Cholecystokinin and Gastrin Receptors

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I. INTRODUCTION

Cholecystokinin (CCK), one of the first gastrointestinal hormones discovered, was originally isolated from porcine duodenum as a 33-amino acid peptide (CCK-33). The sequencing of CCK-33 in 1968 revealed that CCK is structurally related to gastrin, another gut hormone characterized 4 years earlier (Fig. 1) (343, 530). Indeed, the...
two gene products that are generated in multiple molecular forms that differ in length both share five COOH-terminal amino acids (Gly-Trp-Met-Asp-Phe-CO-NH₂). CCK and gastrin are synthesized and secreted by I cells from the upper intestine and G cells from the gastric antrum, respectively. In 1975, a gastrin-like immunoreactive peptide was found in rat brain (537). This peptide was further identified as CCK-8 (133, 399, 416). Since this discovery, large amounts of CCK have been identified in various areas of the central nervous system and in peripheral nerve endings (268, 397, 399). In humans, CCK and gastrin are encoded by two distinct genes located on chromosomes 3p22-p21.3 and 17q21, respectively. They are produced through multi-step processing of large peptide precursors (134, 400, 510, 569). Posttranslational modifications found on biologically active peptides include sulfation of the tyrosine at position 7 from the COOH terminus in CCK, position 6 in gastrin, as well as COOH-terminal amidation. Biologically active gastrins and CCKs also exist in nonsulfated forms. In fact, half of the endogenous gastrins are nonsulfated (134, 183, 184, 400). Because of the sequence similarity in their bioactive region, CCK and gastrin share some biological and pharmacological effects. These peptides, in their mature forms, exert their biological functions by interacting with membrane G protein-coupled receptors named CCK receptors located on multiple cellular targets in the central nervous system and peripheral organs. Nonmaturated forms of gastrin have also been shown to exert several effects (see sect. VII B).

II. THE CHOLECYSTOKININ RECEPTORS

A. Nomenclature

Two types of CCK receptors (type A, "alimentary," and type B, "brain") have been identified on a pharmacological basis. The CCK-A receptor was first characterized in pancreatic acini from rodents (95, 120, 224, 415, 432), whereas the CCK-B receptor was first found in the brain (217, 427). Based on recommendations of the International Union of Pharmacology (IUPHAR) committee regarding receptor nomenclature and drug classification, the CCK-A receptor has been renamed CCK1 receptor (CCK1R), and the CCK-B receptor has been renamed CCK2 receptor (CCK2R). CCK1R binds and responds to sulfated CCK with a 500- to 1,000-fold higher affinity or potency than sulfated gastrin or nonsulfated CCK. The CCK2R binds and responds to gastrin or CCK with almost the same affinity or potency and discriminates poorly between sulfated and nonsulfated peptides. In the periphery, the CCK2R can be considered as the "gastrin receptor" (see sect. VII D).

B. cDNAs Cloning and Deduced Protein Structures

Historically, a cDNA encoding gastric gastrin receptor was originally cloned by A. S. Kopin and co-workers (253) through expression of a canine parietal cell cDNA library in COS-7 cells and isolation of a cDNA clone encoding a 453-amino acid protein. Binding of 125I-Bolton-Hunter-CCK-8 to COS-7 cells expressing this protein was inhibited by CCK- and gastrin-related peptides with potencies in agreement with CCK2R pharmacology. However, the nonpeptide antagonist for the CCK1R (L-364,718) competed with labeled CCK-8 to the recombinant receptor with higher potency (19 nM) than the nonpeptide antagonist of the CCK2R (L-365,260, 130 nM), but this atypical pharmacological feature was further shown to be species specific (38). In addition to binding parameters, intracellular Ca2+ mobilization, inositol 1,4,5-trisphosphate elevation in response to gastrin, and affinity labeling of the 76,000 component confirmed that the cloned receptor was indeed the gastric gastrin receptor. The human brain CCK2R cDNA was then isolated and sequenced, providing evidence that the brain and gastric CCK2R represent a unique molecular entity encoded by a single gene (219, 273, 380).

Report of the cloning of the pancreatic rat CCK1R cDNA by S. A. Wank appeared in the same issue of the Proceedings of the National Academy of Sciences (USA) as that of the canine CCK2R (559). Indeed, Wank and
colleagues purified to homogeneity a sufficient amount of receptor protein to obtain partial sequences of five fragments. From these peptides, degenerate oligonucleotides were designed and used as primers to amplify, by polymerase-chain reaction, a cDNA fragment, which was then utilized as a probe to screen a cDNA library obtained from rat pancreas. A complete cDNA, with an open reading frame encoding a protein of 444 amino acids, was thus cloned. In vitro transcripts of the cloned cDNA were injected into *Xenopus* oocytes and shown to display CCK-induced chloride currents that were inhibited by a specific antagonist of the CCK1R (559).

The CCK1R and CCK2R exhibit a relatively low degree of sequence homology (50%) but present seven hydrophobic segments, likely corresponding to transmembrane domains, with extracellular NH$_2$-terminal and intracellular COOH-terminal ends. Such structures are characteristic of G protein-coupled receptors (GPCRs) in agreement with high-resolution three-dimensional structure of rhodopsin (366). Other sequence signatures of members of the family I of GPCRs that are essential for receptor activation are also present in the CCK1R and CCK2R, such as an E/DRY motif at the bottom of transmembrane domain III, and a NPXXY motif within transmembrane domain VII. Cloning of cDNAs encoding CCK1R in guinea pig gallbladder and pancreas, mouse pancreas, rabbit stomach, and human gallbladder as well as those encoding CCK2R in the rat pancreatic cancer cell line AR4–2J, an enterochromaffin-like carcinoid tumor of *Mastomys natalensis*, rat stomach, and bovine pancreas, demonstrated a high degree of sequence homology well within the range expected for interspecies variations of the same receptor type (122, 140, 165, 345, 534, 560). CCK1R and CCK2R contain three to four potential N-linked glycosylation sites in their amino termini, which is consistent with a high and heterogeneous degree of glycosylation, experimentally noticed in both affinity and photoaffinity labeling experiments (152, 372). For instance, pancreatic native CCK1R, which migrates as a 85,000- to 100,000-Da component in SDS-PAGE, is shifted to a 38,000- to 42,000-Da protein after endoglycosidase-F treatment (152, 372). SDS-PAGE analysis suggested that the degree of glycosylation of affinity labeled CCK2R differs according to the cell type in which the receptor is expressed. Indeed, photoaffinity labeling of canine CCK2R in isolated parietal cells identified a component of 76,000 Da, while photoaffinity labeling of canine CCK2R in pancreatic membranes identified a component of 47,000 Da (153, 305). The bovine CCK2R, which appeared as a 42,000- to 47,000-Da protein in pancreatic membranes, was further identified as a 85,000-Da recombinant protein in COS-7 cells, with both components yielding a 37,000-Da protein by endoglycosidase-F treatment (140, 275). The precise role of the carbohydrate moieties of these receptors remains largely unknown.

C. Genes

The CCK1R gene is on human chromosome 4p15.1-p15.2 and mouse chromosome 5 (216, 430). CCK2R has been assigned to human chromosome 11p15.4 and to distal chromosome 7 in mice (216, 430). The transcriptional start site of human CCK1R was identified 206 bp upstream of the translation start site. In the mouse CCK2R gene, the transcriptional start site was identified at 199 bp upstream of the translation start site in a region devoid of any TATA-like sequences (157, 262). So far, no precise data have been reported on CCKR gene regulation. However, several studies have documented polymorphism in CCKR gene promoters associated with diseases such as panic disorder, alcohol dependence, and obesity (245, 325, 326, 555).

The two receptor genes are each organized into five exons (Fig. 2). In the CCK2R gene, the presence of an exon I variant within intron I was described (317). Ac-

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**FIG. 2.** Schematic representation of genes encoding CCK receptors (CCKRs). The asterisk shows the presence of an exon I variant within intron I. Introns are represented by yellow boxes. Arabic Numbers indicate nucleotide number in introns and exons. Roman numbers indicate transmembrane domains of the CCKRs encoded by the different exons.
cording to the authors, in some cells, especially cancer cells, exon I would be used alternatively to exon I leading to synthesis of transcripts encoding an NH₂-terminally truncated CCK2R (317). Gene organization in exon/intron may theoretically lead to protein diversity. The existence of two mRNA isoforms for the CCK2R, produced by alternate splicing of exon 4 in human stomach, was also reported (491). Although this possibility exists and affects the sequence of the third intracellular loop by introducing a five-amino acid cassette, its functional significance remains controversial. In fact, recombinant human receptors encoded by these two isoforms are undistinguishable in terms of pharmacology and signal transduction features (218). Moreover, the ratio between the expression levels of the two isoforms was found to be ~1:99, supporting the view that the splice site in exon 4 of the human CCK2R gene is dominant (218). However, the nature of the predominant variant differs according to species: small variant in human; long variant in mouse, rat, dog, and calf (140, 219, 227, 253, 273, 380, 384, 559, 560). Additionally, a misspliced cDNA clone that encodes a receptor with retention of intron IV in the third intracellular loop of the murine receptor has been identified from colorectal and pancreatic tumors (130, 199). This misspliced variant seems to exhibit spontaneous ligand-independent oscillatory increases in intracellular Ca²⁺ and increases cell growth rate (199). Concerning CCK1R, the presence of a seven-amino acid glycine-rich cassette in the third intracellular loop of the murine receptor has been reported to contribute to species-specific aspects of signaling (384).

D. Binding Properties and Ligands

Many studies have been devoted to the pharmacological characterization of naturally expressed, and more recently, of recombinant CCK receptors. Radiolabeled analogs of CCK and gastrin as well as tritiated antagonists have been used in such works. Binding experiments using labeled agonists as radioligands yielded the binding parameters of the G protein-coupled state of the receptor, whereas binding studies with labeled antagonists provided binding parameters of the resting and inactive state of the receptor. This explains, at least in part, why binding experiments with antagonists identified a greater number of binding sites compared with experiments using agonists. The binding affinities of CCK and gastrin agonists for the different affinity states of the CCK receptors are different. In general, \( K_d \) values for binding of CCK to high- and low-affinity sites of the CCK1R are in the range of 50–300 pM and 50–200 nM, respectively (222, 225, 432). While high-affinity sites are much less numerous than low-affinity sites, both components are functionally important, at least in pancreatic acinar cells (see sect. IVA). On the other hand, \( K_d \) values for high- and low-affinity binding of CCK to CCK2R are ~100–300 pM and 2–5 nM, respectively (264). In addition to these two sites, there exist very-low-affinity sites as well with \( K_d \) values of ~10 μM in both the CCK1R and CCK2R (213).

The natural ligand with the highest affinity for CCK1R is the sulfated octapeptide of CCK (CCK-8) (Fig. 1). Other natural molecular variants of CCK such as CCK-33, CCK-39, and CCK-58 bind to CCK1R with similar affinity to CCK-8 (396, 490). Gastrin, at physiological concentrations, is likely a poor activator of CCK1R, its affinity being 100- to 500-fold lower than that of sulfated CCK-8. Structure-activity relationship studies with synthetic CCK analogs have indicated that sulfation of the position 2 tyrosine in CCK-8 (Fig. 1) is critical for binding to CCK1R, since its removal causes a 500-fold drop in affinity. The two natural ligands with the highest affinities for CCK2R are sulfated gastrin-17 (often abbreviated G-17II) and sulfated CCK-8 (140, 214). Nonsulfated gastrin-17 (G-17I) exhibits a 3- to 10-fold lower affinity than sulfated gastrin-17. This affinity order and the fact that postprandial blood levels of gastrins are 5- to 10-fold more elevated than those of CCK lead one to consider that sulfated gastrin-17 is the preferred ligand and probably the physiological ligand of most of the peripheral CCK2R. On the other hand, due to the abundance of sulfated CCK-8 in the central nervous system, it is likely the ligand which naturally activates brain CCK2R. Compared with sulfated gastrin-17, the COOH-terminal tetrapeptide common to gastrin and CCK and nonsulfated CCK-8 interacts with the CCK2R with only a 10- to 50-fold decreased affinity.

The variety of physiological functions that can be regulated through the CCK receptors and their potential use as targets for the treatment of several human diseases have stimulated searches for specific, potent agonists and antagonists of these receptors. For most of these molecules, their chemistry and pharmacological properties are extensively described elsewhere (Table 1) (201, 351). Historically, amino acid derivatives such as benzotript and proglumide were the first CCK receptor antagonists reported. They were followed by peptidic analogs of CCK

### Table 1. List of some popular cholecystokinin receptor antagonists

<table>
<thead>
<tr>
<th>Name</th>
<th>CCKR Selectivity</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-364,718 (devazepide)</td>
<td>CCK1R &gt;&gt;&gt; CCK2R</td>
<td>82</td>
</tr>
<tr>
<td>CR-2017 (dexloxiuglide)</td>
<td>CCK1R &gt;&gt;&gt; CCK2R</td>
<td>295</td>
</tr>
<tr>
<td>SR-27,897 (linitrip)</td>
<td>CCK1R &gt;&gt;&gt; CCK2R</td>
<td>190</td>
</tr>
<tr>
<td>L-365,260</td>
<td>CCK2R &gt;&gt; CCK1R</td>
<td>291</td>
</tr>
<tr>
<td>L-369,466</td>
<td>CCK2R &gt;&gt;&gt; CCK1R</td>
<td>51</td>
</tr>
<tr>
<td>YM-022</td>
<td>CCK2R &gt;&gt;&gt; CCK1R</td>
<td>436</td>
</tr>
<tr>
<td>CR-2945</td>
<td>CCK2R &gt;&gt;&gt; CCK1R</td>
<td>296</td>
</tr>
<tr>
<td>YF-476</td>
<td>CCK2R &gt;&gt;&gt; CCK1R</td>
<td>460</td>
</tr>
<tr>
<td>GV150013X</td>
<td>CCK2R &gt;&gt;&gt; CCK1R</td>
<td>536</td>
</tr>
<tr>
<td>PD-134308 (CI-988)</td>
<td>CCK2R &gt;&gt; CCK1R</td>
<td>215</td>
</tr>
</tbody>
</table>
and gastrin and, more recently, by nonpeptide ligands with distinct chemical structures.

Among the peptidic analogs, JMV 179 and JMV 180 display nanomolar affinity for CCK1R (159, 286). JMV 179 is a CCK1R antagonist that corresponds to the COOH-terminal heptapeptide of CCK where the COOH-terminal amidated phenylalanyl residue has been replaced by a phenylethyl ester moiety, and the L-Trp by a D-Trp. Interestingly, unlike in CCK, tyrosine sulfation in JMV 179 is of minor importance for its binding to CCK1R (17, 470). Exchange of D-Trp for a L-Trp in JMV 179 yielded an interesting result: unlike in CCK, tyrosine sulfation in JMV 179 is of minor importance for its binding to CCK1R. JMV 180 is a partial agonist. At any respect to CCK1R-mediated stimulation of inositol phosphate production, JMV 180 is a partial agonist. With partial agonist depending on the species, tissue, or biological event under investigation (159, 571, 589). With partial agonist depending on the species, tissue, or biological event under investigation (159, 571, 589). With respect to CCK1R-mediated stimulation of inositol phosphate production, JMV 180 is a partial agonist. At any concentrations, this CCK analog causes [Ca^{2+}]_i oscillation in pancreatic acini similar to that observed with CCK at picomolar concentrations (see sect. IV).

Although the COOH-terminal tetrapeptide of CCK presents a low affinity for the CCK1R, chemical manipulation of this peptidic fragment successfully generated selective high-affinity agonists of this receptor which surprised scientists in the field who believed that sulfated tyrosine was required for optimal binding and activity of CCK at CCK1R. Substitution of Met by an o-tolylaminocarbonyl-Lys residue and of Phe by (N-Me)Phe in CCK-4 were key modifications that gave these peptides a CCK1R personality (288). Interestingly, high-affinity agonists of CCK2R were obtained by replacement of Met by (NMet) Nle (351). Such work, which yielded a large variety of CCK-4 analogs, also indicated that minor structural differences in CCK-4-based peptides dictate affinity and selectivity for CCK1R and CCK2R.

In the benzodiazepine derivative family, L364,718 and L365,260 appeared as the first potent nonpeptide antagonists of CCK1 and CCK2R, respectively (82, 291). They were followed by a large series of potent compounds (201). Interestingly, from a drug design point of view, CCK1R antagonist L364,718 (MK-329) was generated from asperlicin, a fermentation product isolated from the fungus Aspergillus alliaceus, which presents micromolar affinity for CCK1R (83).

Several of the nonpeptide agonists and antagonists have reached phase I and II clinical trials. Indications for these compounds can be deduced from the pathophysiological role of CCK1R and CCK2R in humans (see sect. vii). In this context, one major question that recently arose with some of the nonpeptide antagonists was whether these were pure antagonists rather than partial agonists. Indeed, some so-called antagonists turned out to present some agonist activity in the stomach and pancreas and on cells transfected with the cDNAs encoding CCK1R or CCK2R (48, 257, 447). Presumably, newly generated molecules are more capable of efficiently blocking CCK1R- or CCK2R-related physiological effects (201).

III. STRUCTURE-FUNCTION RELATIONSHIP OF CHOLECYSTOKININ RECEPTORS

A. Localization of Ligand Binding Sites

A large set of converging data related to binding sites of cholecystokinin receptors is currently available, giving a good picture of the binding mode of natural and synthetic ligands to their cognate receptors. The data were provided using essentially four complementary approaches, site-directed mutagenesis, photoaffinity labeling, NMR-NOE transfer, and three-dimensional modeling. The binding site for CCK on the CCK1R is of particular interest due to the high selectivity of this receptor for sulfated versus nonsulfated CCK and for sulfated CCK versus gastrin. The two key interactions, which account for the 500- to 1,000-fold selectivity of CCK1R for sulfated versus nonsulfated CCK, involve a Met and an Arg in the second extracellular loop (169, 170). Proximity of the sulfated moiety of CCK with Arg was recently confirmed by photoaffinity labeling (17). The NH2-terminal moiety of CCK is tightly linked to extracellular residues of CCK1R, including residues in the second extracellular loop at the top of transmembrane (TM) helix I and within the third extracellular loop (16, 237). The COOH-terminal tetrapeptide of CCK appears to be embedded between TM helices III, V, VI, and VII through a network of both ionic and hydrophobic interactions (16, 145, 168). This binding mode of the COOH terminus of CCK into CCK1R is in agreement with an NMR study of the interactions between CCK and a fragment of CCK1R comprising the top portion of helix VI and the third extracellular loop as well as a fragment including amino acids at the top of transmembrane segment I (173, 373). With the use of photoaffinity labeling, two hits in the CCK1R were identified. The first was a Trp at the top of TM I using a photoprobe with the reactive moiety within the COOH-terminal Phe of CCK and the second was an His within the third extracellular loop using a probe with a benzophenone in the place of the Gly of CCK (Fig. 3) (193, 228). Accordingly, a model of binding of CCK to the CCK1R was proposed in which the COOH terminus of CCK and the tyrosine sulfate were in interaction with Trp39 and Arg197, respectively, and the NH2-terminal moiety was in contact with the third extracellular loop of the receptor (132). This second model of CCK positioning into the CCK1R binding site is divergent from that obtained on the basis of site-directed mutagenesis results (16, 237).

The first precise information available on the CCK2R binding site was the identification of five amino acids of the second extracellular loop of CCK2R that were essen-
tial for high-affinity interaction with gastrin (473). In subsequent mutagenesis studies, the authors showed that within this five-amino acid sequence, an His is crucial for CCK recognition and interacts with the penultimate aspartic acid of CCK (471, 472). Furthermore, several amino acids and key regions that interact with the amidated COOH-terminal phenylalanine of CCK were identified in transmembrane helices IV and VI (161, 264). Photoaffinity labeling experiments using a CCK ligand with a photosensitive moiety attached to the NH$_2$ terminus identified amino acids at the top of helix I, which agrees with results from site-directed mutagenesis and molecular modeling experiments that the sulfate on CCK-8 slightly interacts with an Arg in this region of CCK2R (13, 161). On the basis of these studies, it appears that binding sites of CCK on CCK1R and CCK2R involve residues that are located in homologous regions. However, detailed analysis also shows slightly distinct positioning of CCK within the two receptors. The molecular basis for such divergent positioning is still not clearly understood.

An exciting issue that emerged in the course of binding site studies with CCK receptors was whether synthetic molecules that are structurally divergent with the natural ligands of the receptors share the same binding site or have distinct binding pockets. This issue has been addressed by several authors using site-directed mutagenesis. Regarding CCK1R, it was shown that certain residues of CCK1R were critical for binding and response to CCK but were without any importance for binding and activity of several nonpeptide antagonists. The best illustration
was achieved through mutation of the Arg in the second extracellular loop of CCK1R, which caused dramatic decreases in affinity and potency of CCK but did not change affinity and antagonistic potency of L364,718 and SR27,897. In contrast, mutation of Asn333 at the top of transmembrane helix 6 affected affinity and potency of both CCK, L364,718, and SR27,897 (168, 170).

A large set of data exists concerning the binding sites for nonpeptide ligands of CCK2R that were essentially obtained in the course of studies related to the analysis of the impact of intraspecies and interspecies polymorphisms of this receptor on affinity and partial agonism of a series of CCK2R antagonists. The first striking interspecies difference to be studied was that between dog and human CCK2R. Indeed, the CCK1R antagonist L364,718 binds to the canine CCK2R with an affinity similar to that with which the CCK2R antagonist L365,260 binds to the human CCK2R, and vice versa. Mutation of nonconserved amino acids from transmembrane helices led to the identification of a single amino acid in the canine sequence (Leu355, TM VI) that is responsible for this reversal of specificity (38). In general, studies with so-called nonpeptide antagonists of CCK2R revealed that although the amino acid sequence homology of CCK2R in the different species is near 90%, the efficacy of the nonpeptide molecules to stimulate phospholipase C varied from 0 to 60% of the CCK-induced maximal response according to the species and the compound tested (L365,260, L740,093, YM022, PD135158, PD136,450, PD134,308) (39, 47, 48, 227, 232, 255, 256). Incorporation of nonconservative amino acids in the human CCK2R sequence enabled identification of amino acids in transmembrane helices that account for these variations (47, 48). These studies contributed to our understanding of the structural basis by which CCK2R ligands possess agonist activity. Furthermore, they point out that polymorphisms among receptors from different species can cause alterations in the apparent pharmacological profile of a drug.

Another interesting issue, raised by the generation of nonpeptide ligands for peptide-binding GPCRs, was whether or not compounds with similar structures but opposite biological activities share the same binding site and, so, which intrinsic mechanism(s) govern(s) receptor function at the binding site level. A nonpeptide agonist of the CCK1R, SR146,131, was generated starting from the structure of the agonist SR27,897 (44). Pharmacological analysis of CCK1R mutants using these compounds, together with molecular modeling, agreed with the view that the binding sites of the two nonpeptidic ligands and of CCK are largely overlapping. Within these binding sites, a Leu in TM VII interacts with SR146,131 but not SR27897 or L364,718, suggesting that one underlying mechanism of activation by the nonpeptide ligand resides in this additional site of interaction (145, 182).

B. Activation Mechanism and Regulation

Residues located inside the ligand binding site in GPCRs play a key role in the GPCR activation process. Conserved motifs such as the E/DRY (TM III) and NPXXY (TM VII) motifs found in members of family I of GPCRs are critical as mutation in either of these two respective regions in CCK2R yielded a constitutively active or inactive receptor (160, 162). The constitutively active CCK2R mutant bearing a mutation in E/DRY motif (Asp mutated to Ala) was associated with dramatic alterations in cell morphology in which it was expressed and enhanced cell proliferation and invasion. On the other hand, CCK2R bearing a mutation in the NPXXY motif (Asn mutated to Ala) was unable to activate phospholipase C, it still physically coupled to Gαq protein, thereby demonstrating that the Asn of this motif is essential for G protein activation (160). Other residues, which are conserved in GPCRs, were also shown to be important for activation of phospholipase C and adenylyl cyclase by CCK2R and CCK1R. Transmembrane residues of the CCK2R such as Asp of TM II, triple basic motif (KKR) at the COOH terminus of the third intracellular loop, and a Phe residue of TM VI were shown to play a key role in the coupling of CCK2R to phospholipase C (222, 383, 554). Interestingly, mutation of two of these amino acids or motifs, namely, the Phe of TM VI and the KKR motif of the third intracellular loops, were also demonstrated to be without importance for CCK2R-induced stimulation of arachidonic acid release, supporting distinct mechanisms of CCK2R coupling to phospholipase C and phospholipase A2 (383). The chemical nature of residue 121 within TM III of the CCK1R seems to be very important for activation of the receptor. In fact, biological experiments with mutants as well as dynamic simulations of modeled liganded CCK1R support the conclusion that introduction of hydrophobic residues near position 121 determines positioning of the aromatic ring of the Phe of CCK within the binding pocket (145). This was further documented in a study with JMV 180, a partial agonist of the CCK1R, which partly shares its binding site with that of CCK (15). In this study, it was shown that helices III and VI of the CCK1R are functionally linked through the CCK1R agonist binding site and that positioning of the COOH-terminal ends of peptidic agonists towards Phe330 of helix VI is responsible for extent of phospholipase C activation through Gαq coupling (15). The molecular basis for CCK1R coupling to adenylyl cyclase was investigated based on the data showing that CCK1R, but not CCK2R, stimulates cAMP formation. Chimeric receptors constructed and expressed in HEK cells provided evidence that a small peptidic sequence of the first intracellular loop of CCK1R is essential for cAMP signaling (577). The importance of the first intracellular loop for CCK1R coupling to adenylyl cyclase was further supported by substitution of a single residue...
(Ser) in the CCK2R by an Asn, which confers a full cAMP response to CCK and gastrin (575).

CCK receptors, like other GPCRs, were initially thought to trigger intracellular signals as monomeric membrane proteins. Biochemical and biophysical data have recently challenged this concept and provided evidence that many GPCRs, if not all, can form homo-oligomeric and hetero-oligomeric assemblies within the plasma membrane (65, 368). CCK1R may exist as dimers that dissociate under CCK activation (90). A functional impact of CCK1R/H11001 CCK2R heterodimerization was suggested on the basis of the observations that coexpression of CCK1R and CCK2R in CHO cells yields enhanced signaling and cell growth compared with the expression of a single receptor type (89). This finding could explain why transgenic mice expressing CCK2R in the pancreas together with endogenous CCK1R develop preneoplastic lesions and pancreatic cancers. These intriguing data deserve further investigation to establish the pathophysiological relevance of CCKR heterodimerization.

GPCR-mediated internalization is a biological process that contributes both to the agonist response and to its desensitization. Both CCK1R and CCK2R have been shown to undergo internalization in pancreatic acini and transfected NIH 3T3 or CHO cells (177, 418, 522). Interestingly, in the case of CCK1R, agonist and antagonist-induced desensitization of CCK1R were described (417). Under CCK stimulation, the CCK1R is rapidly phosphorylated, mostly in the third intracellular loop, both by protein kinase C and a G protein receptor kinase. There are at least five distinct phosphorylatable amino acids in CCK1R. While exchange of two phosphorylatable amino acids within the third intracellular loop for alanine completely abolishes CCK1R phosphorylation, they do not alter its signaling and internalization (363). A study supports that an internal region of the COOH-terminal tail of the CCK1R which is devoid of phosphorylation sites is important for normal CCK1R trafficking in CHO cells (177). Analysis of the molecular basis for CCK2R internalization using COOH-terminal truncated receptors indicated that phosphorylation sites involved in CCK2R endocytosis are mainly located on the COOH terminus of the receptor (381).

IV. SIGNALING TRANSDUCTION PATHWAYS ACTIVATED BY CHOLECYSTOKININ RECEPTORS

In this section, signaling pathways that account for short- and long-term activation of CCK receptors are described. A summary is depicted in Figure 4.

A. Phospholipases/Ca2+ Mobilization and Protein Kinase C Activation

Typically, the superfamily of GPCRs is capable of inducing a rapid hydrolysis of phosphatidylinositol bisphosphate by phospholipase C (PLC) family members to generate inositol trisphosphate (IP3) and diacylglycerol (DAG), which respectively induce calcium mobilization and stimulate several protein kinase C (PKC) isoforms. In different cell types CCK1 or CCK2 receptors (CCK1R, CCK2R) activate principally PLC-β isoforms, FIG. 4. Schematic description of the signaling pathways known to be activated by CCK1R and CCK2R. In addition to signaling pathways classically activated by G protein-coupled receptors such as the phospholipase C (PLC)–β/diacylglycerol (DAG)/Ca2+/protein kinase C (PKC) cascade or the adenylyl cyclase (AC) pathway, CCK receptors also induce several other signaling pathways known to be activated by tyrosine kinase receptors: 1) the mitogen-activated protein (MAP) kinase pathways including ERK, JNK, and p38-MAPK; 2) the phosphatidylinositol 3-kinase (PI3K) pathway; or 3) the PLC-γ pathway. Activation of nonreceptor tyrosine kinases including Src, JAK2, FAK, and PYK2 is also an early event in CCK receptor signaling. These tyrosine kinases are involved in particular upstream of the MAP kinase or PI3K pathways and in intracellular events related to cell adhesion (see text for details). Finally, CCK2R are also known to induce epidermal growth factor (EGF) receptor transactivation. Arrows correspond to positive stimulations.
likely through heterotrimeric G proteins of the G\textsubscript{q} family as demonstrated by immunoblocking experiments with anti-PLC-\(\beta\) or anti-G\(_{\text{q/\alpha}}\) antisera (341, 371, 379, 588). However, two publications suggested that \(\beta\gamma\) subunits of G proteins might be also involved in PLC activation by CCK1R (587, 590).

PLC-\(\gamma\) isozyme has also been implicated in IP\(_{3}\) formation through CCK2R (582, 583). Recently, a direct association between CCK2R and PLC-\(\gamma\) has been demonstrated implicating the COOH-terminal phospho-Tyr438 of the receptor and the SH2 domains of the PLC-\(\gamma\)1 (19).

IP\(_{3}\) produced by PLC isoforms leads to the subsequent release of calcium from intracellular stores. In numerous cell types naturally expressing endogenous CCK receptors or stably transfected with CCK1R or CCK2R, CCK and gastrin induce calcium mobilization. In particular, calcium signaling in response to CCK has been extensively studied in rat pancreatic acinar cells, which naturally express CCK1R. In this model, the two affinity states of CCK1R generate different patterns of cytosolic calcium elevations. Activation of the high-affinity binding sites with physiological concentrations of CCK generates calcium oscillations resulting from the complex regulation of different intracellular receptors that control calcium mobilization (374). These oscillations are mediated through the activation of IP\(_{3}\) receptors, although weak IP\(_{3}\) production has been observed in response to physiological concentrations of CCK (158, 526). This primary release of calcium from IP\(_{3}\)-sensitive stores may be relayed through calcium mobilization from IP\(_{3}\)-insensitive pools by a mechanism that involves calcium and other types of receptors (551). Among them, two receptors types, the ryanodine receptors and the nicotinic acid adenine dinucleotide phosphate (NAADP) receptors, which are activated by two different calcium mobilizing messengers, cADP-ribose (cADPr) and NAADP, have also been implicated in calcium oscillations evoked by CCK1R (71–75, 525). The cellular distribution of these receptors, mainly apical for the IP\(_{3}\) receptor (346), basolateral for the ryanodine receptor (274), and likely over the whole cell for the NAADP receptor (70) as well as the cooperation that exists between these receptors may explain the spreading of the calcium wave from the apical region throughout the whole cell in response to CCK. Recently, CCK1R has been shown to activate a cytosolic ADP-riboisyl cyclase that may be responsible for the production of the calcium mobilizing messenger cADPr (260, 500).

In rat pancreatic acini, calcium oscillations play a crucial role in enzyme secretion regulated by low doses of CCK (570).

Several signaling molecules modulate calcium oscillations induced by CCK1R. G\(_{\text{q}}\), G\(_{11}\), G\(_{14}\), as well as the \(\beta\) and \(\gamma\) subunits likely released from G\(_{\text{q}}\) family members, play an important role in mediating the oscillatory calcium response (588, 590). The pattern of calcium oscillations is also regulated by the phosphorylation of IP\(_{3}\) receptors in response to physiological doses of CCK through a mechanism dependent on the protein kinase A (PKA) pathway (271, 502). In addition, the high-affinity binding site of CCK1R is coupled to the phospholipase A\(_{2}\)/arachidonic acid cascade that inhibits IP\(_{3}\) and ryanodine receptors (180, 469). Activation of the low-affinity binding sites by high concentrations of CCK generates a very different pattern of calcium mobilization, which consists of a rapid global elevation of intracellular calcium that decreases to a sustained plateau (394). The initial peak of calcium corresponds to IP\(_{3}\) production and the subsequent release of calcium from IP\(_{3}\)-sensitive intracellular stores, whereas the plateau is dependent on extracellular calcium influx. The mechanisms regulating calcium signals in response to CCK2R activation remain poorly understood. Both a rapid mobilization of intracellular calcium that decreases to a sustained plateau and calcium oscillations have been described in numerous cell types naturally expressing endogenous CCK2R as well as stably transfected cell lines (6, 462, 463, 517).

In addition to \(\beta\)- and \(\gamma\)-PLC isoforms, two other phospholipases are activated by the CCK receptors: cytosolic phospholipase A\(_{2}\) (cPLA\(_{2}\)), an enzyme that produces arachidonic acid from membrane phospholipids (166, 180, 266, 469, 474, 531), and phospholipase D (PLD), which induces a sustained diacylglycerol (DAG) production and activates PKCs (7, 54, 180, 414). Whereas the high-affinity binding site of CCK1R activates cPLA2, the low-affinity binding site is coupled to PLD (180, 454).

The PKC family includes three subgroups: conventional PKCs, which are dependent on calcium and DAG (\(\alpha\), \(\beta\), \(\gamma\)); novel PKCs, which show sensitivity to DAG but are calcium independent (\(\delta\), \(\epsilon\), \(\eta\), \(\theta\)); and atypical isoforms that are unresponsive to calcium and DAG (\(\zeta\), \(\lambda\), \(\iota\)).

Numerous studies have shown the involvement of PKCs in both CCK1R and CCK2R signaling using broad-spectrum PKC inhibitors. In particular, mitogen-activated protein kinase pathways are activated by CCK1R or CCK2R through PKC-dependent mechanisms, although the specific isoforms of PKC involved were not identified in these studies (107, 111, 116, 528). More recently, the activation of several PKC isoforms by gastrin and CCK has been reported. In rodent pancreatic acinar cells, low-affinity CCK1R occupancy leads to the stimulation of PKC-\(\alpha\), -\(\delta\), -\(\epsilon\), and -\(\zeta\) (33, 280, 377, 435, 519). In different human gastric tumor cell lines, CCK2R activates PKC-\(\alpha\), -\(\delta\), -\(\epsilon\), and -\(\eta\) (355, 503).

Like conventional and novel PKCs, protein kinase D (PKD, also called PKC-\(\mu\)) and PKD2, which shows a high homology to PKD, are serine/threonine kinases targets of both DAG and phorbol esters. However, their structures and the regulation of their enzymatic activities can be distinguished from the members of the PKC family. Only CCK2R, stably transfected in fibroblasts or human gastric...
cancer cells, induces phosphorylation and activation of PKD and PKD2 through a PKC-dependent mechanism (93, 503, 504).

B. Adenyl Cyclase and cAMP Production

Although both CCK1R and CCK2R activate the PLC pathway via a Gq/11 protein, only CCK1R is also coupled to Gs. In pancreatic acinar cells, CCK induces adenylate cyclase activity and in CHO cells stably transfected with CCK1R, high doses of CCK increase intracellular cAMP by stimulating this enzyme (480, 589). The structural basis for this activation is described in section III. Although some studies have reported the effect of pertussis toxin on signaling pathways activated by CCK receptors (210, 338, 382), coupling to Gi proteins and adenyl cyclase inhibition remain controversial (378). Numerous publications have shown that CCK1R is not coupled to Gi in pancreatic acinar cells (303, 543, 576), and only one publication has reported that CCK2R inhibits adenyl cyclase activity via a mechanism likely to involve Gi (440).

C. Nitric Oxide and cGMP Pathway

Nitric oxide (NO), a molecule produced from L-arginine by NO synthase (NOS), can initiate cGMP-dependent or cGMP-independent signaling pathways. Production of cGMP, through the stimulation of soluble guanylate cyclase by NO, leads principally to the activation of cGMP-dependent protein kinases but can also indirectly activate cAMP-dependent protein kinases by blocking enzymes involved in the degradation of cAMP (147). The NO/cGMP pathway has been shown to be activated by CCK1R in different cellular models. In CHO cells expressing CCK1R, CCK increases NOS activity, NO production, and the intracellular concentration of cGMP. In this cell model, activation of this signaling pathway by CCK1R is linked to cell proliferation (101, 102). Stimulation of the NO/cGMP signaling cascade in response to CCK has also been observed in rodent pancreatic acini and could be involved in pancreatic secretion in vivo. Indeed, nonselective NOS inhibitors as well as deletion of NOS in transgenic mice decrease pancreatic secretion induced by CCK (2, 125). In addition, the gastroprotective role of CCK on gastric mucosal is dependent on this pathway (63, 64, 568).

D. Mitogen-Activated Kinase Cascades

Mitogen-activated protein kinases (MAPKs) are serine/threonine kinases known to be activated by several families of receptors including tyrosine kinase receptors, GPCRs, or cytokines receptors. They include four subgroups: extracellular signal-regulated kinase 1/2 (ERK1/2 also known as p44MAPK and p42MAPK), c-jun NH2-terminal kinases (JNKs), ERK5, and p38 MAPKs. These kinases are implicated in numerous cellular functions such as cell growth, differentiation, survival, and apoptosis.

Several studies using ERK kinases (MEKs) inhibitors have shown the involvement of the ERK1/2 pathway in different cellular processes regulated by CCK receptors. In particular, this signaling cascade controls cell proliferation and migration induced by CCK2R and the transcriptional regulation of gastrin-sensitive genes (208, 353, 496).

In pancreatic acinar cells, the ERK pathway also regulates protein translation induced by CCK1R, a cellular event involved in digestive enzyme synthesis (433).

Activation of ERK1 or ERK2 by growth factor receptors with intrinsic tyrosine kinase activity has been well documented. The best understood mechanism involves the recruitment of the Grb2/Sos or Shc/Grb2/Sos complexes to tyrosine-phosphorylated receptors. These complexes subsequently activate the following cascade: Ras/Raf/ERK-kinases/ERK1/2.

In pancreas-derived AR42J cells, which are known to express endogenous CCK2R or in stably transfected CHO cells, gastrin stimulates the Ras-dependent ERK1/2 pathway via tyrosine phosphorylation of Shc, which enables interaction with the Grb2/Sos complex. This mechanism is PKC dependent, and Src family kinases, also activated by CCK2R, have been identified as the tyrosine kinases mediating gastrin-induced Shc phosphorylation (111, 113, 465). The Ras-dependent MEK/ERK1/2 cascade is also activated by CCK1R in pancreatic acinar cells (138, 139).

In this cell model, CCK1R stimulates the Shc/Grb2/Sos complex through a PKC-dependent mechanism. Although the tyrosine kinase upstream of this cascade has not been clearly identified, PVK2, a tyrosine kinase related to p125-FAK, which is activated by both high- and low-affinity CCK1R, might be involved (107, 520). In addition, it is possible that Src family kinases, also known to be stimulated by the CCK1R in pancreatic acini, could play a role in Shc phosphorylation (533). CCK1R also activates a downstream effector of the ERKs, the 90-kDa ribosomal S6 kinase (p90rsk), known to play an important role in protein synthesis (56). Alternately, ERK1/2 activation by CCK receptors might be mediated by a Ras-independent mechanism involving the direct activation of Raf by PKC (348, 463).

ERK1/2 activation by CCK2R can occur through a very different mechanism in gastrointestinal epithelial cells. This mechanism involves transactivation of the epidermal growth factor (EGF) receptor. In gastric epithelial cells, gastrin induces the expression and processing of proHB-EGF leading to the release of HB-EGF, tyrosine phosphorylation of EGF receptors and the subsequent activation of downstream signaling pathways (327, 475). In contrast, gastrin induces EGF receptor transactivation
in intestinal cells via an intracellular signaling pathway mediated by Src family kinases (191). The transactivation of a receptor tyrosine kinase has never been reported for CCK1R.

Phosphorylation of GPCRs by specific GPCR kinases (GRKs) is a mechanism that contributes to receptor desensitization. It also plays an important role in regulation of MAPK cascades. Particularly, for the β2-adrenergic receptor, serine and threonine phosphorylated residues bind β-arrestins, which serve as adapter proteins in the recruitment of signaling molecules. The resulting complex is internalized with the β2-adrenergic receptor and leads to activation of MAPKs (315). Although CCK receptors are known to be phosphorylated by serine/threonine kinases such as PKCs or GRKs and internalized (164, 177, 363, 381, 393), MAPK stimulation by a β-arrestins-dependent mechanism has never been described for the CCK receptors.

JNK and p38-MAPK were initially identified as two signaling cascades mediating cellular stress induced by exposure to ultraviolet radiation, proinflammatory cytokines, and osmotic shocks. Afterwards, several studies have shown that they are also activated by receptor tyrosine kinases and GPCRs (192). JNK and p38-MAPK cascades are both stimulated by CCK receptors. Activation of these pathways by CCK2R can be blocked by PKCs or Src kinases inhibitors and is involved in the regulation of cell proliferation and survival by gastrin (115, 116).

In rat pancreatic acini, which naturally express CCK1R, supraphysiological concentrations of CCK activate JNK. In this model, CCK-induced JNK activation is mediated through a Ras-dependent mechanism that does not involve PKCs or calcium mobilization (106). Little is known about the role of the JNK pathway in CCKR-mediated effects. However, it has been suggested that JNK activation by high doses of CCK might be a stress response, since these doses also induce pancreatitis (443, 549). In addition, the JNK pathway may be also involved in DNA synthesis stimulated by CCK1R in pancreatic cells (348).

In contrast, in the same cellular model, the p38-MAPK pathway is induced by physiological doses of CCK and mediates actin cytoskeleton reorganization, likely through the phosphorylation of the small heat shock protein Hsp27 (442, 550).

In addition to ERK1/2, JNK, and p38-MAPK, the ERK5 pathway is activated by gastrin in the intestinal cell line RIE-1 transfected with CCK2R. In this cell line, ERK5 may participate in the activation of the transcription factor NFκB and the regulation of COX-2 downstream of CCK2R (191).

E. Phosphatidylinositol 3-Kinase

Class I phosphatidylinositol (PI) 3-kinases are a family of lipid kinases that play a central role in numerous cellular processes including cell proliferation and survival, protein synthesis, motility, and adhesion. These lipid kinases phosphorylate the D3 position of the inositol ring on phosphatidylinositols which serve as intracellular second messengers and recruits pleckstrin homology (PH) domain-containing proteins, such as AKT, to the plasma membrane. These kinases are heterodimers composed of the p110 catalytic subunit constitutively associated with the p85 adaptor/regulatory subunit.

Class I PI 3-kinases are subdivided into class IA and class IB. The PI-3-kinase γ, belonging to class IB, is mainly activated by GPCRs. Recently, using pancreatic acini isolated from mice deficient for the catalytic subunit of PI 3-kinase γ, Gukovsky et al. (188) have shown the role of this PI 3-kinase isoform in several intracellular events induced by supramaximal concentrations of CCK including calcium mobilization, calcium influx, and activation of NFκB and trypsinogen (188). All these events may play an important role in acute pancreatitis induced by supraphysiological doses of CCK. However, other PI 3-kinase isoforms may be activated by CCK1R.

In pancreatic acinar cells, the PI 3-kinases also play an important role in protein synthesis activated by low doses of CCK. In this model, Bragado et al. (57, 58) demonstrated that activation by CCK1R of two components of the translational machinery, p70S6K and the initiation factor eIF4E, which is involved in cap-dependent translation, can be blocked by PI 3-kinase inhibitors (57, 58). However, the isoform involved in this process remains to be identified. Mechanisms leading to PI 3-kinase activation by CCK1R are poorly understood. They might involve Src family kinases. Indeed, immunoprecipitation studies in pancreatic acini have shown that CCK induces an association between Src and PI 3-kinase (240).

Class IA PI 3-kinases are known to be activated by receptor tyrosine kinases (RTKs) through at least two different mechanisms. First, the SH2 domains of p85 can bind directly to specific phosphotyrosine-containing sequences on tyrosine kinase receptors. Conformational changes in the p85/p110 complex following recruitment by the receptor and the proximity to lipid substrates lead to kinase activation. Another mechanism has been described for insulin and insulin-like growth factor (IGF)-I receptors, in which receptor autophosphorylation following ligand stimulation permits the binding of scaffold proteins called insulin receptor substrates (IRS). Subsequent phosphorylation of IRS proteins by the receptor recruits the p85/p110 PI 3-kinase and leads to its activation.

This class of PI 3-kinase has been reported to be activated in response to gastrin in different cell lines expressing endogenous CCK2R as well as stable transfected (43, 113, 149, 258). The molecular mechanism involves Src phosphorylation of the adaptor protein IRS-1.
on tyrosine residues, which serve as binding sites that recruit and activate p85/p110 PI 3-kinase complex (113, 259). The downstream effector of p85/p110 PI 3-kinase, AKT (also known as PKB), has also been identified to play a role in CCK2R signaling and is rapidly activated by phosphorylation in response to gastrin. The PI 3-kinase/AKT pathway is involved in the proliferative and anti-apoptotic action of CCK2R as well as the regulation of cell adhesion and migration mediated by this receptor (43, 149, 198, 527). Another role of this pathway in CCK2R signaling is to control protein synthesis by regulating components of the translational machinery. In particular, CCK2R activates p70S6K and the initiation factor eIF4E (119, 392, 466).

F. Focal Adhesion Kinase and Associated Proteins

p125-focal adhesion kinase (FAK) is a cytoplasmic protein tyrosine kinase, localized to focal adhesion sites, that controls multiple intracellular signaling pathways involved in cell morphology, cell motility, and invasion. This protein is activated by numerous membrane receptors including integrins, RTKs, and GPCRs. In many cell types, p125-FAK phosphorylation leads to the recruitment of Src kinases and the formation of an activated p125-FAK/Src complex. This complex associates and phosphorylates integrin-associated proteins such as paxillin and talin, as well as adaptor proteins such as Shc and p130Cas (446). Phosphorylation of p125-FAK, p130Cas, and paxillin have been reported for both CCK1R and CCK2R in numerous cell models. These phosphorylations are regulated by the small GTPase Rho that also plays an important role in stress fiber formation and modulation of cell morphology observed in response to activation of CCK receptors (163, 239, 365, 463, 517, 518, 586).

Formation of an activated p125-FAK/Src complex has also been observed following CCK2R activation, and phosphorylation of p130Cas by gastrin has been shown to be Src dependent (112, 115).

PYK2 is also a nonreceptor focal adhesion tyrosine kinase closely related to p125-FAK. In pancreatic acini, CCK, through CCK1R, induces the phosphorylation and the activation of PYK2 by a calcium- and PKC-dependent mechanism. Once phosphorylated, PYK2 can associate with Grb2 or a p130Cas-associated protein, CrkII, leading to the activation of downstream signals (520).

G. The JAK/STAT Pathway

Janus kinases (JAKs) are a family of nonreceptor tyrosine kinases which includes four members: the ubiquitously expressed JAK1, JAK2, TYK2, and JAK3 which is found in hematopoietic cells. They are known to phosphorylate and activate the STAT family of transcription factors (signal transducers and activators of transcription). Once phosphorylated, STAT proteins dimerize and translocate to the nucleus where they bind to target genes. The JAK-STAT signaling pathway that is well known to be activated by cytokines and growth factor receptors is involved in a wide variety of cellular processes including immune response, differentiation, cell survival, proliferation, and oncogenesis. The mechanism of JAK activation by cytokine receptors has been elucidated. Ligand binding induces dimerization of the receptors and a transphosphorylation of the associated JAK tyrosine kinases. Activated JAKs in turn phosphorylate the receptor that recruits the STAT proteins. To date, very few GPCRs have been shown to be connected to the JAK/STAT pathway. Recently, CCK2R has been shown to activate the JAK2/STAT3 pathway in different cell lines in vitro and in vivo, such as in transgenic mice expressing the receptor in pancreatic acini (148, 149). The mechanism of JAK2 activation involves Goq proteins and requires the NPXXY motif, located at the end of the seventh TM domain of CCK2R. This motif, which is critical for Gq-dependent signaling pathways, is also required for STAT3 activation by CCK2R. This signaling pathway participates in CCK2R-mediated growth effects. JAK2 could also be involved in gastrin-induced modulation of cell-cell adhesion.

H. Other Signaling Molecules

1. Small GTPases

The small G protein superfamily, which includes at least five subfamilies (Ras, Rho/Rac/Cdc42, Rab, Arf/Sar1, and Ran), plays a key role upstream of numerous signaling cascades and regulates a large number of cellular processes.

CCK2R stimulates the small GTPase Ras upstream of the ERK1/2 and PI 3-kinase/AKT pathways, mediating the proliferative and antiapoptotic action of gastrin (495).

Ras is also activated by CCK1R, in pancreatic acini. This activation does not appear to be involved in pancreatic enzyme secretion stimulated by CCK but could lead to a stimulation of DNA synthesis through a mechanism independent of the ERK1/2 pathway (139, 348).

Among the Rho family members, Rho, Rac, and Cdc42 have been reported to be activated by CCK2R. Rho and Cdc42 appear to regulate the PI 3-kinase pathway and the proliferative effects mediated by this receptor (495). In addition to their role in regulating cell proliferation, Rho and Rac are able to switch on signaling pathways involved in amylase secretion evoked by CCK1R (41, 354) and stress fiber formation and morphological changes induced by both CCK receptors (see sect. IV).

In numerous cell systems, Rho can be activated through the α-subunits of the heterotrimeric G proteins,
G₁₂ and G₁₃. In intestinal smooth muscle cells, high concentrations of CCK activate G₁₂, G₁₃, and RhoA (342). More recently, it has been reported in NIH3T3 cells transfected with CCK1R that occupation of the low-affinity binding site of the receptor leads to Rho activation and cytoskeletal remodeling mainly through a G₁₃-dependent mechanism (276). In addition to the Ras and Rho families, several members of the Rab family have been implicated in CCK1R signaling. Although the precise mechanisms are not known, a role for Rab3D, Rab4, Rab11, and Rab27b in regulating CCK-induced acinar exocytosis has been reported by several groups (87, 88, 212).

2. NFκB/IκB

Supramaximal concentrations of CCK, known to induce pancreatitis in rats via CCK1R, activate NFκB, a transcription factor that regulates inflammatory processes. In most cells, NFκB is sequestered in the cytoplasm through interactions with the IκB (inhibitor of NFκB) family of inhibitory proteins. The mechanism leading to CCK induction of NFκB involves the activation of an IκB kinase. Phosphorylation of IκB and its subsequent ubiquitination and degradation by proteasome activity dissociates NFκB from IκB. Nuclear translocation of NFκB leads to transcription of target genes such as the mob-1 chemokine. Several signaling pathways triggered by CCK1R are upstream NFκB activation, including calcium mobilization, the novel PKCs δ and ε, and PI3-kinase γ (197, 435, 514).

In gastric epithelial cells, CCK2R also activates NFκB, leading to expression of proinflammatory genes such as interleukin (IL)-8 (206).

V. TARGET GENES OF CHOLECYSTOKININ RECEPTORS

A. Gastrin-Dependent Gene Regulation

Gastrin is known to regulate gastric acid secretion by acting on enterochromaffin-like (ECL) cells that control synthesis and secretion of histamine. This process requires expression of three target genes regulated by gastrin and CCK2R in ECL cells: 1) histidine decarboxylase (HDC), the rate-limiting enzyme for histamine biosynthesis; 2) vesicular monoamine transporter 2 (VMAT2), involved in histamine accumulation into secretory vesicles; and 3) chromogranin A (CgA), which plays a role in vesicle stability and propeptide processing (208). In gastric epithelial cells, CgA expression is regulated by gastrin via the binding of three transcription factors, SP1, CREB and Egr-1, to a GC-rich element of the promoter. In the VMAT2 gene promoter, two sequences are involved in gastrin-mediated effects: a cAMP responsive element (CRE) that binds the transcription factor CREB and an overlapping AP2/SP1 site regulated by an uncharacterized nuclear protein. A gastrin-responsive element has also been identified in the HDC gene promoter that allows the association of two uncharacterized nuclear factors, gastrin responsive element binding proteins 1 and 2. In gastric epithelial cells, gastrin likely stimulates the expression of these three target genes through the activation of a Raf/MEK/ERK1/2 cascade in a ras-independent mechanism involving the direct activation of Raf by PKCs (208).

In glucagon-producing pancreatic cells, CCK2R also regulates glucagon gene expression via activation of the ERK1/2 pathway and binding of the transcription factor Egr-1 to the islet-specific G4 element present in the proximal glucagon promoter (277).

In addition to secretion, CCK2R is known to regulate cell proliferation by stimulating the expression of early response genes and other growth-related genes. In the tumor pancreatic cell line AR4–2J, in ECL cells, and in fibroblasts, c-Fos expression has been shown to be regulated by CCK2R. In particular, the CA-rich G box of the serum response element (SRE) has been reported to play a crucial role in gastrin-induced c-Fos promoter activation. However, maximal activation of the SRE by gastrin also requires the binding of ternary complex factors (TCFs) such as Elk1 and SAP1a to an E26 transformation specific (Ets) motif. This activation is PKC dependent and mediated by the ERK pathway. In addition, specific activation of the CA rich box by CCK2R involves the small G protein RhoA (496, 499). One publication also mentions the importance of the CRE promoter element in gastrin-induced c-Fos expression that might cooperate with the two other binding sites (523). In gastrointestinal cells, CCK2R was also described to induce the expression of the protooncogenes c-Jun and c-Myc, although the mechanisms involved are unknown (517, 556).

Cyclins, that control the G₂/S transition during the cell cycle, play a crucial role in cell proliferation. Several studies have shown an increase in the transcription of cyclin D1, D3, and E in response to gastrin (388, 594). The mechanism by which gastrin induces cyclin D1 transcription has been studied in a model of gastric tumor cells expressing CCK2R. The CRE site of the cyclin D1 promoter was shown to predominantly mediate cyclin D1 induction by gastrin via the binding of two transcription factors CREB and β-catenin (388).

Proteins of the Reg family have been recognized as novel growth factors whose expression is increased under cellular stress, during inflammatory processes and tumor development. Reg-1 gene expression in response to gastrin has been reported in ECL cells and may be involved in gastric mucosal cell growth (20, 156). A C-rich region has been identified in the Reg-1 promoter sequence that binds transcription factors of the Sp-family, SP1 and SP3, in response to gastrin and mediates the effects of
CCK2R activation. This transcriptional regulation of Reg-1 involves, in gastric epithelial cells, the activation of PKCs and the small G protein RhoA (20). In the same cells, CCK2R also increases the expression of plasminogen activator inhibitor-2 (PAI-2), known to be upregulated in gastric cancers. The binding of CREB to a CRE and of c-jun to an AP-1 site have been shown to be responsible for PAI-2 induction by gastrin. As for c-Fos gene expression, the transcriptional regulation of PAI-2 by CCK2R is mediated by Rho, PKCs, and the ERK1/2 pathway (539).

In gastric epithelial cells, the growth stimulatory effect of CCK2R could be partially mediated through the expression of HB-EGF, release of this factor, and stimulation of the EGF receptor. In these cells, the mechanism by which CCK2R induces HB-EGF gene transcription involves activation of PKCs and the EGF receptor and requires a GC-rich region of the promoter (475).

In several cellular models such as gastric and colonic cancer cells, intestinal epithelial cells and fibroblasts transfected with the CCK2R, gastrin has been shown to enhance cyclooxygenase-2 (COX-2) gene expression (100, 191, 482). This key enzyme of prostaglandin synthesis is known to play an important role in inflammation processes and carcinogenesis. In particular, COX-2 has been involved in hyperproliferation, transformation, invasion, and angiogenesis. In colon cancer cells, the ERK1/2 and PI 3-kinase pathways are involved in gastrin-induced COX-2 expression (100), whereas in intestinal cells, multiple MAPK cascades, including ERK1/2, ERK5, JNK, and P38-MAPK, contribute to the increase of COX-2 transcription regulated by gastrin (191). Two cis-activating consensus sequences, AP-1 and MEF2, have been identified within the COX-2 promoter, as responsible for CCK2R-mediated COX-2 expression.

In gastric tumor cells expressing CCK2R, gastrin also regulates the expression of genes associated with cell migration and invasion such as MMP9, a matrix metalloproteinase. This induction requires a pathway including PKCs/Raf/MEK/ERK cascade.

However, gastrin has also been reported to stimulate genes that might counterbalance its proliferative action in some circumstances. In particular, the trefoil factor TFF1 has been identified as a gastrin responsive gene in gastric cells. This factor known to participate in the repair of gastrointestinal mucosa by stimulating cell migration might be involved in the inhibition of cell proliferation as well. A GC-rich region of the TFF1 promoter has been identified as a gastrin-responsive element that binds the transcription factors SP3 and MAZ (238). As seen with genes involved in gastric acid secretion, CCK2R stimulates the expression of TFF1 through the Ras-independent activation of a Raf/MEK/ERK cascade.

B. CCK-Dependent Gene Regulation

In contrast to gastrin and CCK2R, very little information is available on gene regulation by CCK and CCK1R. Intravenous infusion of CCK in the rat increases the expression of pancreatic digestive enzymes such as amylase, chymotrypsinogen B, and trypsinogen I (423). In the same model, CCK also stimulates pancreatic ornithine decarboxylase (ODC) expression, a key enzyme in polyamine biosynthesis, that is potentially involved in pancreatic growth (422). In pancreatic acini, CCK1R has also been shown to induce the expression of immediate early genes including c-fos, c-jun, and c-myc (292). More recently, in the same cell model, CCK1R has been reported to regulate mob-1 chemokine gene expression by activating NFκB through a mechanism dependent on PKCs and intracellular calcium (196, 197).

VI. TISSUE DISTRIBUTION AND PHYSIOLOGICAL ACTIONS OF CHOLECYSTOKININ RECEPTORS IN THE GASTROINTESTINAL TRACT AND OTHER PERIPHERAL ORGANS

All data concerning the expression of CCK receptors and their function in normal (nonpathological) tissues and organs are summarized in Table 2.

A. Gastric Mucosae, Exocrine and Endocrine Pancreas

1. Stomach

The stomach is one of the main targets of gastrin, and several of the physiological functions of the hormone have been well characterized in this organ. Gastrin is produced in gastric endocrine G cells and has been initially identified as the key stimulus of gastric acid secretion by Edkins (142, 143). In addition to the control of gastric secretion, recent observations in mice overexpressing gastrin or in which the gastrin gene has been deleted demonstrate that the hormone has important roles in the control of proliferation, differentiation, and maturation of the different cell types of the stomach (reviewed below). As a matter of fact, CCK2R that is expressed in the stomach is an important part of the system that regulates functions of the gastric epithelium. CCK is also involved and appears to be a negative regulator through binding to CCK1R, also expressed in the stomach.

Mapping of the receptors in human and dog gastric tissues has shown that CCK2R is predominantly expressed in the mid glandular region of the fundic mucosa, corresponding to the location of parietal cells. CCK1R is
<table>
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<th>Tissue/Organ</th>
<th>CCK1R RNA</th>
<th>CCK1R Protein</th>
<th>CCK1R Function</th>
<th>CCK2R RNA</th>
<th>CCK2R Protein</th>
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<td>Guinea pig (IHC) (521)</td>
<td>Trophicity, proliferation of gastric mucosa</td>
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<td>Dog, human, rat (RA) (410)</td>
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<td>Human (IHC) (261, 451)</td>
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<td>Rat (binding) (217)</td>
<td>Enzyme secretion</td>
<td>Human (RT-PCR) (96, 226)</td>
<td>Guinea pig (binding) (153)</td>
<td>Glucagon secretion</td>
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<td>Mouse (binding) (572)</td>
<td>Trophicity and proliferation</td>
<td>Human (Northern blot) (275, 380)</td>
<td>Calf (binding) (272)</td>
<td>Development, beta cell neogenesis</td>
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<td>Calf (binding) (272)</td>
<td>Insulin, somatostatin, pancreatic polypeptide secretion</td>
<td>Human (ISH) (409)</td>
<td>Rat cholangiocyte (RT-PCR) (174)</td>
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<tr>
<td>Liver</td>
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<td>Not determined</td>
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<td>Rat (RT-PCR) (297)</td>
<td>Leptin gene regulation</td>
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<td>Bovine (binding) (494)</td>
<td>Contraction of gallbladder</td>
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<tr>
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<td>Hamster (RA) (14)</td>
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<tr>
<td>Stomach</td>
<td>Guinea pig (cDNA cloning) (123)</td>
<td>Human (RA) (410)</td>
<td>Gastric motility and emptying</td>
<td>Guinea pig (cDNA cloning) (123)</td>
<td>Guinea pig (binding) (340)</td>
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<td>Rat pyloric sphincter (RA) (487)</td>
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<td>Bowel</td>
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<td>Human (RA) (402, 412)</td>
<td>Bowel motility</td>
<td>Not determined</td>
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<td>Adipocytes</td>
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<td>Rat (RA) (297)</td>
<td>Aldosterone secretion</td>
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Physiol Rev • VOL 86 • JULY 2006 • www.prv.org
mostly found in the basal region of the fundic mucosa where chief cells are located (299, 410). In several rat studies, treatment with CCK2R antagonists caused changes in ECL cell activity, indicating the presence of the receptor on these cells (131, 195). Binding and functional studies demonstrated that both CCK1R and CCK2R are present on guinea pig chief cells (91, 389). In addition to detection on parietal and chief cells in the guinea pig stomach, CCK2R was also detected on rare unidentified endocrine cells that were negative for gastrin, histamine, secretin, enkephalin, and gastrin-releasing peptide (521). Precise cellular localization of human stomach CCK2R by in situ RT-PCR and immunohistochemistry demonstrated expression in gastric parietal, ECL, and putative precursor cells (261, 451). Expression of CCK1R was visualized in neuroendocrine D cells in antral mucosa and in nonparietal cells of the oxyntic mucosa, most likely chief cells, mucous cells, and undifferentiated precursor cells of the stem cell compartment of the oxyntic gland (451). Presence of CCK1R and CCK2R in putative precursor cells supports the model that depending on the cell lineage, expression of one or either or even both receptors is switched off.

The cellular distribution of CCK2R within the acid-producing mucosa is in agreement with the major role of gastrin in the regulation of gastric acid secretion. However, the question of whether the parietal cell CCK2R is linked to acid secretion has been a matter of debate (289, 391, 552). A number of physiological studies have demonstrated the predominant role of histamine released from ECL cells on acid secretion (30). Pharmacological studies using histamine-2 blockers support the concept that gastrin stimulates acid secretion via the release of histamine from ECL cells (134, 203). The importance of gastrin was confirmed by studies performed on gastrin or CCK2R-deficient animals that exhibit similar abnormalities in the stomach with reduced acid secretion and elevated basal gastric pH (155, 248, 265, 344). The significance of gastrin was corroborated by partial restoration of gastric secretion following gastrin infusion in gastrin-deficient mice (155). Actually, a gastrin-ECL cell-parietal cell axis is generally proposed for the control of gastric acid secretion. Nevertheless, morphological data showing expression of CCK2R on parietal cells support a contribution of gastrin to the acid secretory response by direct activation of parietal cells (450). However, the population of parietal cells appears to be heterogeneous with regard to CCK2R presence, thus suggesting that direct stimulation of parietal cells is a less efficient stimulus than the gastrin-mediated release of histamine from ECL cells.

In addition to its well-known effect on acid secretion, gastrin has for many years been recognized for its trophic effects in the stomach (229). Targeted disruption of the gene for CCK2R or gastrin in mice demonstrated the importance of gastrin and the CCK2R in maintaining the normal function, cellular composition, and organization of the gastric mucosa. In addition to reduced acid secretion, these mutants exhibit marked atrophy of the gastric mucosa, a decrease in the number of parietal and ECL cells, and an increase in the number of mucous neck cells (84, 248, 265, 344). These studies showed an essential role of CCK2R and gastrin not only in proliferation but also in the regulation of ECL and parietal cell differentiation (84, 246). First, parietal cells are present in gastrin-deficient

<table>
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<tr>
<th>Tissue/Organ</th>
<th>CCK1R RNA</th>
<th>CCK1R Protein</th>
<th>CCK1R Function</th>
<th>CCK2R RNA</th>
<th>CCK2R Protein</th>
<th>CCK2R Function</th>
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</thead>
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<tr>
<td>Blood cells</td>
<td>Human mononuclear cells (RT-PCR) (453)</td>
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<td>Unresolved</td>
<td>Jurkat cells (binding) (135, 285)</td>
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<td>Antiproliferative effects, unclear</td>
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<td>Kidney</td>
<td>Human, rat (RT-PCR) (320)</td>
<td></td>
<td>Unresolved</td>
<td>Guinea pig (Northern blot) (121)</td>
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<td>Potassium and sodium absorption</td>
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<td>Mouse (RT-PCR) (270)</td>
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<td>Vagal afferent fibers</td>
<td>Human, rat (RT-PCR, ISH) (62, 336)</td>
<td>Rabbit (binding) (287)</td>
<td>Satiety</td>
<td>Human, rat (RT-PCR, ISH) (62, 336)</td>
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<td>Pancreatic enzyme secretion</td>
<td>Rat (IHC) (336)</td>
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<td>Leptin secretion</td>
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GI, gastrointestinal; IHC, immunohistochemistry; ISH, in situ hybridization; RT-PCR, reverse transcription-polymerase chain reaction; IS RT-PCR, in situ reverse transcription-polymerase chain reaction; RA, receptor autoradiography; RNase PA, ribonuclease protection assay.
cells that direct histamine synthesis and storage (see sect. 5.5.3). Gastrin also regulates several genes in ECL and in extreme cases the development of ECL carcinoid (23). In hypergastrinemia, there is ECL cell hyperplasia, as it is known to stimulate proliferation of ECL cells in mice, gastrin is an important regulator of ECL cell number, as it is known to stimulate proliferation of ECL cells (23). In hypergastrinemia, there is ECL cell hyperplasia and in extreme cases the development of ECL carcinoid tumors (53). Gastrin also regulates several genes in ECL cells that direct histamine synthesis and storage (see sect. A) (126–129). Moreover, CCK2R blockade with antagonists was linked to changes in ECL cells morphology, indicating that CCK2R activity is needed to maintain the shape, size, and activity of ECL cells (86). It is noteworthy that gastrin does not act as a trophic agent in the rat stomach before weaning (45). In vivo and in vitro data also suggested that CCK2R, in regenerative rat gastric oxyntic mucosa, enhances trophic effects during gastric wound healing (448). Finally, while it is clear that CCK2R is expressed by parietal and ECL cells, the presence of this receptor on the gastric proliferating progenitor cell population remains to be confirmed, and gastrin is actually considered to act indirectly via the release of growth factors such as HB-EGF and Reg (20, 327, 540). Moreover, since the proliferation rates are similar in wild-type and gastrin-deficient mice, other factors must maintain a proliferative balance in the absence of gastrin (248).

CCK acting on CCK1R in D cells inhibits the functions of parietal cells, ECL cells, and gastrin-producing cells via the release of somatostatin. This inhibitory role of CCK is now confirmed in the adult calf exocrine pancreas, where 4% of CCK binding sites are CCK2R (585). Predominance of CCK2R, compared with CCK1R, was observed in the antropyloric region and stimulates acid secretion via the release of histamine from ECL cells. Gastrin stimulates proliferation and differentiation of ECL and parietal cells as well as migration of parietal cells either directly or via paracrine cascades downstream of the CCK2R (induction of HB-EGF, TGF-α, and Reg1α). CCK acting at CCK1R on D cells inhibits parietal cell, ECL cell, and gastrin-producing cell functions via the release of somatostatin from endocrine D cells. CCK1R expressed on chief cells mediates gastric leptin secretion.

2. Exocrine pancreas

A major physiological function of CCK is the stimulation of pancreatic enzyme secretion. This effect has been widely studied in rodents where pancreatic acinar CCK1R was first characterized in the 1980s (95, 120, 224, 415, 432). Functional, pharmacological, structural, and biochemical characterization as well as cloning of CCK1R were performed with rat pancreas. However, several reports suggested variations in the type of CCK receptor expressed, with the presence and even the predominance of CCK2R in the exocrine pancreas. Indeed, a few CCK2R, representing ~20% of the total CCK binding sites, were characterized in the exocrine pancreas of dog, but their presence has not been linked to any physiological event (153). A similar situation was found in guinea pig pancreas where 4% of CCK binding sites are CCK2R (585). Predominance of CCK2R, compared with CCK1R, was demonstrated in the adult calf exocrine pancreas, whereas CCK1R is almost exclusively present during the
physiological functions of the human exocrine pancreas remains unanswered, possibly due to the fact that studies using RT-PCR from pancreas homogenates or binding studies performed on membranes may be contaminated by nonexocrine tissues. However, the presence of CCK2R, identified by receptor autoradiography in the acini of chronic pancreatitis, indicates that human acini have the potential to express CCK2R (409). On the other hand, recent data suggesting that CCK2R is involved in the regulation of pancreatic blood flow and vascular conductance might warrant further localization investigations with regard to human pancreas (185).

As in the rodent pancreas, CCK is the major secretagogue of human exocrine pancreas, and most pharmacological data support that pancreatic CCK-mediated exocrine secretion relies upon activation of CCK1R (78). The participation of acinar CCK1R in human pancreatic secretion has been investigated, but direct functional responses to CCK or gastrin were never characterized (226, 323). In fact, the vagovagal reflex plays an important role in the mediation of pancreatic secretion evoked by CCK. CCK1R is expressed on abdominal branches of the vagus nerve in several species (see sect. viC). These findings, together with experimental evidence, suggest that the major effects of CCK on pancreatic secretion are mediated by receptors on vagus nerve, even in species that bear CCK1R on pancreatic acinar cells (362). When present on pancreatic acini, CCK1R appears to play a minor role in mediating pancreatic secretion. Indeed, earlier canine studies showed inhibition of pancreatic enzyme secretion in response to low doses of CCK by atropine (252). Li and Owyang (283) demonstrated that atropine and hexamethonium abolished the pancreatic enzyme response to physiological doses, but not supra-physiological doses of CCK in rats, thus suggesting that CCK acts on a presynaptic site along the cholinergic pathway. Similar effects were obtained using perivagal pretreatment with capsaicin or gastroduodenal application of capsaicin or transection of afferent nerves. This gives strong evidence for a neuronal action of endogenous CCK, via an afferent vagal pathway originating in the duodenal mucosa, on pancreatic secretion. High-affinity CCK1R binding sites on the vagus nerve were demonstrated to mediate CCK-stimulated pancreatic secretion in the rat (281). At pharmacological doses, CCK can act directly on acinar cells when CCK1R is present. The neural circuitries involving CCK1R of capsaicin-sensitive vagal afferent neurons, central synapses, and vagal cholinergic efferent fibers (represented in Fig. 6) were recently confirmed by Yamamoto et al. (580). Although most of the information on the vagal CCK1R is obtained from research in animals, observations were reported in humans suggesting that CCK stimulates the pancreas via a pathway dependent on cholinergic innervation (35, 493).

In addition to effects on secretion, CCK exerts trophic and proliferative effects in the pancreas. The essential contribution of CCK1R for pancreatic regeneration following pancreatectomy or pancreatic duct ligation has been demonstrated in OLETF rats as well as in a congenic rat carrying a CCK1R null allele (321, 322, 332, 333). The importance of CCK for normal pancreatic growth has also been reported in this rat strain or in control rats after...
injection of agonists or antagonists (118, 150, 319, 387). Conversely, CCK- and CCK1R-deficient mice demonstrate that CCK is not a required growth factor for the murine pancreas (263, 513), nor is CCK1R required for the growth of the guinea pig pancreas (202).

3. Endocrine pancreas

For decades, research with several different species has demonstrated that CCK regulates pancreatic hormone secretion. CCK binding sites have been found in rat islet insulin, glucagon, and blood vessel cells (428). While this study supported the concept of CCK regulation of endocrine pancreas, information about the type of CCK receptor and the nature of the endocrine cell was lacking. Studies using morphological methods, particularly immunofluorescence and confocal microscopy, are scarce and have only recently been performed, yet these studies still result in controversial data. CCK1R transcripts were detected in the center of rat islets, a location that corresponds to the distribution of the insulin cells (236). With the use of immunohistochemistry, CCK1R was detected in glucagon cells in pig pancreas (458). Immunofluorescence and confocal microscopy studies have confirmed the localization of CCK1R in both insulin and glucagon cells in the rat and in glucagon cells in pig and human endocrine pancreas (230, 337). Recent immunofluorescence data combined with in situ hybridization studies support that CCK1R is expressed in both insulin and glucagon cells in rat pancreas. Results of studies aimed at defining the localization of CCK2R are still a matter of debate. Glucagon cells were reported to be the major site of CCK2R expression in adult and fetal human pancreas (426). Other data localized CCK2R in the calf, horse, pig, rat, dog, and human pancreas in somatostatin cells (337). The reason for such discrepancy is at present still unknown and may be due to the specificity of the antibodies that were used. Although their specificity was tested against the peptide used to raise them, a comparative localization test on pancreas from knockout and wild-type mice would be the most appropriate and unequivocal confirmation. Noteworthy, the presence of CCK2R in human islets cells was also recently confirmed by in situ hybridization and in vitro receptor autoradiography using CCK1R- and CCK2R-selective analogs (409). However, in the latter study, the islet cell type expressing CCK2R was not identified, and CCK1R was found in pancreatic nerves.

A number of studies have indicated that CCK plays a role in blood glucose regulation through stimulating insulin secretion as demonstrated in vivo in dogs, sheep, pigs, rats, and mice, as well as in vitro in the rat perfused pancreas and in rat isolated islets (4, 186, 234, 235, 316, 401, 431, 506, 508, 535, 541, 542). In humans, the ability of CCK to stimulate insulin secretion is controversial (5, 141, 151, 249, 284, 424). On the other hand, while CCK might not be a physiological incretin in humans, its pharmacological insulinotropic action might be useful in the treatment of diabetes (3, 34, 349, 457).

The role of CCK1R in mediating insulin release was either assessed using CCK receptor antagonists or confirmed in the genetically diabetic Otsuka Long-Evans Tokushima fatty (OLETF) rats that lack CCK1R (234, 235, 316, 508). In addition to stimulation of insulin release, CCK1R have been shown to be involved in the release of somatostatin and pancreatic polypeptide release in humans (249, 284). In line with the previously suggested role of CCK2R in the modulation of pancreatic endocrine function, expression of CCK2R on human pancreatic glucagon cells was linked to a physiological secretion of glucagon from isolated human islets (4, 401, 426). The role of gastrin in the normal islet glucagon counterregulatory response to hypoglycemia has been recently confirmed in gastrin knockout mice (55).

Gastrin and CCK2R might be important for pancreatic development as well. In mammals, the major site of expression of amidated gastrin is in the fetal pancreas, supporting a specific role for this receptor during the development of this organ (29, 426). Moreover, demonstration that gastrin stimulates glucagon gene expression opens new, interesting prospects concerning its contribution to pancreatic development, as glucagon is the earliest peptide hormone that is expressed in the developing pancreas (277). The fact that glucagon might play a role in the early phase of insulin cell differentiation suggests that gastrin could actively contribute to the paracrine induction of differentiation of pancreatic cells (390). Several studies examining whether gastrin also acts as a beta-cell proliferative/differentiative factor in the adult pancreas demonstrated a role for gastrin in islet neogenesis. Indeed, gastrin is expressed with its receptor during this regenerative process. Hypergastrinemia leads to expansion of islet mass in rat or human pancreas, and combined therapy with EGF and gastrin stimulates functional beta-cell neogenesis of human, rat, and mice pancreas (27, 59, 419–421, 505, 557).

4. Liver and intestine

Although the expression of CCK2R mRNA was reported and related to modulation of rat cholangiocyte function by gastrin, there is until now no other evidence for the presence of CCK receptors in the liver (174, 270, 546). The role of CCK as a stimulant for small bowel and colon growth is unclear. The majority of reports suggest that the normal colon does not appear to express CCK2R, with the exception of detection by RT-PCR in the mouse colon (270). To date, there is also insufficient evidence for the presence of CCK2R in the small intestine.
B. Gastrointestinal Smooth Muscles

Not only do CCK receptors have a physiological role in the stimulation of secretion, growth, and differentiation, but they also participate in the regulation of gastrointestinal motility via mechanical actions. These motor effects include postprandial inhibition of gastric emptying, gallbladder contraction, and inhibition of colonic transit, all of which are related to the expression of CCK1R and CCK2R in smooth muscles.

1. Gallbladder

The gallbladder was the first recognized target of CCK. CCK induces gallbladder contraction via activation of CCK1R located on smooth muscle cells. Presence of CCK1R on the muscularis layer of bovine gallbladder, where binding sites are absent on both the mucosal and serosal membranes, demonstrated a direct myogenic effect of the hormone for the contraction of the gallbladder (494). This localization was further confirmed by autoradiographic studies in several species including human (14, 359, 445, 529, 548). The role of CCK1R in gallbladder contraction was recently confirmed in knockout mice lacking the CCK1R gene (507). It should be noted that several studies have clearly demonstrated that CCK also acts on gallbladder emptying through the cholinergic pathway, further demonstrated by the finding that the contractile effect of CCK is inhibited by atropine (189, 584). Physiological plasma levels of CCK were shown to act via stimulation of presynaptic cholinergic neurons in a vagally mediated pathway (509). The same study reports direct activation of CCK receptors on the smooth muscle at supraphysiological levels of CCK. A dual control of gallbladder contraction was demonstrated through activation of CCK1R in the vagal pathway and stimulation of gallbladder smooth muscle contraction (492).

2. Stomach

CCK1R and CCK2R have been identified by receptor autoradiography on the human gastric muscle tissue, which predominantly expresses CCK1R (410). In contrast to the canine stomach, neurons in human gastric muscles do not express CCK2R (299, 410). On the other hand, labeling of CCK receptors in rat and dog gastric muscles was comparatively weaker than in human tissue, suggesting interspecies differences in gastric emptying mechanisms. Particularly, CCK receptor expression was restricted to the circular smooth muscle of rat pyloric sphincter (487). Both CCK receptors transcripts are also present in guinea pig stomach smooth muscles (123). Localization of CCK1R in cell membranes of rat pyloric muscle cells has been confirmed by immunohistochemistry (370). In addition, CCK1R was localized on mouse and rat pyloric interstitial cells of Cajal that are not smooth muscle cells but nevertheless influence motility and are considered pacemaker cells for gastrointestinal slow-wave activity (69, 370).

CCK released in the duodenum plays an important role in the delay of gastric emptying by modulating the contractile state of the stomach and the pylorus. This effect involves activation of CCK1R, as evidenced by studies with antagonists in rats and humans (131, 154, 437, 459, 591). Reduction of gastric emptying by a specific CCK1R agonist is also consistent with a CCK1R-mediated mechanism (80).

In contrast to the expected enhancement resulting from the lack of CCK1R, OLETF rats have delayed gastric emptying compared with controls (468). This was linked to several alterations of smooth muscle and autonomic transmission (511). However, gastric muscular contraction in the OLETF rat was found to be undeteriorated in vitro (356). Studies of gastric emptying in CCK1R, CCK2R, and CCK1,2R knockout mice yielded similarly contradictory results. Gastric emptying was enhanced in mice lacking CCK2R and unchanged in CCK1R−/− mice, raising the question of the mechanism underlying CCK2R regulation of gastric emptying (320).

3. Bowel

There is no report to date of morphological studies aimed at localizing CCK receptors in intestinal smooth muscle at the cellular level. Two studies evaluated CCK receptors by in vitro receptor autoradiography in human nonmalignant tissues adjacent to resected colonic tumors (402, 412). CCK1R was found at moderate-to-low density in colonic longitudinal smooth muscle and on nerve cells of the myenteric plexus. CCK2R binding activity was more rarely characterized. Results of ligand binding performed on dispersed cells suggested the presence of both CCK1R and CCK2R in cecal circular smooth muscle cells of guinea pig (340). Other data supporting the presence of CCK1R on intestinal smooth muscle mostly arise from bioassays. With the use of human muscular strips, contraction of the ascending colon was demonstrated to result from exclusive activation of CCK1R (339). These data suggest that CCK exerts its action on bowel motility, at least in part, directly on CCK1R from smooth muscle cells in addition to acting via the myoenteric plexus. As observed in vitro, experiments with the CCK1 receptor antagonist loxiglumide indicate that CCK affects human colonic motility in vivo. Indeed, this compound accelerates colonic transit in normal volunteers (313, 438, 449, 538). Moreover, demonstration that small intestinal motility is in part mediated by CCK1R-induced signaling was recently supported by significantly lower transit rates in CCK1R null mice (553).
C. Other Peripheral Organs and Tissues

1. Adipocytes

The demonstration of saturable CCK binding on rat adipocytes membranes was correlated with the detection of transcripts for CCK2R, but not for CCK1R, and linked to the long-term regulation of leptin expression and secretion by gastrin (21). In contrast to rat adipocytes, RT-PCR and binding experiments demonstrated the lack of CCK2R as well as CCK1R in murine adipocytes (M. Dufresne, unpublished observations).

2. Adrenal gland

Human and rat adrenal zona glomerulosa cells, but not inner adrenocortical cells (zona fasciculata-reticularis cells), express mRNAs for CCK1R and CCK2R (297, 307). CCK-stimulated aldosterone secretion was related to the activation of CCK1R and CCK2R in rats but is exclusively through activation of CCK2R in humans (307). Although the physiological relevance of these observations requires further investigations, they suggest new functions for CCK and/or gastrin in the control of adrenocortical secretion.

3. Blood mononuclear cells

The relationship between CCK and the immune system has been scarcely studied. In fact, pharmacological demonstration of the existence of CCK2R in human mononuclear cells was only performed in the human Jurkat lymphoblastic T cell line. Other studies reported the expression of CCK2R mRNA in blood mononuclear cells (453) or only in polymorphonuclear cells (221). Moreover, according to the data in the literature, no conclusion can be drawn concerning the presence or absence of CCK1R mRNA in these cell types (105, 453). The antiproliferative effect of gastrin on human phytohemagglutinin-pretreated peripheral mononuclear cells was reported but whether CCK2R or the CCK2R45sv missspliced variant of CCK2R mediated the effect was unclear (453). Negative modulation of murine lymphoproliferation and mobility by CCK has also been described, but the receptor subtype mediating this effect has not been identified (117). Further elucidation of the biological functions of CCK receptors in the regulation of immunohematopoietic systems is required.

4. Kidney

Evidence for the presence of CCK2R in the guinea pig and rat kidney is given by immunohistochemical or binding data demonstrating the presence of the protein with supporting RT-PCR or Northern-blot RNA analysis. In contrast, while CCK1R has been detected by RT-PCR in the murine and human kidney, there are actually no data reporting expression of the CCK1R protein. CCK2R, expressed in the rat kidney on tubules and collecting ducts, mediates changes in renal potassium and sodium absorption (546). The role of gastrin as a growth factor has been proposed due to its ability to induce proliferation of a renal cell line (121, 497).

Surprisingly, impact of CCK2R invalidation on these effects was not explored in knockout mice. Furthermore, the physiological relevance of renal CCK2R needs to be determined in humans.

5. Vagal afferent fibers

A large number of studies, mostly performed in rats, implicate vagal afferent mechanisms in the control of several CCK actions such as relaxation of proximal stomach combined with increased antral and pyloric contraction, increased duodenal motility, gallbladder contraction, sphincter of Oddi relaxation, and pancreatic enzyme secretion. Expression of both CCK1R and CCK2R has been demonstrated in rabbit vagus nerve, rat vagal afferent fibers, nodose and dorsal root ganglia, and human nodose ganglion (62, 287, 336). Indirect evidence strongly suggests CCK1R localization on vagal nerves in the duodenal mucosa, in the vicinity of CCK endocrine cells (40), mediates CCK satiety signals (60, 413). A role for the hepatic vagal branch innervating the proximal duodenum, which contributes to this effect, was also suggested (103). Several studies indicate that CCK acts via an afferent vagal pathway originating in the duodenal mucosa to stimulate pancreatic secretion in rats as well as in humans (282, 493). Modulation of pancreatic secretion by leptin was also postulated to involve CCK1R on duodenal afferent vagal fibers (187). Furthermore, activation of these receptors is thought to initiate a vagovagal reflex inhibition of gastric motor function (175, 176). However, recent attempts to characterize CCK1R immunoreactivity yielded surprising and unexplained negative results in the duodenal mucosa with positive results in cell bodies from the nodose ganglia (369, 501).

Data demonstrating CCK receptors on gastric vagal afferents are less convincing. Gastric CCK-responsive vagal afferent fibers have been identified on in vitro isolated stomach-vagus nerve preparations (564). A population of CCK1R-immunoreactive fibers, localized in the gastric mucosa, markedly decreased following subdiaphragmatic vagotomy, suggesting a vagal origin (501). However, expression of these receptors was not confirmed in other studies (369, 370).

VII. PATHOLOGICAL ACTIONS OF PERIPHERAL CHOLECYSTOKININ RECEPTORS AND THEIR RELEVANCE TO CLINICAL DISORDERS IN HUMANS

A. Digestive and Metabolic Diseases

1. Peptic ulcer

The links between Helicobacter pylori, gastrin, and gastric ulcers were recognized several years ago (278).
Gastric ulcers are primarily caused by \textit{H. pylori} infection. \textit{H. pylori} infects the antrum and is associated with G-cell hyperfunction (278, 573). The increased gastrin secretion is a consequence of the action of cytokines on the G cells (68). Cytokines also inhibit D-cell function, thus contributing indirectly to enhanced G-cell function. Patients with \textit{H. pylori} infection restricted to the gastric antrum show an increased secretion of gastrin that is thought to lead to increased acid output and tendency to ulceration. However, in patients with infection in both the antrum and corpus of the stomach, inflammation of the latter can lead to depressed acid secretion and atrophic gastritis. Atrophy may affect the antrum, leading to a loss of G-cell number, but where the antrum is spared, there is a further tendency to enhanced plasma gastrin concentrations due to lower acid inhibition of G cells (308, 309, 573). The relationship between \textit{H. pylori}, increased gastrin synthesis, secretion, and gastric cancer is an important issue (covered in sect. \textit{viiB}).

2. Irritable bowel syndrome

Because 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 and irritable bowel syndrome (IBS) that is associated with motor abnormalities in the small intestine and colon. It has been suggested that exaggerated and prolonged CCK release in IBS patients could contribute to intestinal dysmotility (481). Increased colonic response to CCK in vivo and in vitro has been observed (92). On the other hand, CCK1R antagonists have been evaluated clinically to stimulate transit and treat IBS (438, 538). Loxiglumide caused significant improvement of symptoms in IBS while, in contrast to previous studies, dexloxiglumide was very recently reported to delay transit in the ascending colon in patients with constipation-predominant IBS (76, 104, 109). This indicates that further studies are still required to characterize the response to CCK1R antagonist therapy for this pathology.

3. Gallbladder diseases

Gallbladder muscles from patients with cholesterol stones exhibit contractile defects that are related to altered binding properties of CCK1R, which result from decreased membrane fluidity (578). In this pathology, abnormalities are reversed when excessive cholesterol is removed from the plasma membrane. CCK1R gene expression was found to be significantly decreased, and genetic polymorphism was detected in the receptor promoter region in gallbladders from patients with gallstones (324). The CCK1R-null mouse provides a good model for the study of cholesterol cholelithiasis. Indeed, absence of CCK1R impairs gallbladder contraction as well as absorption of cholesterol resulting in enhanced biliary secretion, bile crystallization, and finally agglomeration of solid crystals in gallbladders (324, 434, 553).

Although the mechanisms underlying impaired gallbladder emptying have not been completely elucidated, the existence of a general defect in the contractile apparatus, not a specific abnormality of CCK1R or impaired nerve-induced contractile responses, has been recently hypothesized in impaired gallbladder function of patients with acalculous cholecystitis (11, 312). The muscle cell defect appears to be specific for this condition and not for gallbladder disease with cholesterol gallstones.

A CCK provocative test has been developed, in combination with either cholecystography, cholescintigraphy, or ultrasonography, for assessment of gallbladder motility. This test is based on the concept that induction of gallbladder dyskinesia, and/or the reproduction of biliary pain by hormonal stimulation, would help to identify patients more likely to benefit from cholecystectomy. However, many parameters can affect the response of the gallbladder including the kind of stimulus (intravenous bolus injection, slow infusion of CCK, or a fatty meal to release endogenous CCK) (31, 314, 360, 595–597). Moreover, several studies question the value of measuring gallbladder emptying with the CCK provocative test as a method to select patients with acalculous gallbladder disease for surgery (267, 395). Further studies are required to clearly characterize acalculous cholecystitis and the role of CCK in this pathology.

4. Pancreatitis

Evidence based on animal studies implicates that CCK acts as an agonist towards low-affinity acinar CCK1R in the induction and development of acute pancreatitis (429). Moreover, hyperstimulation of rodent acinar CCK1R was widely used as an experimental model for the human disease and thus contributed greatly to the current knowledge and understanding of the pathophysiology and cell biology of this disease. Although this model is closely related to some aspects of the human disease, it is important to note that the absence of functional CCK1R in human acini excludes a direct action of CCK in the human pathology. Notwithstanding, one multicenter, controlled trial evaluated therapeutic efficacy of the CCK1R antagonist loxiglumide in the treatment of acute pancreatitis. Decrease of pain and serum levels of digestive enzymes in this study suggested the usefulness of loxiglumide in the treatment of these patients (467). Of note, CCK2R has recently been detected in the acini of a few samples of chronic pancreatitis, yet the biological significance remains to be determined (409).
5. Obesity

CCK belongs to the growing list of factors modulating food consumption, and CCK molecules are actually promising targets for antiobesity drugs. Since the initial discovery of its property as a food-intake inhibitor, CCK was demonstrated to be a short-term, meal-reducing signal in most mammalian species including humans (243, 485, 486). Besides the demonstration that exogenous CCK significantly decreases meal size, a number of experiments examined the actions of endogenous CCK with administration of CCK receptor antagonists (24). The inhibitory action of CCK was shown to depend on the presence of CCK1R on vagus nerve, which relays to the hypothalamus via brain stem areas such as the nucleus tractus solitarius and the area postrema (62, 335, 456). Increased gastric distension induced by slowing gastric emptying by CCK constitutes a contributing aspect of the satiety response (242). This signal is transmitted via CCK-responsive vagal afferent fibers that express mechanoreceptors and also respond to CCK (114, 455). Moreover, synergistic interactions with leptin were demonstrated (32). While the satiety actions of peripheral CCK are well characterized, a role for brain CCK has been more controversial. While CCK was recently confirmed to centrally act as a neuro-modulator to produce satiety, the central mechanisms are still partly resolved (49, 50). Most works implicate that CCK1R is involved in the mediation of the control of food intake. The satiety role of CCK1R was confirmed in CCK1R-deficient mice, which in contrast to wild-type animals showed no change in food consumption after administration of CCK (254). These mice have normal total daily food intake and normal body weight, suggesting that CCK is not essential for these regulatory processes. A different phenotype was observed in CCK1R-deficient OLETF rats. OLETF rats exhibit hyperphagia, resulting from the lack of an intact peripheral satiety system, which leads to obesity. Comparison of both species with respect to CCK1R distribution led to the demonstration that mice are in contrast to rats that have CCK1R in the dorsomedial hypothalamus, which controls anorexigenic neuropeptide Y gene expression, thus highlighting important interspecies variation in body weight regulation and raising the important question of satiety control in humans (334).

Polymorphisms in the promoter region of the human CCK1R gene were detected in 1.9% of individuals in a cohort study and were related to increased body fat content (157). The functional significance of this genetic factor is actually unknown, since the reported polymorphism has no role in the transcriptional regulation of CCK1R (512). Another mutation detected in obese subjects and affecting the coding region of CCK1R was demonstrated to significantly decrease the expression and function of the receptor, but further studies are needed to validate its role in human obesity (300). On the other hand, abnormal plasma CCK levels have been characterized in several eating disorders. Basal and postprandial CCK was found to be either increased or decreased in anorectic individuals and reduced in women with polycystic ovary syndrome secretion who tend to binge eat and become overweight (28, 207, 376). Lack of a CCK response to a high-fat meal was reported to contribute to the hyperphagia-mediated obesity of Prader-Willi subjects (67). Increased sensitivity to the satiating effect of CCK was observed and may be related to the decrease in appetite and food intake that occurs with aging (293). Increased plasma concentrations of CCK were also suggested to contribute to the inhibition of appetite in acquired immunodeficiency syndrome patients (18). Diverging results of plasma CCK measurements must be interpreted with caution as they might be due to inaccurate immunoassays that prevailed between 1965 and 1995 and thus require further confirmation (367, 398).

To date, the hypothesis that CCK2R may mediate the modulating action of CCK on food intake cannot be totally excluded. Indeed, CCK2R is expressed in the vagus nerve brain stem complex that mediates the satiety effect of peripheral CCK (62, 336). Moreover, CCK2R is the predominant form found in the brain, particularly in hypothalamic areas, and is therefore an ideally positioned candidate for the mediation of the action of centrally released CCK (66, 77, 298, 311, 352). Increased food intake observed after central or peripheral administration of CCK2R antagonist gives support to this possibility (136, 137). On the other hand, circulating gastrin can activate neurons in the nucleus tractus solitarius, the area postrema, and specific brain regions including hypothalamic areas involved in food intake (110, 579). Finally, the enhanced gastric emptying that is observed following CCK2R gene invalidation in mice also supports an inhibitory role for feeding behavior (320).

B. Cancers

An increasing body of evidence supports the fact that CCK and gastrin act via their receptors as growth and invasiveness factors, thus promoting the development and progression of cancers. While cell lines are indeed valuable tools for the elucidation of mechanistic pathways that are switched on during carcinogenesis, the expression of CCK receptors in cancer cell lines has mostly given rise to controversial results with regard to the type and density of CCK receptors. Nevertheless, several studies aimed at the determination of the levels of CCK receptors protein expression in human tumors were recently reported. In addition to clarifying whether or not CCK receptors might contribute to the tumorigenic process, these data now open new perspectives to the fields of diagnostic and therapeutic applications such as recep-
tor scintigraphy, tumor therapy with radioactive or cytotoxic analogs, and long-term therapy with nontoxic analogs. Importantly, a role for CCK receptors in the early steps of cancer development, with possible loss of expression at later stages, must also be considered.

1. Gastric cancer

Gastric cancer is one of the most frequent malignancies in the world and one of the leading causes of cancer death worldwide (124). Epidemiological evidence indicates that environmental factors play a major role in gastric carcinogenesis in association with immunologic, genetic, and immunogenetic factors that are thought to contribute to the pathogenesis of gastric carcinoma. In the multifactorial model of human gastric carcinogenesis, *Helicobacter pylori* (Hp) is a major environmental risk factor that contributes to the development of this cancer (301). Hp infection is associated with increased plasma gastrin release from G cells, which in turn stimulates the growth of Hp (94). The precise mechanisms by which gastrin promotes gastric carcinogenesis probably involves upregulation of HB-EGF, COX-2, cyclin D1, and Reg gene expression as well as intracellular, proliferative signaling pathways. Regulation of these gastrin-sensitive genes and signals is described in detail in section V of this review. The possibility that elevated gastrin is only a consequence and not a causative factor remains an open question, as demonstrated in the report describing a synergistic action with Hp infection in hypergastrinemic transgenic mice (558).

Expression of CCK2R in gastric cancer samples indicates that gastrin stimulates tumor growth by autocrine mechanisms (96, 200, 250, 310, 358, 412, 592). False-positive detection of CCK1R gene expression was also reported to correspond to sample contamination with noncancerous tissue (412).

Gastric carcinoid tumors are distinct from adenocarcinomas and in general exhibit a more favorable outcome. These tumors arise from proliferating ECL cells, a major proliferative target for gastrin. The reason for the recent increase in gastric carcinoid tumor incidence is unclear (328). One theory is that the use of acid-suppressive therapy has led to an increased incidence of hypergastrinemia, although no overt relationship has yet been identified in humans. Hypergastrinemia results in ECL cell proliferation, which initiates and maintains neoplastic changes in these cells (172, 181, 350). The basis of hypergastrinemia is usually a low-acid state such as atrophic gastritis/pernicious anemia, but it can also be associated with a gastrin-secreting neoplasm. Up to 30% of gastrinoma patients on a background of multiple endocrine neoplasia-1 (MEN-1) and ~5% of patients with atrophic gastritis/pernicious anemia may develop ECL cell carcinoid tumors (52, 167). Therefore, the effect of gastrin in promoting the development of these tumors is enhanced by an acquired inflammatory condition or by an inherited susceptibility. Mutations of the growth factor Reg have also been reported in some ECL cell carcinoid tumors (204). The contribution of a CCK2R-mediated role of gastrin in tumor formation is well demonstrated in the case of the African rodent *Mastomys natalensis*. This animal exhibits an increased susceptibility to ECL cell growth and an elevated rate of gastric carcinoid formation even with normal gastrin circulating levels. This susceptibility was linked to an interspecies polymorphism within the coding region of the CCK2R gene, which confers a constitutive activity upon the receptor (444).

2. Pancreatic adenocarcinoma

A number of data from transgenic animals or chemically induced pancreatic carcinogenesis support the idea that CCK2R plays a role in tumor development. The asparagus-induced rat pancreatic carcinoma DSL-6 and the derived cell line AR42J as well as transgenic mice bearing the elastase I promoter-SV40T-antigen fusion gene show pancreatic expression of CCK2R (386, 439, 593). Importantly, pancreatic tumors develop with ageing in ElasCCK2 transgenic mice expressing functional human CCK2R under the control of the elastase I promoter in pancreatic acinar cells (97, 98, 425). Alteration of cellular morphology and differentiation was observed before tumor formation, indicating an evolution from an acinar to a ductal phenotype (42). Recently, increased sensitivity to an acinar carcinogen was also demonstrated in this transgenic strain (302). Moreover, the upregulation of emergence of pancreatic progenitor cells expressing Pdx1, now acknowledged as potential sites for initiation of carcinogenesis, was also observed in ElasCCK2 mice (205, 302).

The role of CCK1R and CCK2R in human pancreatic carcinogenesis is unclear and remains a much debated question. The upregulation of gastrin and its receptor that was localized immunohistochemically in human pancreatic adenocarcinoma suggests mechanisms involving autocrine or paracrine stimulation (79). Others have reported the expression of CCK2R, gastrin, and CCK1R, but not CCK, in tumors, but the cellular localizations remain imprecise (178). On the other hand, CCK1R was selectively detected in ductal cells from human pancreatic adenocarcinoma by in situ hybridization (330, 565, 566). Expression of a splice variant of CCK2R (CCK2Ri4sv), which has constitutive activity, was also characterized in human pancreatic cancers (130, 199). Although the mRNAs of the receptors have been described, most reports have failed to provide precise localization of the proteins in tumors. A recent morphological evaluation of normal and diseased pancreatic tissue samples demonstrated a low amount of CCK2R in chronic pancreatitis and carcinoma...
with no positive signals for CCK1R (409). Of importance, chronic pancreatitis is associated with a high risk of developing cancer and exhibits genetic and morphological lesions similar to those seen in pancreatic adenocarcinoma (81, 294). Together with the fact that CCK2R is present in some transitional acinar cells, typical lesions of chronic pancreatitis, these data suggest that this receptor plays a role in the initiation steps of carcinogenesis.

3. Barrett’s esophagus and esophageal adenocarcinoma

Recent studies have demonstrated the presence of CCK2R and gastrin in Barrett’s metaplasia, a premalignant condition that predisposes one to developing esophageal adenocarcinoma. Increased expression of the CCK2R gene was demonstrated in endoscopic biopsy specimens and linked to stimulation of DNA synthesis (1, 194, 198). Signal pathways activated by gastrin were identified in several esophageal cell lines (OE19, OE21, OE33, SEG-1, BIC, SKGT-4) that expressed CCK2R. Activation of Erk pathways and PI 3-kinase was linked to proliferation in response to gastrin (194, 331). High basal levels of activated PKB/Akt were observed in samples from Barrett’s metaplasia and linked to the activation of CCK2R in esophageal cell lines (198). Based on these studies, hypergastrinemia resulting from long-term administration of proton pump inhibitors for the treatment of Barrett’s esophagus could play a role in the growth of esophageal adenocarcinoma via activation of CCK2R. Of note, a significant increase in gastrin gene expression was characterized in Barrett’s metaplasia biopsies in addition to the de novo expression of the constitutively active CCK2R splice variant (CCK2Ri4sv) (1, 198). This suggests that an autocrine signal or constitutive activity of CCK2R could be involved in the pathogenesis of Barrett’s esophagus before the development of dysplasia and cancer. This involvement may be in part through CCK2R induction of COX-2 expression (1, 251).

4. Other tumors expressing CCK receptors

The incidence and density of CCK receptors, detected using in vitro receptor autoradiography in more than 100 neuroendocrine tumors, has been recently summarized (407). Some of these tumors are promising targets for the use of CCK/gastrin analogs for detection and/or therapeutic purposes. Indeed, a high expression level of CCK2R is found in vipomas (tumors which secrete excessive amounts of vasoactive intestinal polypeptide), thus confirming previous reports that studied insulinomas as well as bronchial and ileal carcinoid (516). While CCK1R was absent in all insulinomas and rare in vipomas and bronchial carcinoids, they were highly but heterogeneously expressed in ileal carcinoids. Interestingly, their expression pattern was related to specific areas in these tumors. The incidence of CCK1R was ~50% in gastrinomas that were devoid of CCK2R. High CCK2R density and incidence has been shown in medullary thyroid carcinomas. The high sensitivity and specificity of the pentagastrin stimulation test, which detects the presence or recurrence of medullary thyroid cancer (MTC), suggested that CCK2R is present in malignant calcitonin-producing C cells (567). Their presence with a very high incidence (92%) in these tumors and their absence in nonmedullary thyroid carcinomas as well as normal thyroid glands was demonstrated by binding assays, autoradiography, and RT-PCR (12, 408). However, CCK2R expression was detected more recently in nonmalignant, normal thyroid C cells (46).

The presence of CCK2R and gastrin has been shown in a high percentage of small cell lung cancers, but not in lung adenocarcinomas, supporting a possible autocrine growth regulation (304, 306, 406). In contrast to earlier studies performed in small cell lung cancers, the presence of CCK1R was not detected in human biopsies (461). To our knowledge, only one study has investigated neuronal, renal, and myogenic stem cell tumors for CCK receptor expression (441). An unexpected high incidence of gastrin mRNA was found in these tumors. CCK mRNA was present in Ewing sarcomas and leiomyosarcomas. Only a minority of the tumors expressed the receptors except for Wilms’ tumors, where CCK2R was found, and leiomyosarcomas, which expressed both CCK1R and CCK2R. CCK2R and gastrin were also detected in all stromal ovarian cancers (406).

Activation of CCK2R also modulates proliferation of cells of the immune system, and it has been clearly established that gastrin stimulates growth and growth-associated genes in human lymphoblastic Jurkat T cells and leukemia cells (221, 357). In the latter study, in addition to expression of CCK2R, the presence of gastrin-like immunoreactivity was demonstrated in the culture media of several leukemia cell lines, suggesting the existence of an autocrine loop promoting the growth of nonepithelial cells.

5. Colon cancer and gastrin precursors

Hypergastrinemia has been associated with an increased risk of colorectal carcinoma (108, 489, 524). However, most recent data support the view that gastrin and CCK2R do not play an important role in colon cancer, whereas precursors of gastrin could contribute to neoplastic progression in the colon by acting through receptors that are distinct from CCK2R and CCK1R. First, CCK receptors do not seem to be expressed in normal colonic epithelial cells and are found in a minority of human colonic tumors (452). In contrast, high concentrations of gastrin precursors, such as glycine-extended gastrin (G-gly) and progastrin, have been observed in colon tumors and in the blood of patients with colorectal cancer (244, 347). These precursors represent 90–100% of the gastrin peptides.
produced by colon tumors and are found in 80–90% of colorectal polyps in humans (484). In addition, numerous groups have demonstrated that unprocessed forms of gastrin act as growth factors for colon cancer cell lines that do not express CCK1R or CCK2R (25, 211, 290, 477, 498).

Trophic effects of gastrin precursors on colonic mucosa have also been confirmed in vivo. Gastrin-deficient mice perfused with G-gly or progastrin showed a marked increase in proliferation of colonic epithelial cells, whereas mature amidated gastrin had no effect on the same model (247, 361). Similarly, G-gly perfusion into rats results in proliferation of colonic mucosal cells and the formation of aberrant crypt foci and increases the sensitivity to azoxymethane, a colon procarcinogen (10). In addition, transgenic mice overexpressing progastrin (hGAS mice) or G-gly (MTI/G-Gly mice) exhibit hyperplasia of the colonic mucosa and increased epithelial proliferation, and hGAS mice treated with a chemical carcinogen display an increased predisposition to develop pre-neoplastic lesions or even colonic adenocarcinoma compared with wild-type, control mice (99, 247, 478, 479).

On the basis of binding studies, several receptors for gastrin precursors, with different pharmacological profiles, have been characterized. The first one to be described was a receptor with a high affinity for G-gly, which does not bind gastrin or the antagonists of the CCK receptors (211, 231, 464, 498). Afterwards, a second receptor, binding with a strong affinity both amidated gastrin and gastrin precursors but not the CCKR antagonists, was described by Singh and co-workers (220, 476, 477). Both receptor types were shown to mediate the proliferative effects of gastrin precursors. However, a receptor with these pharmacological characteristics has not yet been cloned. Baldwin et al. (26) have purified a gastrin binding protein (GBP) that is a member of the enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase family of enzymes but displays a low affinity for gastrin precursors. In addition, whether this GBP mediates the proliferative effects of gastrin precursors remains to be confirmed (581).

The constitutive splice variant of CCK2R, retaining intron 4 in the third intracellular loop, has recently been identified in certain human colon tumors (199). However, this CCK2R splice variant binds gastrin precursors with a very low affinity. In addition, the presence of this variant in colon cancer remains controversial since it has not been found by other groups to be present in human colorectal tumor samples (452).

C. Peptide and Receptor Targeting

1. Gastrimmune

Gastrimmune or G17DT is an immunoconjugate of the NH₂-terminal sequence of gastrin-17 linked via a spacer to diphtheria toxin. It raises antibodies that neutralize both the carboxy-amidated and glycine-extended forms of gastrin17 but shows no cross-reactivity with other forms of gastrin or CCK. Preliminary testing of a potential therapeutic benefit was provided by in vivo studies using animal models. Data from this evaluation gave evidence for adequate targeted inhibition of primary tumor growth and metastasis of a colorectal tumor. In addition, it showed a significant improvement in survival using a xenograft model of gastric cancer (561–563). These positive results further led to recent phase II clinical studies in patients with gastric, colic, or pancreatic cancer (61, 171, 483). In these studies, G17DT was well tolerated in the majority of patients, who developed an antibody response. Phase III studies with G17DT alone or in combination with chemotherapy are ongoing.

2. CCK receptors

Peptide receptors are targets for in vivo cancer diagnosis and therapy. While the most frequently targeted peptide receptor is the somatostatin receptor, some tumors expressing CCK2R with a high incidence and density represent adequate targets for CCK receptor labeling in vivo. Different groups have developed indium-111, technetium-99m, or iodine-131 radiolabeled CCK and gastrin analogs suitable for in vivo receptor scintigraphy and therapy (8, 37, 144, 269, 411, 545). An overview of data obtained in this field is given in References 9, 36, 404, 405. As reviewed in the above section, medullary thyroid carcinomas, small cell lung cancers, stromal ovarian cancers, insulinomas, vipomas, and bronchial and ileal carcinoids are good candidates for CCK2R labeling in vivo.

VIII. CONCLUSION/PROSPECTS

From this overview and the referenced works, it appears that available data related to CCK receptors clearly establish their importance and relevance to human physiology and pathophysiology. Actions of peripheral CCK/gastrin receptors have largely extended beyond their initial spheres where they were first identified and isolated, namely, the pancreatic acinar cells and the gastric parietal cells. In addition to their peripheral functions, one must keep in mind the extreme importance of CCK receptor functions in the central nervous system, which are not described in this paper. As a corollary of the recently recognized long-term actions of CCK and gastrin, recent works have documented that CCK receptors are capable of using signaling molecules common to growth factor receptors having tyrosine kinase activity. Although the use of specific nonpeptide ligands of the two CCK/gastrin receptors as well as the availability of knock-out mice have greatly contributed to this knowledge, undesired activities of initial and still popular molecules, as
well as interspecies differences concerning the structure, expression, and localization of CCK receptors, may have contributed to questions regarding the relevance of these receptors as targets for human diseases. With all these data in mind, one can consider that there is still much to discover about the physiological functions, regulation, and functioning of these two members of the G protein-coupled receptor family.

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