-OP (blue) in isolated human gallbladder. Each point represents the mean and standard error of the values obtained from 35 individual experiments. Adapted from Maselli *et al*. (2001).

Binding of the peptide to high-affinity CCK_1 receptors leads to synchronized activation of tyrosine kinase, phosphatidylinositol 3-kinase, and phospholipase D (Rivard *et al*., 1994; Tsunoda & Owyang, 1995), while occupancy of low-affinity receptors induces activation of phospholipase C and enhanced phosphoinositide breakdown (Bianchi *et al*., 1994; Tsunoda *et al*., 1996). Whereas CCK_1 receptors in the pancreas are present in both high- and low-affinity states, those in gastrointestinal smooth muscle exist only in the low-affinity state (Maubach *et al*., 1991; Taniguchi *et al*., 1995; Varga *et al*., 1998; Kisfalvi *et al*., 2001) and mediate CCK-induced contraction (Figure 6). In line with these results, Moran *et al*. (1994) suggested that CCK-8 induces pyloric contraction and delays gastric emptying *via* interaction with receptors functionally similar to low-affinity pancreatic receptors. Furthermore, similar results have been obtained with gallbladder smooth muscle from guinea-pig and rabbit (Maubach *et al*., 1991; Taniguchi *et al*., 1995), suggesting that the absence of high-affinity CCK_1 receptors on gastrointestinal smooth muscle cells might be a general phenomenon, at least in rodents.

Data regarding CCK_1-receptor affinity states in nerves are controversial. It has been reported that activation of gastric mechanosensitive vagal afferent fibers (Schwartz *et al*., 1994) is mediated by low-affinity CCK_1 receptors in rats. More recent studies, however, have confirmed the presence of both high- and low-affinity receptors on vagal nerves in the same species (Li *et al*., 1999; Lu & Owyang, 1999; Simasko *et al*., 2002).

We have to emphasize at this point that it is very difficult to predict *ab initio* the functional consequences of CCK_1 receptor stimulation. In guinea-pig pancreas, both high- and low-affinity CCK_1 receptors mediate the stimulation of bicarbonate and fluid secretion (Szalmay *et al*., 2001). In rat pancreas, high-affinity CCK_1 receptors induce pancreatic growth while activation of low-affinity CCK receptors results in interstitial pancreatitis and cell destruction (Varga *et al*., 1988). In the rat acinar cell line AR4-2J, both high- and low affinity-receptors elicit growth-promoting effects (Hoshi & Logsdon,

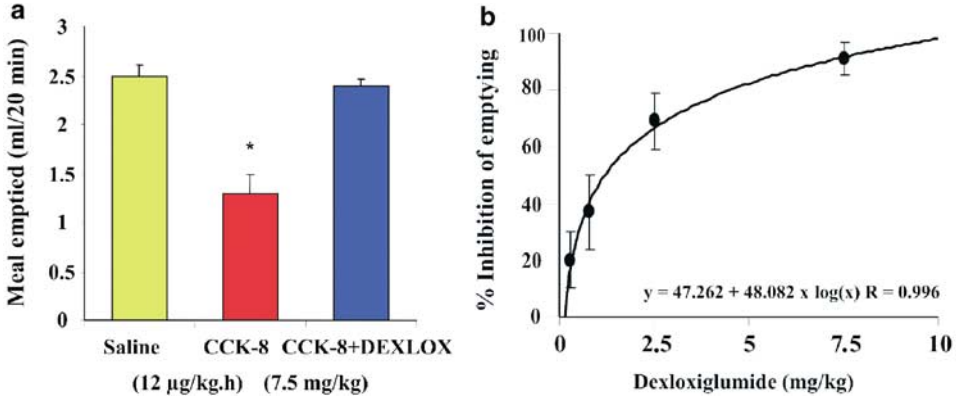

Figure 5 Effect of CCK_1 receptor blockade on gastric emptying of liquids in rats. (**a**) Inhibitory action of 7.5 mg kg^{-1} dexloxiglumide, administered intravenously 15 min before the agonist, on the CCK-induced delay in gastric emptying. (**b**) Dose-dependent inhibition of the CCK-induced delay in gastric emptying by dexloxiglumide (DEXLOX). Each column or point represents the mean and standard error of the values obtained from 8 to 10 individual experiments (*$P<0.05$). The curve shows a computer-generated logarithmic plot of percentage inhibition as a function of the antagonist dose. Adapted from Scarpignato *et al*. (1996).

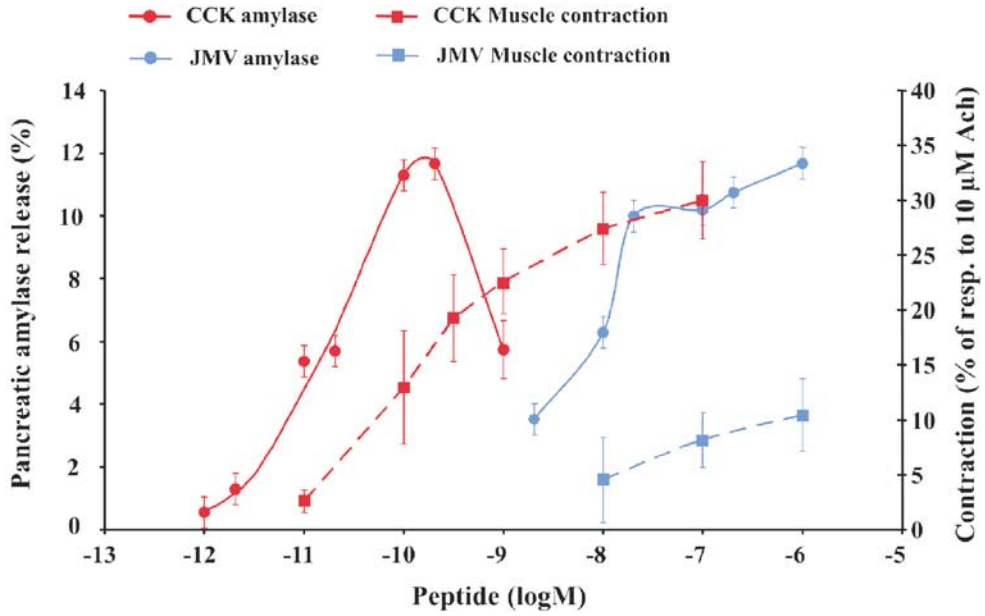

Figure 6 Concentration-dependent effect of CCK-8 (red) and JMV-180 (blue) on amylase release from rat pancreatic acini (solid line, left axis) and on contractile activity of isolated rat pyloric rings (broken line, right axis). Amylase release is calculated as a percentage of the initial amylase content. Values for the contractile activity are expressed as a percentage of the contractile effect of 10^{-5} M acetylcholine on the same preparation. Each point represents the mean and standard error of the values obtained from at least five to six individual experiments. Adapted from Kisfalvi *et al.* (2001).

1993). Finally, in human Panc-1 cells transfected with CCK_1 receptors, activation of both high- and low-affinity CCK receptors leads to an arrest of cell proliferation (Detjen *et al.*, 1997). In tissues where more than one cell type bears CCK_1 receptors, the functional responses can be quite complex and may actually derive from a mixture of opposing effects.

CCK_1 receptor polymorphism

Although CCK_2 receptor polymorphism is well characterized (Kopin *et al.*, 2000), CCK_1 receptor polymorphism is relatively unexplored. The CCK_1 receptor gene is mapped to chromosomal location 4p15.2-15.1 (Inoue *et al.*, 1997) and polymorphisms have been detected both in the coding regions and in the promoter region. First, two missense variants were identified in the coding region of the gene: a G-to-C base mutation in exon 1, resulting in a glycine-to-arginine substitution in codon 21, and a G-to-A base mutation in exon 5 that introduced an isoleucine for valine in codon 365 (Inoue *et al.*, 1997). Marchal-Victorion *et al.* (2002) characterized the isoleucine for valine mutation in codon 365 and demonstrated a decreased level of expression (26%) and reduced efficacy in generating inositol phosphates (25%). The authors suggested, therefore, that in humans bearing this or other mutations, decreases in CCK_1 receptor expression and coupling efficiency may influence CCK-induced regulation of satiety, and might be involved in the development of type II diabetes mellitus and obesity (Marchal-Victorion *et al.*, 2002). Novel polymorphisms (201A>G, 246G>A in the promoter region, 1260T>A, 1266T>C in intron 1 within the 3′ mRNA splice acceptor site consensus sequence, and Leu306Leu in exon 5) were found in addition to the variants (608G>A in intron 1, 3849C>T [Ile296Ile] in exon 5) reported previously (Tachikawa *et al.*, 2001). The analysis suggested that the 201A allele frequency was higher in the schizophrenic group, especially in the paranoid type (Tachikawa *et al.*, 2001). CCK_1 receptor polymorphism has also been described in other studies: no correlation was found with panic disorder (Ise *et al.*, 2003), but there were indications of an association with Parkinson's disease (Wang *et al.*, 2003) and with chronic alcoholism (Wang *et al.*, 2003).

Although evidence for the possible significance of CCK_1 receptor polymorphism is growing, no published data are available on the correlation between CCK receptor polymorphism and gastrointestinal motility. No other pharmacogenetic or pharmacogenomic studies have investigated the potential role of polymorphisms in genes encoding other regulatory systems, which might interact with CCK_1 receptors in the control of gastrointestinal motility under normal conditions or in functional disorders. Such studies may, however, provide new opportunities for predicting how patients will respond to particular treatments according to their genetic make-up.

CCK in colon motility

CCK has been known to affect human colonic motility for more than three decades (Harvey & Read, 1972). In spite of the large number of functional investigations since then, only one study (Rettenbacher & Reubi, 2001) has provided data regarding the distribution of CCK_1 receptors in human colon. This study, using receptor autoradiography, showed that the main target of CCK is the myenteric plexus, which has predominantly CCK_1 receptors. In addition, CCK_1 receptors were present at moderate-to-low density in the longitudinal muscle, while the circular muscle was negative for both CCK_1

and CCK_2 receptors. Neither CCK_1 nor CCK_2 receptors were expressed in the blood vessels, lymphoid tissue, mucosa, and muscularis mucosa. CCK receptors on nerve cells of the myenteric plexus had a high affinity for CCK over gastrin, which is characteristic of CCK_1 receptors (Rettenbacher & Reubi, 2001). These data suggest that CCK affects colonic motility by two fundamentally different pathways: acting on neurons in the myenteric plexus and directly on the smooth muscle cells.

Direct contractile effects of CCK on longitudinal and circular colonic muscle have been demonstrated in a number of studies. Experiments on human colon specimens revealed a substantial variation in agonist-stimulated contractions in terms of the number of preparations that responded to CCK and also in the maximal responses obtained (D'Amato *et al*., 1990; 1991; Morton *et al*., 2002b). This may have been due to genetic factors, the extent of tissue damage, the ages of the patients, and the medication that they had received.

Using selective CCK_1 receptor antagonists, it was also revealed that the contractile effects of CCK on human gallbladder and colon are solely mediated by CCK_1 receptors (D'Amato *et al*., 1991; Morton *et al*., 2002b). A recent study, however, has indicated some heterogeneity of the CCK_1 receptors in the colon in contrast to the homogeneity of those in the gallbladder (Morton *et al*., 2002a). A two-site analysis of the colon data revealed that one of the two sites is indistinguishable from that characterized in the gallbladder (Morton *et al*., 2002a). The molecular basis of the apparent receptor heterogeneity in the colon remains to be established, but it calls our attention to the fact that the actions of CCK on colonic smooth muscle are more complex than previously thought.

As observed *in vitro*, CCK also affects human colonic motility *in vivo*. It has long been suggested that endogenous CCK release increases colonic transit time (D'Amato & Rovati, 1997; Scarpignato & Pelosini, 1999). In the colon, the peptide stimulates electrical spike activity associated with segmenting contractions (Renny *et al*., 1983). In accordance with this observation, more recent data suggest that endogenous CCK exerts its inhibitory effect on propulsive motility in the ascending colon (Fosatti-Marchal *et al*., 1994). However, physiological concentrations of either endogenous or exogenous CCK in the circulation have been found not to affect phasic contractility, tone or transit in healthy subjects (Niederau *et al*., 1992; O'Brien *et al*., 1997), suggesting that CCK does not play a major physiological role in the control of interdigestive and postprandial human colonic motility. However, experiments with the CCK_1 receptor antagonist loxiglumide indicate that this compound can accelerate colonic transit in normal volunteers (Meyer *et al*., 1989). This raises the possibility that a CCK antagonist can act as pro-kinetic compound and may therefore be useful in treating constipation. Indeed, it was found that loxiglumide was able to significantly improve chronic constipation in geriatric patients (Meier *et al*., 1993).

Furthermore, it has been shown that CCK infusion can provoke abnormal reactions of gallbladder motility in IBS patients (Kellow *et al*., 1987), higher pain scores in patients with functional abdominal pain (Roberts-Thomson *et al*., 1992), and it can also unmask dysmotility (Kellow *et al*., 1988). An amplified release of CCK in IBS patients has also been shown in one study (Sjolund *et al*., 1996), although it was not confirmed by another, more recent one (Simren *et al*., 2001). In addition, loxiglumide has been shown to interfere with the gastro-colonic reflex and ileal motility and is able to selectively slow colonic transit time in patients suffering from IBS (Barrow *et al*., 1994). In a recent study, motility patterns were compared between healthy volunteers and IBS patients with abdominal pain and frequent defecation or diarrhea (Chey *et al*., 2001). The motility index, the frequency of high-amplitude propagating complexes, and also the responses to CCK were significantly greater in this subset of IBS patients. The high-amplitude propagating complexes coincided with the appearance of pain in the vast majority of observations. The effects of CCK were profoundly inhibited by both CCK_1 receptor blockade with loxiglumide and by muscarinic receptor blockade with atropine. These data indicate that the action of CCK on the colon in IBS is mediated at least in part *via* the enteric nervous system (Chey *et al*., 2001).

In summary, CCK_1 receptors are present in the human colon both on the smooth muscle cells and also on neurons. CCK is effective at both sites and the CCK_1 receptors are involved both in pain perception and in the regulation of motility offering multiple targets for potential beneficial effects. They are therefore important effectors in the control of colon function both in health and disease.

Clinical development of CCK_1 receptor antagonists as a potential treatment for IBS

Since CCK is involved in sensory and motor responses to distention in the intestinal tract, it is conceivable that CCK may contribute to symptoms like constipation, bloating, and abdominal pain that are often characteristic of IBS. It is therefore, not surprising that CCK receptor antagonists are being developed for the treatment of different functional gastrointestinal disorders, including IBS (Scarpignato *et al*., 1993; D'Amato & Rovati, 1997; Varga, 2002).

So far, six CCK_1 receptor antagonists have been tested in humans. Among these, to the best of our knowledge, only two are still under development for potential clinical applications. They are the two proglumide derivatives, loxiglumide and its active enantiomer dexloxiglumide (presently in phase III). No updated information is available for the indolyl derivative lintitript (Sanofi Synthelabo and reported to be in phase II). The substituted benzodiazepine derivatives devazepide (Merck & Co Inc) and FK-480 (Fujisawa Pharmaceutical Co Ltd), and the aspartic acid derivative 2-NAP (James Black Foundation, U.K.), have been discontinued because of gallstone formation and acute renal failure, respectively (D'Amato & Rovati, 1997). As we are concerned here with a potential clinical application, we will focus mainly on the effects of the two compounds still undergoing clinical development. It is hoped that these will provide a template for future therapeutic candidates and that they will help in defining the mechanistic role of CCK and its antagonists in this therapeutic area.

IBS is associated with increased sensitivity to gut distension, resulting in alterations of intestino-intestinal reflexes and pain perception. In a recent animal study, the blockade of CCK_1 receptors by the CCK_1 antagonist dexloxiglumide (5 and $20\,mg\,kg^{-1}$) was investigated in colonic motor alterations

(colonic spike bursts) and abdominal pain (abdominal contractions) induced by rectal distension in conscious rats under normal conditions and following intracolonic trinitrobenzene sulfonic acid-induced inflammation (Bonnafous *et al*., 2002). In control conditions, rectal distension progressively inhibited the occurrence of colonic spike bursts and increased the frequency of abdominal contractions. In both control and inflamed conditions, dexloxiglumide increased the threshold of the recto-colonic inhibitory reflex, and reduced hyperalgesia and the threshold of pain (Bonnafous *et al*., 2002). These data indicate that CCK_1 receptor blockade can modulate rectal-distension associated viscero-motor and pain responses. In another experimental model in dogs, blockade of CCK_1 receptors accelerated gastric emptying of a standard meal and reduced the inhibition of emptying rate induced by distension of the proximal colon (Fioramonti *et al*., 1996), indicating the potential therapeutic usefulness of CCK_1 receptor antagonists in delayed gastric emptying and in IBS.

In humans, ingestion of fatty acid reduced the tolerance of intragastric liquid load by delaying gastric emptying, and this action could be effectively antagonized by CCK_1 receptor blockade (Lal *et al*., 2004). In another study, duodenal lipid caused a dose-related appearance of nausea and other dyspeptic symptoms during gastric distention and release of CCK in healthy subjects and of patients with functional dyspepsia (Fried & Feinle, 2002). CCK_1 receptor blockade abolished the increase in intragastric volume induced by duodenal lipid infusion, significantly increased the highest tolerable intragastric pressure and significantly attenuated symptoms severity, suggesting that CCK_1 receptor blockade is able to modulate visceral hypersensitivity and to decrease dyspeptic symptoms (Feinle *et al*., 1999).

As described in detail above, it has long been known that endogenous CCK release increases colonic transit time (CTT) and that CCK_1 receptor antagonists are able to shorten CCT (Meyer *et al*., 1989). However, by virtue of their activity as selective CCK_1 receptor antagonists, these compounds also affect the function of the gallbladder. Owing to the potential contribution of bile stasis to the formation of gallstones, the inhibitory effect of dexloxiglumide on gallbladder emptying has been carefully evaluated in healthy volunteers with the aim of selecting doses of the antagonist that would provide maximal therapeutic effects on intestinal motility while minimizing the negative effects on gallbladder emptying.

In eight male volunteers the effect of dexloxiglumide (200 mg bid) on a liquid diet supplemented with soluble fiber induced changes in CTT (Meier *et al*., 1994). Ingestion of the liquid diet significantly increased mean CTT above normal values. Dexloxiglumide administration partly reversed this effect, thus confirming the involvement of CCK_1 receptors. Its effects ($200\,mg\,kg^{-1}$ bid or tid) on gallbladder emptying were investigated in a subsequent study in which neither antagonist dose regimen was found to significantly impair postprandial gallbladder kinetics (Meier *et al*., 1997a, b). These results suggest that the CCK_1 antagonist dexloxiglumide, at putative therapeutic dose regimens that accelerate CTT, does not interfere with gallbladder contractions.

The therapeutic potential of CCK_1 receptor antagonists as a treatment of functional gastrointestinal disorders has recently been explored in a proof-of-concept trial in patients suffering from IBS (D'Amato *et al*., 1999a, b; 2001). This multi-centre, randomized, placebo-controlled, double-blind trial involved 405 IBS patients (328 females and 77 males) of all subtypes of altered bowel habit characteristics. Patients were prospectively stratified to investigate the safety and efficacy of the CCK_1 receptor antagonist dexloxiglumide (200 mg tid). Dexloxiglumide treatment was well tolerated in all subtypes of patients. The proportion of responders after 12 weeks of treatment was statistically significantly higher to the CCK_1 receptor antagonist than to placebo in the female constipation-predominant IBS (C-IBS) subgroup for whom the drug tended to normalize bowel function. In addition to proving the clinical efficacy of a CCK_1 antagonist in IBS, this study also provided clinical evidence that this can be achieved at doses that do not promote gallstone formation.

Despite extensive research, the etiology and pathogenesis of gallstones remains uncertain and it is currently thought to involve an interplay among several processes, each with many endogenous and exogenous modifiers (Dowling, 2000). The present theory considers several genetic and environmental factors, which include, among others, the physicochemical properties of the bile, the motility of the biliary tree and gallbladder, and the enterohepatic circulation of bile salts and several possible dietary variables as being important in the genesis of gallstones (Meier *et al*., 1997b). On one hand, CCK_1 receptor antagonist activity of dexloxiglumide on gallbladder might lead to 'stasis' of bile, thereby possibly promoting gallstones formation. On the other hand, its ability to accelerate colonic transit would be expected to reduce the enterohepatic recirculation of deoxycholic acid and, as a consequence, lower its concentration in the bile salt pool and its detrimental effect on the solubility of cholesterol (Thomas *et al*., 2000). In addition, dexloxiglumide is also able to increase the biliary flow (Watanabe & Otsuki, 1994), which would lead, by a dilution effect on the bile, to an increase of the solubility of bile salts and cholesterol. It is therefore possible that its action to antagonize gallbladder contraction and its effects on intestinal motility (shortening transit time) and physicochemical properties of the bile might cancel each other out.

In conclusion, the discovery and development of CCK_1 receptor antagonists has allowed a more precise definition of the role(s) of CCK among the major determinants of gastrointestinal function. The results obtained in clinical studies examining motility and symptoms are also promising. In these studies, CCK_1 receptor antagonists are effective facilitators of gastric emptying and inhibitors of gallbladder contraction and have also been shown to accelerate colonic transit time in healthy volunteers and improve symptoms in patients with IBS. A very important clinical aspect of the potential clinical application of CCK_1 antagonists is that a dose can be identified that accelerates colonic transit time without significantly inhibiting gallbladder emptying. These encouraging findings suggest that CCK_1 receptor antagonists may offer an effective treatment for IBS and other disorders of colonic motility.

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