

The gastrointestinal cholecystokinin receptors in health and diseases

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gene in different species, their localization and the results of their specific occupation under normal and pathological states.

Introduction

Over the years, cholecystokinin (CCK) has been accepted as the gastrointestinal hormone mainly responsible for the control of gallbladder contraction, pancreatic enzyme secretion, growth of the pancreatic gland and gut motility. On the contrary, its sister hormone gastrin is recognized to regulate gastric acid secretion and proliferation of the acid secreting portion of the gastric mucosae as well as that of the upper intestine and colon.

These two hormones share the same carboxy-terminal pentapeptide amide sequence but differ in their sulfation sites on the active C-terminal portion of their molecule; indeed, gastrin is sulfated on its sixth tyrosyl residue and CCK on its seventh residue [1]. Because of their structure similarities, both peptides share common biological activities; indeed, CCK possesses weak gastrin activity and gastrin shares slight CCK activity [2].

CCK and gastrin initiate their biological effects through their binding to two subclasses of CCK receptors; the CCK-1 receptor, formerly characterized as the CCK_A receptor (A for alimentary), is highly selective for sulfated CCK [3] whereas the CCK-2 receptor, earlier called CCK_B receptor (B for brain), does not discriminate between sulfated or nonsulfated CCK and gastrin [4].

In this review, we will briefly summarize the different molecular forms and cellular localization of CCK and gastrin. The emphasis will, however, be put on the CCK-1 and CCK-2 receptors describing their biochemical characteristics, their

Cholecystokinin

A. Molecular forms

Shortly after his discovery of CCK-33 in pig intestine [1], Mutt purified the slightly larger form CCK-39 from the same species' intestine [5]. Later on, smaller and larger molecules were isolated from several species' brain and intestine. CCK-58, 8, 5 and 4 were found in porcine brain [6] whereas the molecular forms 58, 39, 33, 25, 18, 8, 7 and 5 were all identified in dog intestine [7,8]. Some of these same peptides were also identified in bovine intestine, 39 and 33, in rat intestine, 58, 22, 8 and in guinea pig intestine, 22 and 8 [9-11]. Early studies indicated that CCK-58 was the major form of CCK in human intestine [12] although it had been previously identified in the blood of dogs [13] and humans [14]. More recently, Reeve demonstrated that the only detectable form of CCK in rat blood was CCK-58 using a new method of extraction to minimize loss and degradation [15]. It was also demonstrated that this CCK-58 peptide exists under sulfated and non-sulfated forms in porcine and dog intestines [16,17]. This CCK-58 molecule was found much less potent than CCK-8 in releasing amylase from dispersed rat pancreatic acini [18]; later on, it was demonstrated that this decreased activity of CCK-58 depended upon its amino terminus shielding the carboxyl terminus responsible for its biological activity [19].

B. Localization

As indicated above, CCK is a peptide that exists in several different molecular forms and all these biologically active molecules contain the same carboxyl terminal phenylalanine amide. CCK is produced and released by the endocrine I cells located within the small intestine mucosae [20]. These I cells are present in larger amounts in the duodenum and proximal jejunum with decreased concentrations further down the gut [21]. CCK was also found in a subpopulation of pituitary cells

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[22] and in the brain, its highest concentration was measured in the cerebral cortex [23]; the hormone has been associated in the pathogenesis of schizophrenia [24] and satiety control [25]. CCK was also localized in the celiac plexus and in the vagus nerve in an efferent direction toward the gut [26]. In the nervous system, central and peripheral, CCK is considered as a neurotransmitter. The major question raised at this point regards the form of CCK stored in the intestine and brain; this is being pointed out because of the recent demonstration that CCK-58 appears to be the predominant intestinal form in dogs [8], humans [12] and rats [15] and was found the major molecular form in dog [13] and human [14] plasma following its release. With all these different forms of endocrine CCK circulating in the blood or acting through paracrine and autocrine mechanisms, it is important to characterize these molecular forms because they operate via only two different receptor subtypes, the CCK-1 and CCK-2 receptors, and have different physiological effects on various target tissues.

Gastrin

A. Molecular forms

Although postulated in 1905 by Edkins [27], the existence and nature of gastrin were confirmed by Gregory and Tracy in 1964 when they isolated, sequenced and pharmacologically evaluated the hormone they had purified from hog gastric mucosae [28]. Later on, two heptadecapeptides were isolated with identical amino acid sequences; they were named gastrin I, the non-sulfated form, and gastrin II the sulfated form [29]. Similar forms of gastrin were later found in cat, cow, sheep, dog, goat, rat, guinea pig and rabbit. Few years later, Besson and Yalow identified a 34 amino acid peptide they named "big gastrin" [30] which was later confirmed to exist also in human as an extended form of the G-17 molecule [31]. With the advent of molecular biology and cloning techniques gastrin cDNA was characterized initially from pig antrum in 1982 [32] and a year later it was done for human [33]. From these cloning studies, it was established that from the porcine gastrin precursor cDNA rich of 620 nucleotides, emerged an mRNA of 312 bases coding a 104 amino-acid preprogastrin molecule while the human preprogastrin peptide contained 4 less amino acids [34]. This preprogastrin precursor is further converted to progastrin which through hydrolysis by specific endopeptidases gave glycine-extended Gastrin-34 and then amidated Gastrin-17 [35]. In search for other gastrin molecules, minigastrin (Gastrin-14) was identified [36] along with smaller forms including a c-terminal hexapeptide from porcine antrum [37] and a pentapeptide from canine brain and intestine [38]. Finally, a C-terminal tetrapeptide was purified from the gut [39]. One interesting feature of the gastrin peptide is that in neonatal rat pancreas, the major source of gastrin in the newborn rat, gastrin is totally sulfated; this complete sulfation of gastrin was also observed in the feline pancreas and in the small intestine of human fetus [40]. Later on, the antrum was shown to contain approximately equal proportions of non-sulfated and sulfated gastrin [29].

Human plasma contains the two major forms of gastrin, G-34 and G-17, along with two minor components identified as little gastrin I and II and minigastrin I and II; these forms also

circulate as sulfated and non sulfated [41]. Recently, progastrin and glycine-extended gastrins were found to possess biological activities [42,43] and be among the blood circulating forms of gastrin although comprising less than 10% of the circulating hormones [35]. Over the last ten years, Reeve and colleagues claimed that CCK-58 was the major circulating form of CCK in the blood of many species including man and that other forms could be products of degradation happening right after blood collection. Gastrin-34 is also the most abundant form of gastrin into circulation [44] and one may also ask the pertinent question whether the other identified forms can result from degradation.

B. Localization

In the gastrointestinal tract, gastrin is synthesized and released from the endocrine G cells [45] and specific antibodies identified G-34 and G-17 in the same gastric antral cells [46]. During development, at least in the rat, it was observed that patterns of gastrin expression differed in the pancreas, antrum and duodenum [47]. In the pancreas, gastrin is transiently expressed during fetal life before the expression of any other islet hormone; immediately after birth, pancreatic gastrin mRNA expression is fading away to become almost undetectable by day 10 after birth [48]. In the duodenum, gastrin expression remains constant during neonatal development while its expression begins 3 days after birth in the stomach [47].

The highest concentrations of gastrin are present in the antrum of adult mammals whereas the duodenum has the highest concentration in the fetus [49]. Contrary to the duodenum of dog, cat and hog, which are almost depleted of gastrin, that of human is quite rich and contains as much hormone as the antrum [50]. It was also established that around 90% of gastrin found in the human antrum was G-17 while this form found in the duodenum represents between 40 to 50% of its total content [30]. Contrary to human foetal intestinal gastrin which is totally sulfated, only 50% is so in adults [51]. According to Reeve, small amounts of gastrin were found in the pituitary gland [52] with most of the gastrin-like immunoreactivity being CCK-8 or CCK-58 [53].

CCK receptors

The cholecystokinin and gastrin family of peptides share a common carboxy terminal pentapeptide-amide amino sequence and interact with two CCK receptor subtypes, CCK-1 and CCK-2, belonging to the family of seven transmembrane domain receptors.

CCK-1 receptor

A. Gene structure

The genes for the CCK-1 receptor have been cloned from guinea pig gallbladder, pancreas and gastric chief cells [54], human gallbladder [55,56], rabbit gastric [57] and mouse [58] cDNA libraries. This receptor gene consists in all species of five exons and four introns and there is a conserved structural homology in this CCK-1 receptor among different species; as an example, the human gallbladder CCK-1 receptor exhibits 92% identity and 95% similarity with its rat pancreatic counterpart

[59]. The human CCK-1 gene is localized to chromosome 4, that of the mouse has been mapped on chromosome 5 [60] whereas the rat CCK-1 gene was identified on chromosome 14 [61].

B. Biochemical characteristics

Over the years, the CCK-1 receptor structure has been evaluated by at least four different techniques starting with radioligand affinity cross-linking then receptor purification; more recently, deduction of the primary amino acid sequences was done from cDNA structure and the production of more specific receptor antibodies helped in confirming the protein mass. Initial studies on CCK-1 receptor characterization using the cross-linking technique with CCK-33 led to the identification of a major protein of 75 to 95 kDa and minor proteins of 47 and, 140 kDa [62,63]. Using labeled CCK-9 as tracer, CCK receptors proteins of 84 kDa were identified on guinea pig pancreatic acini [64] and some of 78, 45 and 28 kDa were located on dog pancreatic acini [64]. On human gastric smooth muscle membrane, a major band was noticed at 75 kDa and a minor one at 200 kDa [65]. For more details on the CCK-1 receptor biochemical characteristics, consult the review by Silvente-Poirot [66].

From radio labeled CCK agonists binding competition studies, their analysis led to an accepted model that the CCK-1 receptor consists of two binding sites, one of high affinity and low capacity and the other of low affinity and high capacity [67]. Further analysis of competition curves led to a model with three affinity states with high, low and very low affinity states [68]. The two sites model has been the most documented and studies using pancreatic acini from guinea pig and dog [64] have reported high and low affinity states of 0.1 and 35 nM (guinea pig) and 0.5 and 25.0 nM (dog); in rat pancreatic acini, values of 0.06 and 21 nM were reported [3] whereas the receptor from gastric smooth muscle membrane exhibits affinities of 0.07 and 8.4 pM for its high and low affinities, respectively [65]. The concentration of these receptors sites is relatively low with values of 20, 25 and 4 fmol mgpr⁻¹ for the high affinity sites on guinea pig, dog [64] and rat [3] pancreatic acini and 500, 600 and 640 fmol mgpr⁻¹ for the low affinity sites in these same three species.

C. Localization

Studies on the location of CCK-1 receptors in target cells have been performed using at least four different techniques. Initially, receptors were located from binding studies using labeled specific CCK agonists or antagonists; at the same time, also with labeled hormone, quantitative electron or regular microscope autoradiographs were displayed. More recently, northern blot and *in situ* hybridization and RT-PCR using species-specific radiolabeled full-length coding sequence cDNA probes or specific primers has been performed using poly (A)⁺ mRNA from each tissue. Finally, the development of specific CCK receptor antibodies was of great help in the identification of the specific cells containing the receptor; these antibodies were used in association with immunofluorescence and confocal microscopy techniques. As examples, the radiolabeled CCK agonist CCK-8 and antagonist L-364,718 binding studies revealed the presence of the CCK-1 receptor in human gallbladder [55], guinea pig gallbladder and pancreas [54] and rat pancreas [59]. The, ¹²⁵I-labeled BH-CCK33 hormone also allowed identifica-

tion of CCK receptor on mouse [69] and rat [70] pancreatic acini by electron microscopy. Autoradiographs of whole pieces of tissue pictured the CCK-1 receptor in human gallbladder [71], pancreatic nerves [72], in the basal region of the human antral and fundic mucosae as well as in the muscularis propria of the antrum, fundus, and gallbladder [73]. Quantitative RT-PCR experiments indicated that the message levels for the CCK-1 receptors are very low [74] or not expressed at all [75] in human normal pancreas. However, the message was detected in normal gallbladder, intestine, colon, spleen, ovary, cerebellum and frontal lobe [75]. Northern blot and *in situ* hybridization analysis which detect specific mRNAs actually present in a tissue indicate that the CCK-1 mRNA are absent from adult human pancreas [74] whereas detected in rat fundus mucosae and pancreas [76]. From all these binding studies and those utilizing molecular biology techniques, it seems that the northern blot and *in situ* hybridization assays give you a better and more accurate estimation of the true presence of the receptor mRNA in enough amount to expect the presence of the receptor protein. Caution has to be taken with the *in situ* hybridization especially with the pancreas of any species because of the presence of large quantities of RNase hydrolyzing the probes used.

Once the receptor mRNA has been identified in any given tissue or organ, it is important to establish the presence of the receptor protein for biological function evaluation. This can be done with accuracy and specificity with potent receptor antibodies which have been well characterized and checked by preabsorption of the antiserum with the synthetic peptide used for immunization. These specific antibodies can discriminate any other receptor proteins in any given cell using immunofluorescence or confocal microscopy in co-localization studies with hormones, specific enzymes or proteins. Development of such CCK receptor antibodies is quite recent and already the first published results raised controversies mainly because some of these antibodies were not as specific as they should have been.

The initial studies with such specific CCK-1 receptor antibodies revealed the presence of this receptor on rat myenteric neurons and on fibers in the muscle and mucosae of their stomach, colocalized with VIP or substance P in different neurons [77]. Similar location was also suggested in calf intestine [78]. With another specific antibody, our group described the CCK-1 receptor on rat and mouse pancreatic acinar cells as well as on central cells of rat, mouse and pig pancreatic islets [79], these cells were later confirmed to be the alpha and beta cells in the rat [80]. Although Schweiger [81] failed to visualize the CCK-1 receptor on pig islets' beta cells, he reported its location on the glucagon cells as we previously indicated but not on the acinar cells [79]. We later indicated that their antibody raised in chicken eggs lacked specificity when tested on pig pancreas [80]. Future experiments in this area of research should focus on the development of new specific receptor antibodies that will allow a better mapping of these CCK-1 receptors in target tissues and cells and the use of new sets of "receptor antagonists" for better understanding of their physiological responses.

D. CCK-1 receptor occupation and physiological responses

Occupation of the CCK-1 receptor by CCK and its analogues leads to stimulation of pancreatic enzyme secretion, pancreatic

endocrine hormones release, motility of the gut organs, contraction of the gallbladder, growth of the pancreas and control of satiety and pain in the central and peripheral nervous systems.

1. Secretion of the pancreatic enzymes

One of the first physiological role attributed to CCK was its implication in the control of pancreatic enzyme secretion; this is why one of its initial name was pancreozymin as its action was on the pancreas. Rodents have been the experimental model of choice for such studies and preparation of fresh acini has been the selected technique. Indeed, incubation of pancreatic acini with increasing concentrations of CCK leads to maximal amylase release [82], an effect totally inhibited by the selective and potent CCK-1 receptor antagonist L-364,718 [83]. This CCK-1 receptor antagonist was also very efficient in inhibiting pancreatic enzyme release *in vivo* in rat [83] and dog [84]. Although Cuber showed that CCK-8 increased pancreatic enzyme secretion in pig [85] and Cantor in human [86], it was later demonstrated that in these two species, CCK via its CCK-1 receptor had no direct effect on pancreatic acinar cell secretion [74,87]. In light of these data, we should consider that in large mammals including human, CCK would bind to its CCK-1 receptor present in afferent neurons and activate enzyme secretion by way of a vagal-vagal loop releasing acetylcholine as the final mediator [88]. This is supported by observations in conscious calves that intraluminal administration of CCK-8 resulted in pancreatic enzyme release, an effect atropine-sensitive [89]. It was later confirmed that the calf pancreas does not possess the CCK-1 receptor on its acinar cells [90].

2. Release of pancreatic islet hormones

In the endocrine pancreas, CCK was shown to stimulate insulin release in many species *in vivo* and also from rat isolated islets [91,92]; this insulinotropic effect of CCK is inhibited by the specific CCK-1 receptor antagonist, L-364,718 [93]. Furthermore, it was recently shown that the CCK-1 but not the CCK-2 receptor transcript was detected in rat islets [94], a location later confirmed by immunofluorescence [79]. CCK is also involved in glucagon release [95] and its effect through CCK-1 receptor occupation was confirmed in CCK-1 receptor deficient rats whose islets did not secrete glucagon in response to CCK [96]. This CCK-1 receptor on glucagon cells was also confirmed by immunofluorescence in rat islets [80]. These data disagree with those of Saillan-Barreau [97] who showed that glucagon release from human purified islets in response to CCK resulted from CCK-2 receptor occupation as they showed co-localization of this CCK-2 receptor with glucagon in alpha cells. Our laboratory was unable to confirm such a co-localization and also unable to establish the specificity of their antibody [80].

3. Motility of the gut organs

STOMACH: It has been known for many years now that the presence of medium chain fatty acids in the upper gut liberates endogenous CCK which can relax the proximal stomach [98] and delay gastric emptying [99]. Recently, it was demonstrated in humans that endogenous CCK release by fatty acids reduced the tolerated volume of liquid delivered into the stomach via

a CCK-1 receptor-mediated delay in gastric emptying by either reducing the proximal gastric tone, antral peristalsis, and/or by increasing pyloric or intestinal tone [100]. These effects on stomach functions were also confirmed in healthy volunteers using GI 181771X, a full specific CCK-1 receptor agonist with no CCK-2 receptor agonist activity [101]. This agonist delayed gastric emptying of solids and increased fasting gastric volumes [102].

COLON: Using receptor autoradiography, it was demonstrated in human colon that the main target of CCK was the myenteric plexus which is rich in CCK-1 receptors. These CCK-1 receptors were also located, at moderate to low density, in the longitudinal muscle [103]. It was then suggested that CCK can affect colonic motility via two different routes involving the neurons of the myenteric plexus and directly on the smooth muscle cells. In the colon, CCK increases colonic transit time [104], thus exerting its inhibitory effect on propulsive motility in the ascending colon [105]. Although physiological serum concentrations of endogenous or exogenous CCK did not affect phasic contractility, tone or transit in healthy subjects, suggesting no CCK physiological implication in the control of interdigestive and postprandial human colonic motility [106], it remains that loxiglumide, a known CCK-1 receptor antagonist, can accelerate colonic transit in normal volunteers [107]. This may suggest that local CCK release can modulate colon motility via paracrine actions involving higher CCK concentrations than those observed in the circulation.

4. Gallbladder contraction

It has to be remembered that CCK was initially discovered for its ability to contract gallbladder. It is now well recognized that CCK is the major hormonal physiological regulator of gallbladder contraction. Indeed, this was established from the demonstration that physiological concentrations of CCK in the blood after a meal were able to cause postprandial gallbladder contraction [108], an effect totally prevented by the administration of a specific CCK-1 receptor antagonist, devazepide [109]. In addition, CCK was postulated to stimulate hepatic bicarbonate secretion into bile [100] and relaxation of the sphincter of Oddi [111]. It thus seems that CCK can physiologically coordinate bile circulation towards its final destination, the duodenum.

5. Growth of gut organs

CCK given to induce enzyme secretion in rats in amount comparable to that in response to a meal, induced growth of the pancreatic gland as indicated by major increases in gland weight, in total enzymes and proteins contents as well as RNA and DNA [112,113]. This trophic action of CCK can be obtained whether CCK was given exogenously [114] or endogenously released either by feeding a high protein diet [115] or in response to pancreatic juice diversion [116]. These growth effects of CCK on the pancreas involve the CCK-1 receptor subtype as its response was totally abolished by the CCK-1 receptor antagonist L-364,718 [115,116]. These trophic effects of CCK on the pancreas have also been observed in the mouse [117] and Syrian hamster [118]. Liver growth however, remained insensitive to the action of either gastrin or CCK at least in the rat [119]. Recently, it was demonstrated that CCK, via its CCK-1 receptor, played a role in

the intrinsic gastric mucosal defense system against injury from luminal irritants, effects which seem to involve the production of nitric oxide from the constitutive form of nitric oxide synthase [120].

6. Control of satiety and pain

SATIETY: CCK is now well recognized as an hormonal inhibitor of food intake in many species including humans. It was observed that this suppressive effect of CCK on food intake was enhanced with age in rats, but the observed enhanced sensitivity to the central administration of CCK could not be explained by changes in gene expression of CCK nor of the CCK-1 receptors [121]. The implication of the CCK-1 receptor in satiety control was clearly demonstrated in OLETF (CCK-1 receptor deficient) rats who are hyperphagic during dark and light periods with increased meal size and a concomitant reduction in total number of meals; this decrease in meal number did not compensate for the increased meal size [122]. It was also recently observed that the dorsomedial hypothalamus is one of the few hypothalamic sites in the rat containing CCK-1 receptors and it is exactly there that local CCK injection has the greatest inhibitory effect on food intake [122]. This brain area is also rich in neuropeptide Y (NPY) which was also associated with satiety control. It was then hypothesized that CCK, via its CCK-1 receptor in the dorsomedial hypothalamus, would play a suppressing role on NPY. In the absence of CCK-1 receptor, this inhibition of NPY is absent and therefore we observe failure to compensate for the increased meal size [124].

GUT PAIN CONTROL: Infusion of CCK to patients with IBS (Irritable Bowel Syndrome) caused higher pain scores in patients with functional abdominal pain [125]; higher plasma CCK was also determined in these IBS patients [126] although this finding was not unanimous [127]. Recently, motility patterns were compared between healthy volunteers and IBS patients with abdominal pain and frequent defecation or diarrhea; in the IBS patients, the motility index, the frequency of high-amplitude propagating complexes, and the responses to CCK were all significantly greater than in the control patients. In most patients, the high-amplitude propagating complexes coincided with pain appearance and the effects of CCK were significantly inhibited by a CCK-1 receptor antagonist and also by atropine, suggesting participation of the enteric nervous system [128].

One of the major problems with the use of CCK-1 receptor antagonists in the management of abdominal pain in IBS patients remains their alteration of the normal functions of the gallbladder owing to potential bile stasis and gallstones formation. In a recent study [129] however, it was shown in healthy male volunteers that dexloxiglumide added to a liquid diet partly reversed the increase in colonic transit time caused by the liquid diet without impairing postprandial gallbladder responses [130]. These new data strongly suggest that CCK-1 receptor antagonists can be used to control gut motility and pain without detrimental effects on the gallbladder. This suggestion was later supported in a broader study in which dexloxiglumide, well tolerated by patients, tended to normalize bowel function in a group of female constipation-predominant IBS without promoting gallstone formation [131].

E. CCK-1 receptor occupation under pathological conditions

1. Effects on the stomach

In the early nineties, a Japanese group [132] reported that Otsuka Long Evans Tokushima Fatty (OLETF) rats were spontaneous mutants with little or no expression of the CCK-1 receptor gene [61]. In these CCK-1 receptor deficient rats, TRH which increases vagal efferent activity, indomethacin which decreases mucosal prostaglandin levels, protective molecules for the stomach, HCL and ethanol, known to increase the severity of gastric mucosal damage, all increased the severity of gastric mucosal lesions when compared to control animals. It is known that CCK exhibits anti-ulcer action on the gastric mucosae through its CCK-1 receptor occupation in rats [133]. Recently, CCK-1 and CCK-2 receptors were located in the human stomach [73] and the low degree of variability of CCK receptor density found in human endoscopic biopsy specimens from various individuals supports analysis of their status in pathological states, which has not yet been done to our knowledge.

2. Effects on the exocrine and endocrine pancreas

In CCK-1 receptor deficient rats, pancreatic protein release in response to exogenous CCK-8 or endogenous CCK release by pancreatic juice diversion were significantly impaired [134]; failure to respond to CCK was also observed in isolated acini from these OLETF rats [135].

In CCK deficient mice, the concentration of CCK-1 receptor mRNA remained normal suggesting that agonist binding to its receptor does not regulate receptor gene expression. Moreover, in these CCK-deficient mice, growth of their pancreas and their enzymes adaptation to different diets remained also comparable to control values [136]. These data on CCK-deficient mice agree in some way with findings in rats without CCK-1 receptor [137]. Indeed, in OLETF rats, pancreatic wet weight increases were significantly lower than those in normal LETO rats at all ages examined while total DNA contents in the whole gland and protein concentrations were comparable in both strains. These studies suggest that CCK-1 receptors might not be an absolute requirement for normal pancreatic growth at least in rodents. Although the pancreas develops normally in CCK-1 receptor deficient rats, its regeneration was significantly delayed following 30% pancreatectomy, suggesting its potential need in the gland regeneration process [138].

In the obese Zucker rats, their pancreatic protein secretion in response to increasing doses of CCK-8 was significantly reduced at all doses tested, this impaired release was also associated with significant decreases in CCK-1 receptor high and low affinity sites without any effect on their affinity [139].

Stimulation of the pancreatic gland by supraphysiological doses of caerulein, a CCK analogue, resulted in oedematous pancreatitis with destruction of the pancreas architecture and an important loss of the pancreatic gland, about 40%. Regeneration of the gland can be totally achieved within 5 days following a caerulein treatment at a physiological doses [140], during which the CCK-1 receptor mRNAs were tremendously increased [141]. This regeneration of the pancreatic gland undoubtedly involves the CCK-1 receptors because it can be

prevented by a concomitant treatment with a specific CCK-1 receptor antagonist, L-364,718 [142].

The absence of CCK-1 receptors also impairs functions of the endocrine pancreas; although pancreatic insulin contents were not affected in CCK-1 receptor deficient rats, its release in response to CCK-8 remained at basal level; similarly, CCK-8 failed to induce glucagon secretion while it increased its release in normal rats. In response to a meal, plasma insulin was reduced and associated with transient hyperglycemia in CCK-1 receptor deficient rats. These data clearly demonstrate the importance of the CCK-1 receptor in the control of insulin and glucagon release along with glycemia [96], and agree with the recent co-localization of the CCK-1 receptor with insulin and glucagon in many species by immunofluorescence [80].

3. Effects on the gallbladder

A deletion of a 262-base pair coding region of the human gallbladder CCK-1 receptor led to obesity and cholesterol gallstone disease in a patient with this mutation [143]. After sequencing, the majority of the mRNA produced from this gene was abnormally processed, resulting in deletion of its third exon; this mRNA encoded an inactive receptor unable to recognize CCK agonists. Although not yet proven in substantial number of patients, it is tempting to associate this peculiar mutation with gallstone formation and obesity.

CCK-2 receptor

A. Gene structure

The genes for the CCK-2 receptor have been cloned in human [144], rat [145], mouse [146], dog [147] and rabbit [148]. In human, the nucleotide sequence plus the 3' noncoding region of the human frontal cortex clone is 1969 bp in length [144]. Using *Mastomys* gastrin receptor cDNAs containing the second and third transmembrane domains and the entire coding region, respectively, five cDNA clones were isolated from a human brain cDNA library with the h CCKB3 clone being the most compatible with the major transcript size of the human brain CCK-2 receptor [149]. From screening a cDNA library constructed from AR42J cells from a rat pancreatic acinar carcinoma cell line, a 2243-base pair clone was isolated from a rat brain cortex cDNA library with identical cDNA sequence to the clone isolated from the AR42J cell cDNA library [145]. The mouse CCK-2 receptor gene was cloned from a 129/SVJ λ genomic library using a rat cDNA probe [146]. Restriction mapping and DNA sequencing revealed the gene structure to be comprised of five exons distributed over, 11 kb. The coding region contains 1362 nucleotides. The canine CCK-2 receptor was cloned from a parietal cell cDNA expression library and its cDNA has an open reading frame encoding a 453 amino acid protein [147]. Finally, the rabbit CCK-2 receptor was cloned by screening a rabbit EMBL phage library with a cDNA probe based on the nucleotide sequence of the human gastrin/CCK-2 receptor. The gene contained a 1356-bp open reading frame consisting of five exons interrupted by 4 introns and encoded a protein of 452 amino acids [148]. According to published data, the human CCK-2 receptor has 90% identity to rat and canine receptor. The predicted mouse CCK-2 receptor shares

87% and 92% amino acid identity with the human and rat receptor, respectively. The rabbit protein coding region of the gene exhibits 93 to 97% amino acid similarity with corresponding cDNA identified in human, canine and rat brain or stomach. Fluorescent *in situ* hybridization of human metaphase chromosomal spreads localized the human CCK-2 receptor gene to the distal short arm of chromosome, 11 [150].

B. Biochemical characteristics

The biochemical characterization of the CCK-2 receptor has been done using cell membrane fractions, cells, fixed tissue on slides, group of cells (acini) or transfected cells, usually the COS-7 cells. The binding studies were usually performed using the following radioligands: ^{125}I -CCK-8, ^{125}I -Nleu¹¹gastrin13, ^{125}I -BH-CCK-8, ^{125}I -BH(Thr.Nle)CCK-9, ^3H -L365,260 and ^{125}I -BH(2-17)G17NS.

Pharmacological characterization of the human brain CCK-2 receptor expressed in COS 7 cells indicated agonist affinities consistent with occupation of the CCK-2 receptor. Indeed, calculated IC_{50} values for CCK-8, gastrin 1 and CCK-4 were 0.14, 0.94 and 32 nM, respectively, values comparable to those previously obtained using isolated brain membranes. The CCK-B receptor antagonist L-365,260 bound with approximately 40-fold higher affinity than L-364,718, the CCK-1 receptor antagonist [151]. In another comparable study, L-365,260 was 50-fold more potent than L-364,718 [144]. In tissue sections of the human gastric mucosae expressing the CCK-2 receptor, L-365,260 presented an IC_{50} of 68 nM compared to an IC_{50} greater than 1 μM for L-364-718 [73]. The binding characteristics of ^3H -L-365,260 to six different human pancreatic cancer cell homogenates were comparable with K_{DS} in the nM range, 2.0 to 4.3, and receptor concentrations of 125 to 280 fmol/mg protein. Similar binding data were obtained from tumors grown in nude mice [152]. According to binding studies done on pancreatic tissue sections using ^{125}I -BH-CCK-8, it would seem that the human pancreas possesses CCK-2 receptor as the receptor did not discriminate between CCK-8 and gastrin-17-1 binding and bound L-365,260 with higher affinity than lorglumide a known CCK-1 receptor antagonist [71].

In rat adipocytes, CCK-2 receptors were identified on membranes and binding studies were consistent with a single class of high affinity sites with a K_{D} of 0.2 nM; the absence of CCK-1 receptor was established by RT-PCR in these cells [153]. On dog pancreatic acini, a population of CCK-2 receptor with high affinity sites for G-17 ns and G/CCK-4 was identified which was not associated with amylase release. This population of receptor recognized equally CCK-39, CCK-8 and G-17 ns with IC_{50} of 1 nM [154]. On rabbit isolated gastric mucosal cells, ^{125}I -(Nle¹¹)-HG-13 bound specifically to a receptor population with a K_{D} of 70 pM, binding displaced by gastrin analogues and specific gastrin antagonists [155]. The pig pancreas demonstrated a single class of high affinity sites with a K_{D} of 0.22 nM established from a saturation analysis of ^{125}I -BH-[Thr.Nle] CCK-9 binding to pancreatic membranes. However, competition binding by specific CCK-1 and CCK-2 agonists and antagonists indicates the presence of both CCK receptor subtypes with CCK-2 receptor being predominant [156]. In general, the CCK-2 receptor cloned from different species exhibits a comparable high affinity for CCK-8

and gastrin-17-1 with IC_{50} in the nM range. On the contrary, they have higher affinity for the CCK-2 receptor antagonist L-365,260 (IC_{50} around 10 nM) than for the CCK-1 receptor antagonist L-364,718 (IC_{50} around 1 μ M).

C. Localization

The search for the CCK-2 receptor localization was done using the following techniques: RT-PCR, Northern Blot, immunological staining and autoradiography.

In human

PCR amplification identified a CCK-2 receptor DNA fragment in the human brain, stomach and pancreas but not in the kidney [76]; in a more exhaustive study using the same technique, this CCK-2 receptor was also identified in brain, stomach, pancreas, small intestine, liver, colon, spleen, lung, thymus, ovary, breast, prostate, testes, adrenal and in the kidney [75] contrary to what was observed in the previous study [76]. Still by RT-PCR, in human purified pancreatic acini, messages for the CCK-2 receptor were observed; however, *in situ* hybridization could not confirm this expression probably because of its insufficient level of expression [74]. The CCK-2 receptor mRNA was also identified in human pancreas and in the islets of Langerhans [97] as well as in human gastric mucosae, more precisely in parietal and neuroendocrine cells and in epithelial cells within the neck of the gastric gland [157].

Using Northern blot analysis detecting the mRNA present in a gland or tissue, the CCK-2 receptor was initially detected in human brain, stomach fundus, pancreas and gallbladder [144,151] but not in heart, placenta, lung, liver, skeletal muscle and kidney [149]; some of these organs indicated positive CCK-2 messages by RT-PCR [75].

By autoradiography analysis of 125 I-BH-CCK-8 binding, it was shown that the human pancreas predominantly expresses the CCK-2 receptor subtype present all across the gland [71]. Using the same technique but with 125 I-[Leu15]-gastrin-1, high concentrations of CCK-2 receptors were detected in the mid glandular region of the human fundic mucosae and circular muscle [73] as well as in pancreatic islets but not in normal acini [72]. Our own study more precisely identified the CCK-2 receptor on human foetal and adult pancreatic islets, specifically on the somatostatin delta cells using a specific CCK-2 receptor antibody; the presence of the receptor was also confirmed by Western blots [158]. These data do not agree with a previous location of this CCK-2 receptor on the human islet's glucagon cells [97] with an antibody we could not evaluate its specificity [80].

In the rat, mouse and guinea pig

In rat and mouse, we encountered the same location problems with the RT-PCR and Northern Blot techniques. By RT-PCR, the CCK-2 receptor was identified in rat total pancreas homogenate, in purified islets [158] and in the antrum mucosae [76] while it could not be detected in two other studies [159,160]. However, by Northern blot analysis, the CCK-2 receptor mRNA remained absent from the rat pancreas and islets [76,145,159,160] and from the rat muscle, kidney, liver and guinea pig gallbladder [76,145]; On the other end, the receptor was detected in the rat brain sub-cortex and cortex and in the

fundic mucosae [76,145]. In the adult mouse, the CCK-2-subtype transcripts were detected by Northern Blot analysis in brain and stomach, but not in the pancreas, liver or colon; however, using RT-PCR, a more sensitive technique, transcripts were confirmed in brain and stomach and others were also present in colon, pancreas, kidney and ovary and remained absent in heart, duodenum, small intestine, liver, gallbladder and testis [146]. In the pig [87], the CCK-2 receptor has been identified by Northern Blot in the brain, pancreas and gallbladder.

By immunohistochemistry and electron microscopy, the CCK-2 receptor was localized in guinea pig parietal cells, on chief cells and in endocrine cells of the stomach but not in the lamina propria [161]. By immunocytochemistry, the CCK-2 receptor was shown to be transiently expressed in foetal rat pancreas (E17-E18), expression which disappeared on E20-E22 and after birth. In adult pancreas, the receptor was localized on glucagon islet cells. By immunohistochemistry using fluo-CCK-8, the CCK-2 receptor was identified on gastric ECL cells but surprisingly not on parietal cells; the bioactivity of this agonist was confirmed by its ability to induce histamine release from the ECL cells [162]. In the guinea pig and dog stomach, a specific CCK-2 receptor antibody identified the receptor in a few, small epithelial cells in the bottom part of the corpus mucosae and in the antral mucosae with co-localization with the somatostatin cells [163] as observed in the rat pancreatic islets [158]. Also with a specific CCK-2 receptor antibody, the protein was identified with mucosae neural components of the bovine small intestine [78].

An analysis of all these data on the CCK-2 receptor localization using different techniques suggests that safe localization at the cellular level will come with the use of standardized and specific antibodies ultimately with colocalization with known cellular protein, enzymes or hormones.

D. CCK-2 receptor occupation and physiological responses

The presence of the CCK-2 receptor has been observed in the esophagus, stomach, upper gut, pancreas and on adipose tissue.

1. Growth of the esophagus

CCK-2 receptors have been identified in both lower and mid esophageal mucosae in human [164] and it was previously reported that gastrin can exert trophic effects on esophageal mucosae whether the hormone was infused or endogenously release [165,166].

2. Effects on the stomach

Administration of graded doses of pentagastrin, a gastrin-17 analogue, caused graded increases in both peak acid output and duration of response in rats [167]. With the discovery of the CCK receptor subtypes, explanation of acid release in response to gastrin has moved from a direct effect on the parietal cells to a rather indirect effect through the enterochromaffin-like (ECL) cells. It is now believed that gastrin stimulates acid release primarily through activation of the CCK-2 receptor on the ECL cells via histamine release [168]. The importance of the gastrin-enterochromaffin-like cell axis was strengthened recently by the observation that in gastrin KO mice, histidine

decarboxylase, the enzyme responsible for histamine synthesis (HDC) mRNA was reduced along with a concomitant decrease in HDC activity. In these ECL cells, the number of secretory histamine vesicles was also decreased. Overall, gastric acid output in gastrin KO mice was only 20% of that in the wild type mice [169]. The involvement of the CCK-2 receptor in the control of gastric acid secretion is also supported from data obtained with a new CCK-2 receptor agonists, the diketopiperazine analogues. Indeed, one of these, compound 1, dose-dependently increased gastric acid output in an anesthetized rat, an effect totally blocked by a CCK-2 receptor antagonist, CI-988 [170].

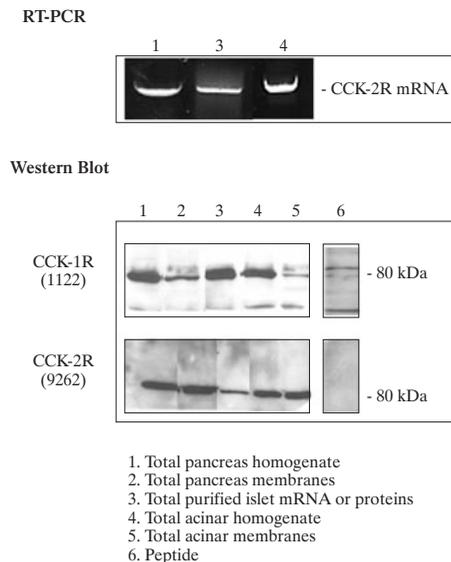
Gastrin is generally considered to be a trophic factor for the oxyntic gland area mucosae of the stomach and may also be involved in regulating mucosal growth in small intestine and colon [171]. However, an extensive study in fed rats using relatively high doses of pentagastrin, 0.25, 1.0 and 4.0 mg kg⁻¹ for 5 days did not have any significant effects on thymidine incorporation into oxyntic gland area, duodenum and colon, total organ weight of these three organs as well as their total DNA contents [167]. More recently, it was shown that gastrin elicited increased [³H] thymidine incorporation into ECL cells but failed to do so on parietal cells. Furthermore, gastrin increased tyrosine phosphorylation and activation of MAP kinase and c-fos and c-jun gene expression only in ECL cells. In these respective cells, gastrin dose-dependently increased histamine release and [¹⁴C]-aminopyrine uptake [172]. These data clearly indicate that gastrin can activate intracellular pathways related to cell growth in the ECL cells but not in the parietal cells and suggest that gastrin may act to promote commitment or differentiation of precursor cells to parietal cells [173].

3. Effects on the pancreas

Although previous pharmacological data in humans support the involvement of CCK-1 receptor in the regulation of pancreatic exocrine secretion based on the potent response to CCK and the limited one to gastrin [86,174], it was later suggested that the CCK-1 receptors were present on neurons, rather than on pancreatic acinar cells, and that the final mediator would be acetylcholine [175]. This hypothesis was recently supported by the observation that the human pancreatic acinar cells lack functional responses to cholecystikinin and gastrin; in that study, however, it was clearly demonstrated that the CCK-2 receptor has the potential to induce enzyme secretion when transiently transfected into human acini with a secretory response to CCK-8 comparable to that induced by carbachol [74]. This potential of the CCK-2 receptor coupled to pancreatic enzyme secretion was also demonstrated in transgenic mice expressing the human CCK-2 receptor in their acini as they secreted amylase in response to CCK and gastrin in a dose-dependent manner in the presence of a CCK-1 receptor antagonist; the pharmacological characteristics of these secretory responses to both stimuli reflect occupation of the CCK-1 rather than that of the CCK-2 receptor with EC₅₀ in the pM range [176]. In the rat, our own data [177] (*Fig. 1*) indicate that their pancreas expresses both CCK receptors subtypes with secretion in response to gastrin being the result of CCK-1 receptor occupation; indeed, it is inhibited by the CCK-1 receptor antagonist, L-364,718 but not by the CCK-2 receptor antagonist, L-365,260. In pig acini, amylase

Figure 1. Amylase release in response to pentagastrin (PG) and PG plus the CCK-1 (L-364,718) and the CCK-2 (L-365,260) receptor antagonists

Freshly prepared acini were incubated 30 min at 37°C with increasing concentrations of pentagastrin, L-364,718 and L-365,260



release was stimulated by caerulein concentrations above 1 nM, remained insensitive to the high affinity CCK-1 receptor agonist JMV-180 and caerulein-induced amylase secretion was inhibited only by the CCK-1 receptor antagonist MK-329 at concentrations above 100 nM [87]. These data are difficult to reconcile with the absence of either CCK receptor subtype on the pig acinar cells [79,80]; one possibility remains that the acini preparation contained functional nerve ending sensitive to CCK as part of the short reflex system described by Konturek [175]. In response to CCK and gastrin in the presence of SR27,897, a CCK-1 receptor antagonist, glucagon secretion from purified human pancreatic islets reached its maximal release at 13 and 8 pM, respectively, a response inhibited by RPR-101048, a specific CCK-2 receptor antagonist [97]. Again, maximal secretory responses to CCK in the pM range pleads for CCK-1 receptor occupation [3] and such high affinity of gastrin for its CCK-2 receptor are unusual [177].

The implication of the CCK-2 receptor in the control of pancreas growth via its agonist gastrin is far from being established. Indeed, in the rat, under conditions which increased serum gastrin levels, gastrin infusion or chronic injections, omeprazole treatment, and fundectomy, all were without effect on the weight and DNA content of the pancreatic gland [167,178]. Earlier studies, however, claimed that gastrin exerts trophic effects in the pancreas [179,180].

In normal rats, levels of circulating leptin observed 2 and 6 h after feeding were significantly reduced by treatment with YM022, a specific CCK-2 receptor antagonist. By reducing plasma leptin, the antagonist simultaneously increased epididymal fat tissue leptin content after refeeding. Concomitantly, the antagonist also dose-dependently inhibited leptin mRNA recovery observed after refeeding. This study also indicated that

the CCK-2 receptor was present in all the adipose tissues tested: mesenteric, epididymal and perirenal [153].

E. CCK-2 receptor occupation under pathological conditions

1. The esophagus

Over recent years, there has been an enormous increase in the incidence of Barrett's metaplasia and esophageal adenocarcinoma. Since gastrin plays an important role in the regulation of gastrointestinal organs proliferation and differentiation, it became important to evaluate its implication in the regulation of Barrett's metaplasia and a search for its CCK-2 receptor.

In SEG-1 cells, a human esophageal adenocarcinoma cell line, the presence of the CCK-2 receptor has been established by RT-PCR and gastrin shown to cause a dose-dependent increase in their proliferation when compared to controls; this proliferative effect of gastrin was abolished in the presence of the specific CCK-2 receptor antagonist L-365,260 [181]. In analyzing human tissue samples, the relative expression levels of gastrin and the CCK-2 receptor were significantly increased by 29 and 8 fold, respectively in the Barrett's samples when compared to their paired normal; in esophageal cells, high basal levels of activated PKB/Akt were associated to endogenous gastrin expression and reduced in the presence of YMO22, a specific CCK-2 receptor antagonist. It is then suggested that gastrin acting in an autocrine manner may aid progression of Barrett's metaplasia through amplification of antiapoptotic pathways [182]. In another study, CCK-2 receptors were detected in 30% of normal patients, in 80% of patients with esophagitis, in 100% of patients with Barrett's metaplasia and in 70% of patients with esophageal adenocarcinomas. In the Barrett's group, all patients expressed CCK-2 receptors twice as much as in controls. Furthermore, in Barrett's mucosal biopsies, gastrin significantly increased DNA synthesis, an effect blocked by the CCK-2 receptor antagonist L-740,093. It thus seems that overexpression of the CCK-2 receptor in Barrett's metaplasia may have implications in the management of patients in whom gastrin is elevated by acid suppression therapy [164]. However, an earlier study indicated that in esophageal cancers, only one sample exhibited a low level of CCK-2 receptor mRNA; in that study, the CCK-1 receptor was overexpressed in esophageal cancers [183]. These contradictory results definitely plead for new studies to clarify which CCK receptor subtypes are overexpressed in Barrett metaplasia and cancer of the esophagus.

2. The stomach

In the human gastric adenocarcinoma cell line, amidated gastrin caused a dose-dependent increase in DNA synthesis, an effect attenuated by the CCK-2 receptor antagonist L-365,260; this growth-promoting effect of gastrin was associated with increased levels of cyclin D1 transcripts, protein and promoter activity [184].

In rat, sustained hypergastrinaemia resulted in ECL cell hyperplasia and later on lead to ECL cell carcinoid; in human, after 5 years of proton pump treatment, ECL cell hyperplasia rather than carcinoids was observed [185]. In the African rodent *Mastomys*, induced hypergastrinemia by histamine-2 receptor blockade, resulted in ECL cells carcinoids associated with slight

elevation of the CCK-2 receptor mRNA but an 8 fold increase in histidine decarboxylase (HDC) mRNA not influenced by CCK-2 receptor inhibition. These data suggest that HDC mRNA expression in neoplastic ECL cells is not under the influence of the CCK-2 receptor [186].

Many studies indicate that gastrin is an important factor in the progression to gastric cancer; however, the presence of gastrin and its CCK-2 receptor in gastric adenocarcinomas remains a controversial issue. Indeed, gastrin and its receptor have been detected in human gastric adenocarcinomas by immunohistochemistry [187]. However, the CCK-2 receptor expression was detected in only 7% of gastric cancer samples by RT-PCR [188], or by receptor autoradiography [189]. In another study using RT-PCR, transcripts of CCK-2 receptors were present in 7 out of 8 specimens of gastric adenocarcinoma [183]. Again, the expression of the CCK-2 receptor in gastric cancer cells and tissues has to be reevaluated with standardized techniques.

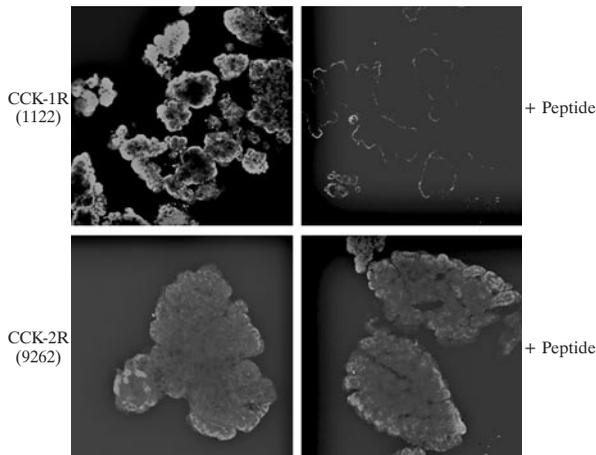
3. The pancreas

In the rat with caerulein-induced pancreatitis, CCK-2 receptor mRNA expression was tremendously increased above control values, and its overexpression was maintained only in animals treated with growth-promoting doses of caerulein used to induce pancreas regeneration [141].

Transfection of the human CCK-2 receptor in mouse pancreatic acinar cells, a location where it does not normally belong [80], led to larger pancreas at age 50 days. When these mice were crossed with gastrin-mice expressing gastrin in their pancreatic β cells, some of these animals (3 out of 20 homozygous), developed malignant transformations through an acinar-ductal carcinoma sequence [190]. On the contrary, transfection of the CCK-2 receptor in human pancreatic cells, the MiaPaCa-2 and Panc-1, led to inhibited anchorage-dependent growth upon CCK stimulation along with decreased DNA synthesis [191]. These data suggest that occupation of overexpressed CCK-2 receptors on at least two pancreatic tumor cell lines can result in cell growth inhibition. However, similar MIA-PaCa-2 cells were shown to possess specific CCK-2 receptors whose occupation by CCK resulted in their proliferation [192]. This inhibitory effect of CCK is also totally opposite to growth stimulation of the hormone on normal acinar cells in culture [193]. These opposite responses are not yet quite understood; the ductal origin of the pancreatic cancer cells could be the reason; however, this possibility is not supported by the observation that ductal complexes induced in duct-ligated rat pancreas are stimulated to grow in response to gastrin; these cells have a higher expression of the CCK-2 receptor absent on normal ductal cells [194]. Furthermore, CCK-2 receptors have been identified by binding assays with the CCK-2 receptor antagonist L-365,260, on six different human pancreatic cancer cells of ductal origin [152].

Controversy also exists in the distribution of the CCK receptor subtypes in human tumors, mostly those of the pancreas. According to Reubi using his autoradiography technique for identification, 22% of the gastroenteropancreatic tumors express the CCK-2 receptor with 38% expressing the CCK-1 subtype [195]. In another study [72], the same authors indicated that ductal pancreatic carcinoma rarely expressed CCK receptors; the CCK-2 receptor mRNA and protein were identified in

Figure 2. RT-PCR of CCK2-R mRNA and Western blots of the CCK-1 and CCK-2 receptor proteins from different pancreatic preparations using the specific CCK-1 (1122) and CCK-2 (9262) receptor antibodies. Conditions of both experiments are described in [158]



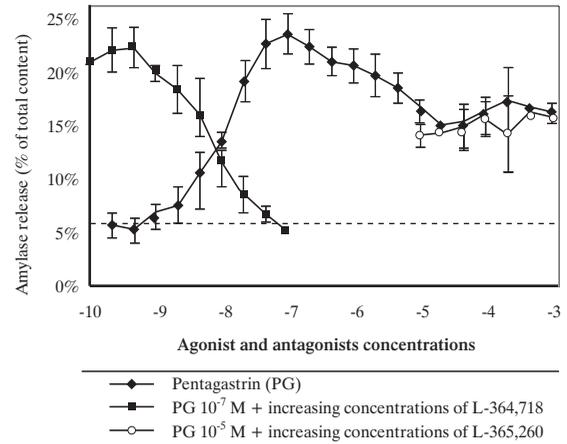
a few tumors characterized by neuroendocrine differentiation. The major source of CCK-2 receptors in pancreatic tumor was found in islets [72].

Using RT-PCR, expression of the CCK-2 receptors was detected in all normal pancreatic tissue samples and adenocarcinomas whereas the CCK-1 receptors were observed in gallbladder, intestine, brain, ovary, spleen, thymus and in all pancreatic adenocarcinomas but not in any normal pancreas specimen [75]. Using the same technique, CCK was undetectable in all tumors and normal pancreas; the CCK-2 receptor mRNA was detected in all pancreatic tumors, all resection tissue, and all normal pancreatic tissue. Furthermore, the CCK-1 receptor mRNAs were detected in 12 of 18 tumors, in 5 of 10 resection margins and in all normal tissue samples [196], this last result being different from those of the previous study [75]. In order to solve the dilemma of CCK receptors presence in normal and tumoral pancreatic tissues and identify the specific cells expressing these receptors, an immunohistochemical study with specific CCK receptor subtype antibodies has to be done.

4. The colon

Until recently, the predominant thought was that the CCK-2 receptor could play a role in the proliferation of colon cancers; this view was deduced mostly from studies performed on cancer cell lines. However, the presence of the CCK-2 receptors in human colon cancer still remains controversial. In one study using receptor autoradiography, the CCK-2 receptors could not be detected in 22 specimens of colorectal carcinomas [195]. In another study using RT-PCR, a low level of CCK-2 receptor mRNA was found in only 2 out of 12 colon adenocarcinoma samples [183]. From binding studies, very low CCK-2 receptor binding sites were detected in some primary human colon tumors [197] whereas others failed to detect specific binding in the majority of colon cancers [198]. The present overall view is that gastrin and its CCK-2 receptor do not play a major role in the growth of

Figure 3. Localization of the CCK-1 and CCK-2 receptors on purified rat pancreatic acini by confocal microscopy. Conditions of the experiment and the use of the specific antibodies are described in [158]



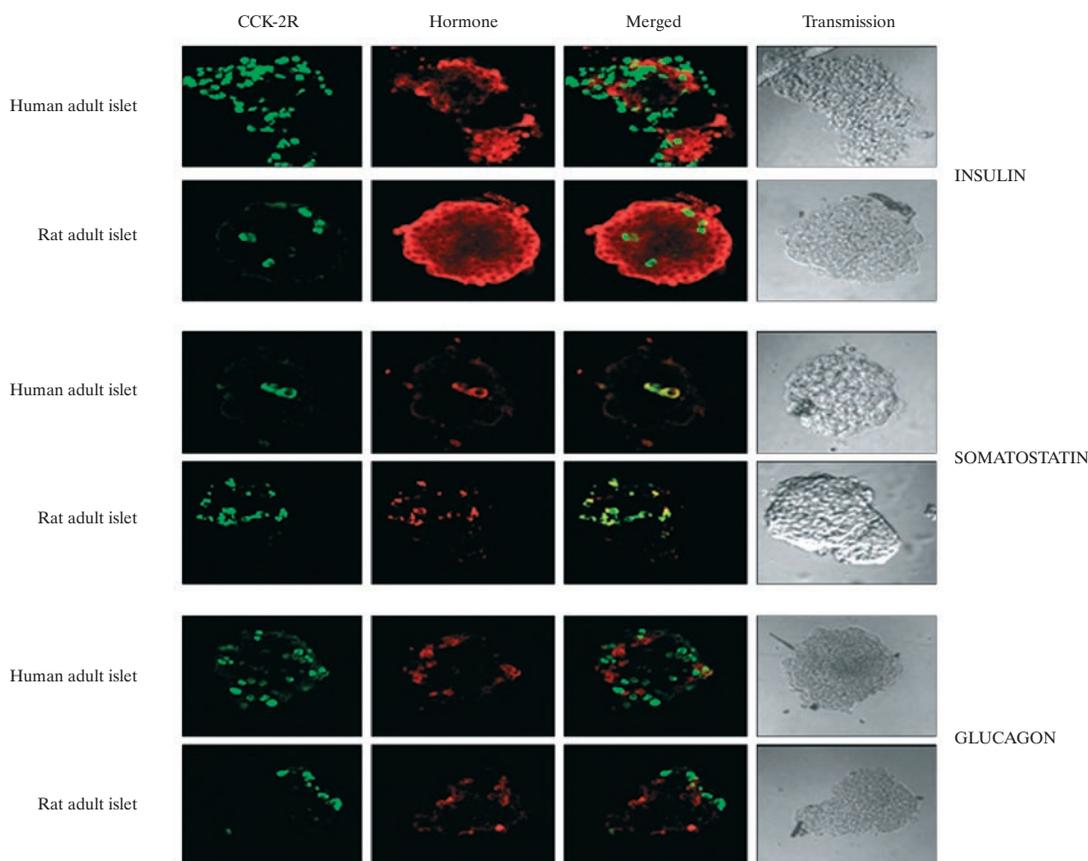
the majority of colorectal carcinomas. It is also evident that future research will have to be focused on the receptor protein cellular localization using specific potent antibodies.

5. Cellular co-localization of the CCK-1 and CCK-2 receptors

Characterization of the CCK-1 and CCK-2 receptors by binding studies on pancreatic acinar cells led to the discovery that those acini may possess both receptor subtypes. An initial study indicated that dog pancreatic acini expressed high affinity sites for G17 and G/CCK-4 and that their occupation was not related to amylase release [154]. Similarly, gastrin receptors were also characterized by binding studies on guinea pig acini and shown to be distinct from the previously described CCK receptors [199]. In purified human acini, using quantitative RT-PCR, it was shown that the CCK-1 receptor was expressed at less than one copy/acinar cell and approximately at five copies/acinar cell for the CCK-2 receptor. These acini did not release amylase in response to CCK-8 and gastrin and this failure was assumed to result from insufficient level of receptor expression [74].

As shown in *Fig. 2*, our own data indicate that the rat pancreas expressed the CCK-2 receptor mRNA in the whole gland (band 1), in purified islets (band 3) and in hand-picked acini (band 4). This message corresponds to the CCK-2 receptor expressed as an 80 kDa protein present in all fractions; acinar cells and islets have the CCK-1 receptor also present as an 80 kDa protein. Specificity of both antibodies is demonstrated in band 6 with preincubation of each antibody with its specific peptide used for its development. The presence of both CCK receptor proteins on purified acini is also confirmed by confocal microscopy with the same antibodies used for the Western blots. As shown in *Fig. 3*, it is evident that the CCK-1 receptor proteins are more abundant on purified rat acinar cells than the CCK-2 receptors which also seem to be present on all cells. Amounts of this CCK-2 receptor are evidently more important in islets delta cells as observed in *Fig. 4*.

Figure 4. Colocalization of the pancreatic CCK-2 receptor with three islets hormones. Islets purification from human and rat pancreas was performed as described in [158]



The major question raised following the demonstration that individual cells contain both subtypes of the CCK receptor, remains its physiological relevancy. The phenomenon is not unique to the CCK receptors as oligomerization [200] and heterodimerization [201] of the somatostatin receptor subtypes have been previously observed. One result of such heterodimerization of the SST3 and SST2A receptors is the resistance of the SST2A to agonist-induced desensitization [201]. Heterodimerization of the CCK-1 and CCK-2 receptors was observed following their stable transfection in CHO cells and such complexes exhibited enhanced agonist-stimulated cellular signaling, delayed agonist-induced receptor internalization and increased cell growth [202]. We previously demonstrated [177] (*Fig. 1*) that the presence of the CCK-2 receptor on rat pancreatic acini was not associated with enzyme release confirming what was obtained in dog acini [154].

Conclusions

The CCK receptor subtypes remain one of the key elements to all the unanswered questions and controversial responses to CCK and gastrin related to their effects in normal cell physi-

ogy and cancer development. It is therefore mandatory to establish their respective cellular location in normal and cancerous organs for potential therapies with selective and appropriate hormones and receptor antagonists. In the near future, we should look forward for a better understanding of CCK receptors' homo- and heterodimerization and their consequences on potential integrated physiological responses and/or association to cancer development.

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