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International Union of Pharmacology. XXI. Structure, Distribution, and Functions of Cholecystokinin Receptors

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I. Introduction

The peptide cholecystokinin (CCK)[2] was originally discovered in the gastrointestinal tract (Ivy and Oldberg, 1928) and has been shown to mediate pancreatic secretion and contraction of gallbladder. Then, CCK was described in the mammalian central nervous system (CNS) as a gastrin-like immunoreactive material (Vanderhaeghen et al., 1975), and it is now generally believed to be the most widespread and abundant neuropeptide in the CNS. This peptide, initially characterized as a 33-amino-acid sequence, is present in a variety of biologically active molecular forms derived from a 115-amino-acid precursor molecule (prepro-CCK; Deschenes et al., 1984), such as CCK-58, CCK-39, CCK-33, CCK-22, sulfated CCK-8 [Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2] and CCK-7, unsulfated CCK-8 and CCK-7, CCK-5, and CCK-4 (Trp-Met-Asp-Phe-NH_2; Fig. 1; Rehfeld and Nielsen, 1995). The presence of CCK in both gut and brain raises the intriguing issue of the evolutionary significance of separate pools of a peptide in two systems originating from different embryonic zones (i.e., endoderm and ectoderm, respectively).

Receptors for CCK have been pharmacologically classified on the basis of their affinity for the endogenous peptide agonists CCK and gastrin, which share the same COOH-terminal pentapeptide amide sequence but differ in sulfation at the sixth (gastrin) or seventh (CCK) tyrosyl residue. Two types of CCK receptors (type A, "alimentary", and type B, "brain") have thus been distinguished. The CCK-A receptor was first characterized using pancreatic acinar cells (Sankaran et al., 1980), whereas the CCK-B receptor, with a different pharmacological profile, was discovered in the brain (CCK-B; Innis and Snyder, 1980b). The gastrin receptor mediating acid secretion in the stomach was initially thought to constitute a third type of high-affinity receptor on the basis of its location and small differences in affinity for CCK and gastrin-like peptides (Song et al., 1993). However, subsequent cloning of gastrin and CCK-B receptors revealed their molecular identity (see later). CCK-A and CCK-B receptor types have been shown to differ by their relative affinity for the natural ligands, their differential distribution, and their molecular structure. The CCK-A receptor binds sulfated CCK with a 500- to 1000-fold higher affinity than sulfated gastrin or nonsulfated CCK

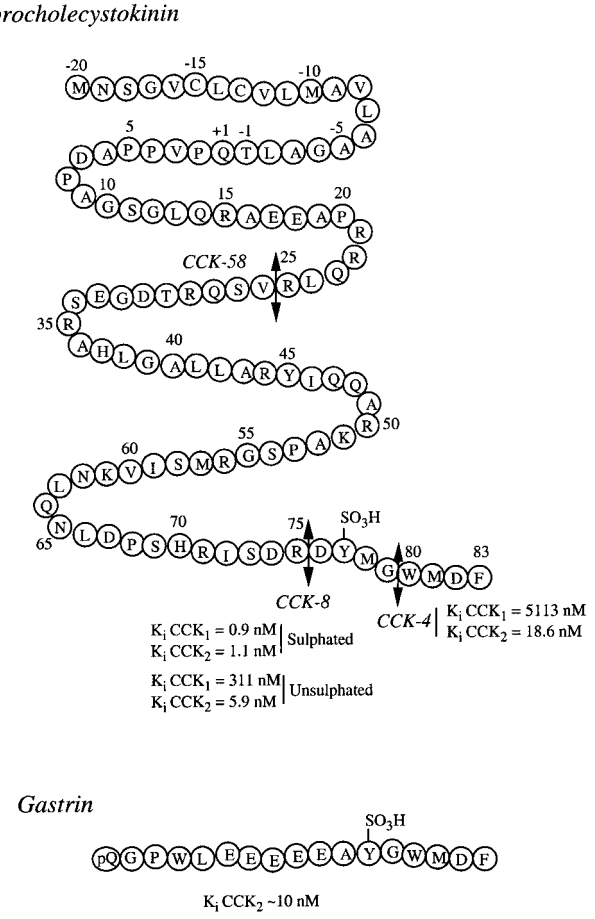


FIG. 1. Predicted structure of human preprocholecystokinin. The signal peptide consists of residues −20 to −1. The amino terminal flanking peptide consists of residues 1 to 25. The largest characterized form from brain and intestine, CCK-58, consists of residues 26 to 83. Other active molecular forms are derived from this precursor, such as CCK-39, CCK-33, CCK-22, CCK-7, and CCK-5.

[2] Abbreviations: CCK, cholecystokinin; IUPHAR, International Union of Pharmacology; CNS, central nervous system; PKC, protein kinase C; Hpa, 4-hydroxyphenylacetyl; DRG, dorsal root ganglia; PLC, phospholipase C; IP_3, inositol triphosphate; GPCR, G protein-coupled receptor; PLA_2, phospholipase A_2; MAPK, mitogen-activated protein kinase; BH, Bolton-Hunter; TM, transmembrane domain; ECL, extracellular loop; SOS, the product of son of sevenless.

(Silvente-Poirot et al., 1993a). The CCK-B/gastrin receptor binds gastrin and CCK with almost the same affinity and discriminates poorly between the sulfated and nonsulfated CCK analogs (Saito et al., 1980). The distribution of CCK-A and CCK-B/gastrin receptors is tissue dependent (see below).

Based on pharmacological and biochemical studies, the existence of subtypes of CCK-A and CCK-B receptors has been postulated. Nevertheless, only two genes have been cloned. The initial nomenclature of the receptors as CCK-A and CCK-B receptors is generally accepted by pharmacologists and molecular biologists. Based on the guidelines defined by the International Union of Pharmacology (IUPHAR) Committee on Receptor Nomenclature and Drug Classification, receptors should be named after their endogenous ligands and identified by a numerical subscript corresponding to the chronological order of the formal demonstration of their existence by cloning and sequencing (Vanhoutte et al., 1996). Because the CCK-A receptor was the first to be cloned, it should be renamed CCK_1, and the CCK-B receptor should become CCK_2. According to these guidelines, new splice variants, if pharmacologically relevant, should be indicated by subscript lowercase letters, in parentheses, such as $CCK_{1(a)}$, $CCK_{1(b)}$, $CCK_{2(a)}$, and $CCK_{2(b)}$ receptors. This new nomenclature would allow any newly discovered CCK receptor to be logically named according to the same informative guidelines (see Vanhoutte et al., 1996).

This rational nomenclature has been adopted in the present review, which is devoted to the two CCK receptors whose existence has been firmly established through cloning.

II. Characterization of Cholecystokinin (CCK) Receptors

A. CCK_1 (CCK-A) Receptors

1. CCK_1 Receptor Clones. The size of the CCK_1 receptor demonstrated by ligand affinity crosslinking studies varied depending on the ligand, the crosslinking reagent, the species, and the tissue expressing the CCK receptor (Svoboda et al., 1982; Rosenzweig et al., 1983; Miller, 1984; Fourmy et al., 1987; Pearson and Miller, 1987; Pearson et al., 1987a,b; Shaw et al., 1987; Schjoldager et al., 1988; Powers et al., 1991). In rat pancreatic acinar cells, the CCK_1 receptor was found to be an 85- to 95-kDa, *N*-linked glycoprotein with a 42- to 44-kDa protein core.

The CCK_1 receptor was purified to homogeneity from rat pancreas. The purified receptor had a molecular mass of 85 to 95 kDa consistent with previous crosslinking studies (Wank et al., 1992a). Microsequencing of five peptide products derived from either enzymatic digestion or chemical cleavage of the protein receptor allowed the design of degenerate oligonucleotide primers for cloning the cDNA of the CCK_1 receptor from a rat pancreatic cDNA library. The deduced sequence of the rat CCK_1 receptor corresponds to a 429-amino-acid protein with a calculated molecular mass of 48 kDa. Hydropathy analysis predicts seven transmembrane-spanning domains (TM) as expected for a member of the G protein-coupled receptor (GPCR) superfamily (Dohlman et al., 1991; Fig. 2). The sequence contains at least three consensus sites for *N*-linked glycosylation (Asn-X-Ser/Thr), consistent with the heavy and variable degree of glycosylation reported using ligand-affinity crosslinking techniques (de Weerth et al., 1993b). The CCK_1 receptor has three consensus sequence sites for protein kinase C (PKC) phosphorylation in the third intracellular loop (Graff et al., 1989), consistent with previous data showing that CCK-8- and 12-*O*-tetradecanoylphorbol-13-acetate-stimulated phosphorylation of serine and threonine residues involves predominantly the third intracellular loop and to a minor extent the cytoplasmic tail of the rat pancreatic CCK_1 receptor (Kawano et al., 1992; Ozcelebi and Miller, 1995). In addition, there are conserved cysteines in the first and second extracellular loops (ECLs) of both CCK_1 and CCK_2 receptors (Figs. 2 and 3), which may form a disulfide bridge required for stabilization of their tertiary structure (Silvente-Poirot et al., 1998), and another cysteine in the C terminus may serve as a membrane-anchoring palmitoylation site (O'Dowd et al., 1988; Ovchinikov et al., 1988).

The CCK_1 receptor cDNA has subsequently been cloned from guinea pig gallbladder, pancreas, and gastric chief cell (de Weerth et al., 1993b), human gallbladder (de Weerth et al., 1993a; Ulrich et al., 1993), and rabbit gastric (Reuben et al., 1994) cDNA libraries using either low-stringency hybridization or polymerase chain reaction methods. The CCK_1 receptor is highly conserved among these species with an overall amino acid homology of 80% and a pairwise amino acid sequence identity of 87 to 92% in humans, guinea pig, rat, and rabbit (Table 1).

2. Antagonists of CCK_1 Receptors. Several structurally different CCK_1 receptor antagonists have been synthesized. They belong to various series of chemicals, including dipeptoid, benzodiazepine, pyrazolidinine, and amino acid derivatives, and have both excellent selectivity and high affinity for CCK_1 receptors.

The first CCK antagonists were derived from a naturally occurring benzodiazepine, asperlicin (Table 2), which has been isolated from the fungus *Aspergillus alliaceus* (Chang et al., 1985). The demonstrated high in vitro and in vivo potency of asperlicin at CCK_1 receptors conferred clear advantages over previously reported CCK antagonists as a tool for investigation of the physiological and pharmacological actions of CCK. The first analogs of asperlicin were designed to assess which structural features of asperlicin could be modified to further enhance its CCK inhibitory potency without compromising its CCK_1 selectivity. Unfortunately, this approach failed to overcome the key defects of asperlicin (Bock et al., 1986). Interestingly, asperlicin contains

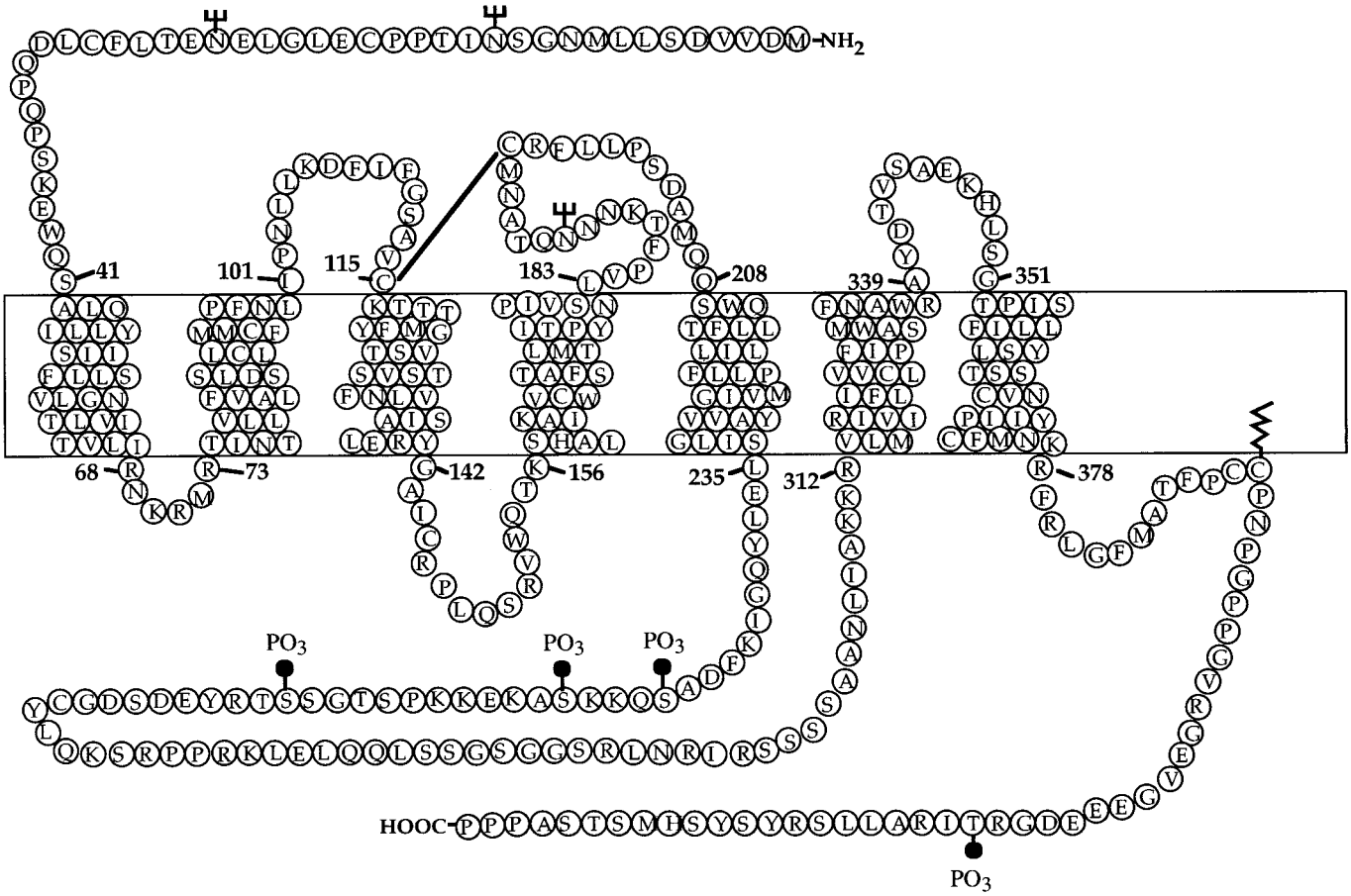


FIG. 2. Schematic representation of the rat CCK_1 receptor showing the postulated transmembrane topology, sites for putative NH_2-linked glycosylation (tridents), serine and threonine phosphorylation by PKC and protein kinase A (PO_3), and conserved cysteines in the first and second ECLs, possibly forming a disulfide bridge, and a possible palmitoylated conserved cysteine in the cytoplasmic tail. NH_2—, N terminus; COOH—, C terminus.

elements of the 1,4-benzodiazepine ring system found in antianxiety agents such as diazepam. On the other hand, several studies support the concept that the natural ligand for the antianxiety benzodiazepine receptor is a peptide (Guidotti et al., 1983; Alho et al., 1985), suggesting that the 5-phenyl-1,4-benzodiazepine ring is in fact a chemical structure that recognizes a peptide receptor. This explains why the 5-phenyl-1,4-benzodiazepine ring was proposed as the basis for the design of improved CCK receptor antagonists (Evans et al., 1986). Indeed, the 3-amino-5-phenyl-1,4-benzodiazepin-2-one derivatives, typified by L-364,718 (MK-329, devazepide; Tables 2 and 3), remained for several years the most potent CCK antagonists described with a good selectivity for CCK_1 receptors (IC_{50} CCK_2/CCK_1 = 3750).

Various tricyclic 1,4-benzodiazepine derivatives were also developed. On the basis of structure-activity relationship studies, as well as the stability and availability of the starting materials of those compounds, (*S*)-*N*-[1-(2-fluorophenyl)-3,4,6,7-tetrahydro-4-oxo-pyrrolo[3,2,1-*jk*][1,4]benzodiazepin-3-yl]-1*H*-indole-2-carboxamide (FK-480; Satoh et al., 1994; Tables 2 and 3) was selected as a candidate for further evaluation. The results obtained showed that FK-480 is a highly selective and potent CCK_1 receptor antagonist (Akiyama and Otsuki, 1994; Ito et al., 1994a).

Several other potent and selective antagonists of the CCK_1 receptor have been described, including glutamic acid derivatives such as loxiglumide (CR-1505) or lorglumide (CR-1409; Makovec et al., 1985; Table 2), and partial sequences of the C-terminal region of CCK. The dipeptide, *N-tert*-butyloxycarbonyl-aspartyl-phenylalaninamide (Boc-Asp-Phe-NH_2), representing the two-amino-acid C-terminal fragment common to both CCK and gastrin, is a low-affinity partial agonist at CCK_2 receptors but has no activity at CCK_1 receptors. This selectivity is abolished by removal of the C-terminal amide. Replacement of the *N-tert*-butyloxycarbonyl group in this dipeptide with an analog, the 2-naphthalene sulfonyl group, gave 2-naphthalenesulfonyl 1-aspartyl-(2-phenethyl)amide (2-NAP; Tables 2 and 3), which behaves as a competitive antagonist at CCK_1 receptors. Interestingly, this compound has a 300-fold greater affinity for CCK_1 than CCK_2 receptors (Hull et al., 1993).

On the other hand, further development of "dipeptoids", initially characterized as CCK_2 receptor antagonists (see below), led to a molecule that has a 100-fold selectivity for the CCK_1 receptor, where it acts as a

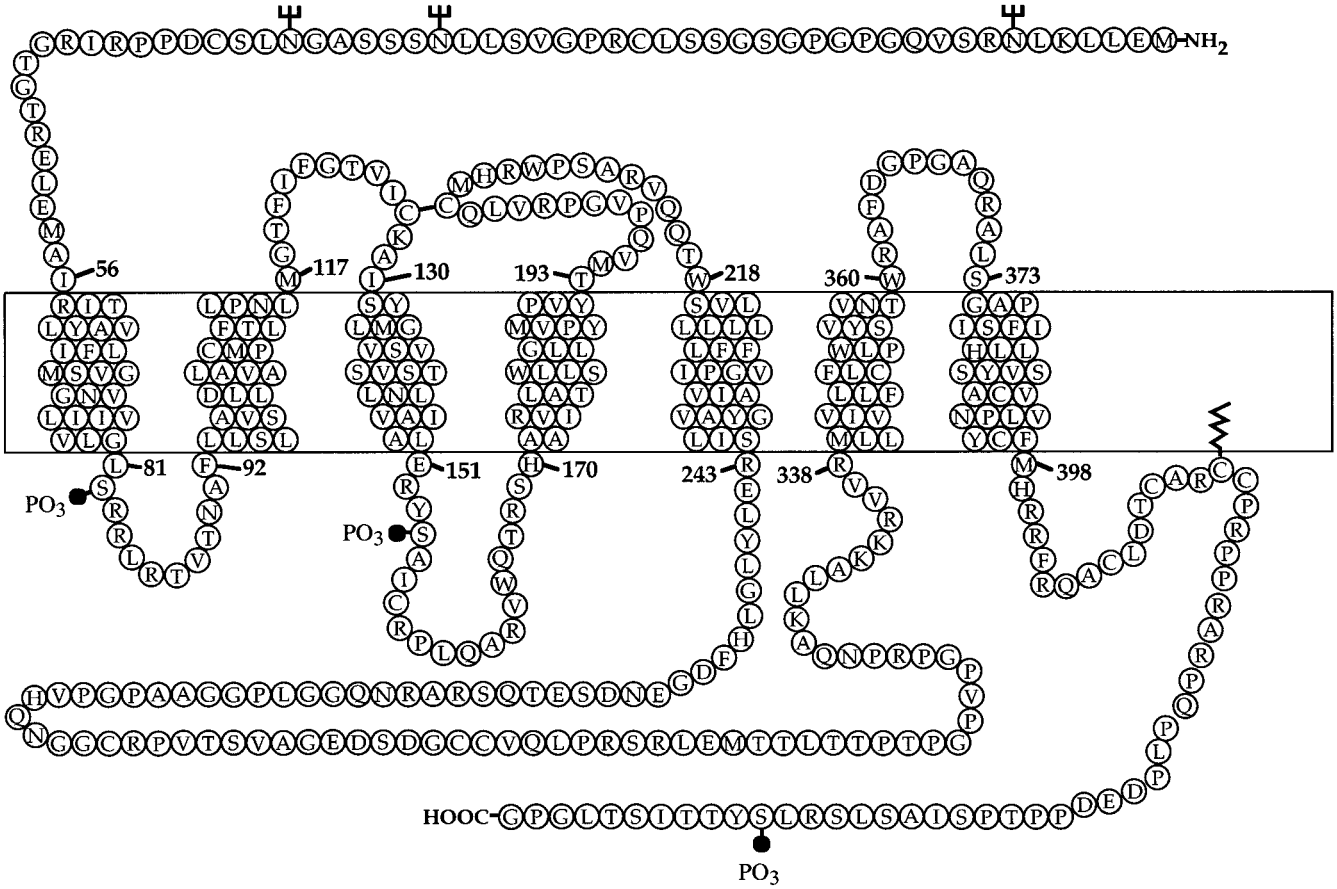


FIG. 3. Schematic representation of the rat CCK_2 receptor showing the postulated transmembrane topology, sites for putative NH_2-linked glycosylation (tridents), serine and threonine phosphorylation by PKC and protein kinase A (PO_3), and conserved cysteines in the first and second ECLs, possibly forming a disulfide bridge, and a possible palmitoylated conserved cysteine in the cytoplasmic tail. NH_2—, N terminus; COOH—, C terminus.

TABLE 1
SwissProt accession numbers for the cloned receptors from various species

Receptor	Species	Accession No.
CCK_1	Human	P32238
	Rat	P30551
	Guinea pig	Q63931
CCK_2	Human	P32239
	Mouse	P56481
	Rat	P30553
	Bovine	P79266
	Dog	P30552
	Rabbit	P46627

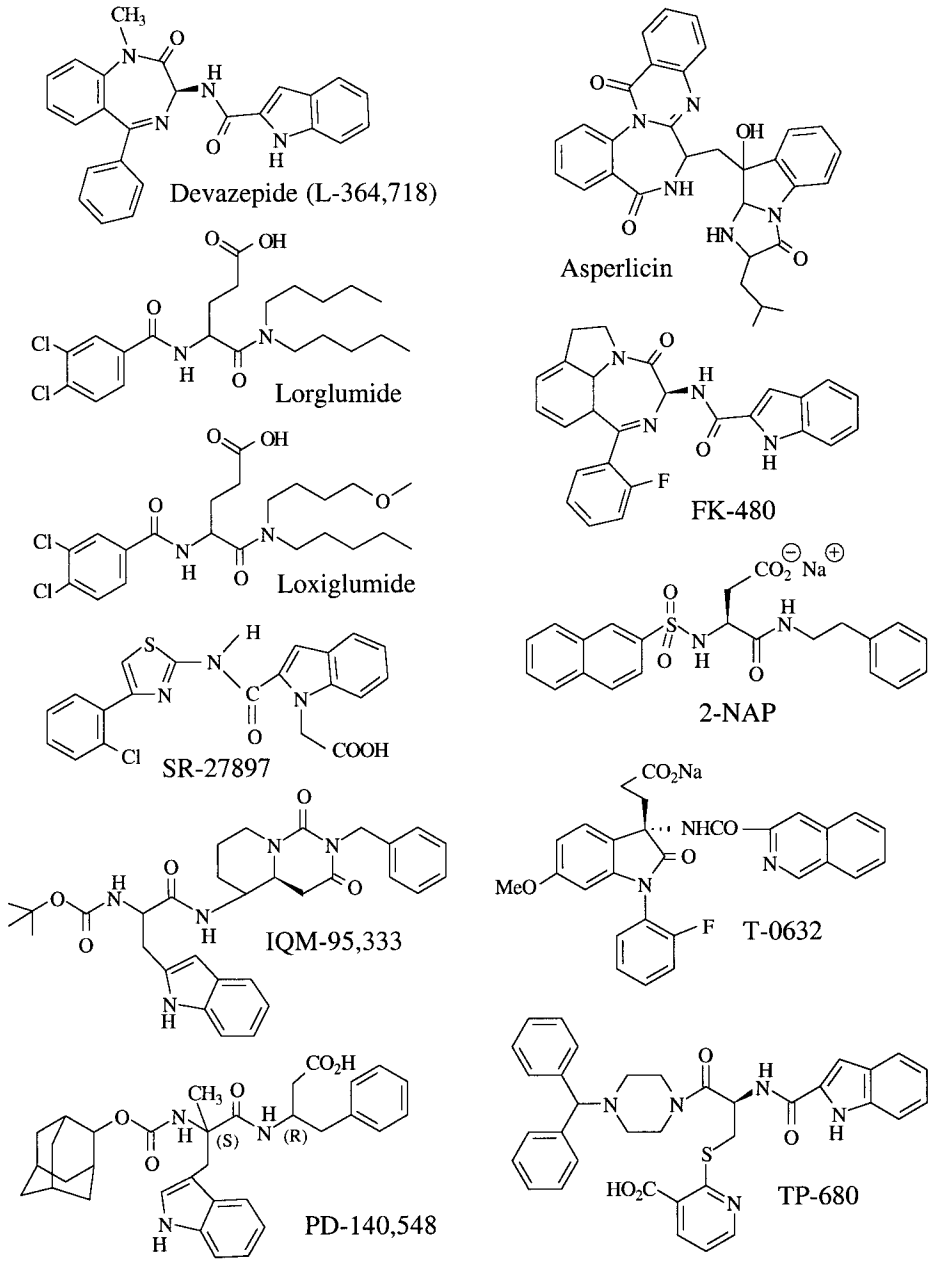
potent competitive antagonist (PD-140,548; Boden et al., 1993).

Several years ago, synthetic peptides with CCK_1 receptor antagonist properties were described (Lignon et al., 1987). One of these compounds, designated JMV-179 [Tyr(SO_3H)-Ahx-Gly-D-Trp-Ahx-Asp-phenylethylester], corresponds to the C-terminal heptapeptide of CCK in which the phenylalamide and the L-tryptophan residues were substituted by a phenylethyl ester and a D-tryptophan, respectively. In addition, to protect the peptide against oxidation, the two methionines were replaced by a 6-aminohexanoic acid (Ahx) residue. The pharmacological results obtained demonstrated that JMV-179 is a full CCK_1 receptor antagonist. In contrast, JMV-180 [Boc-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-phenylethylester] appeared to be an agonist of the stimulatory phase of the amylase release by pancreatic acini (low concentration range) and an antagonist of the inhibitory phase (high concentrations; Galas et al., 1988).

A new serine derivative, (*R*)-1-[3-(3-carboxypyridine-2-yl)-thio-2-(indol-2-yl)carbonylamino]propionyl-4-diphenylmethylpiperazine (TP-680)] has been recently developed (Akiyama et al., 1996; Tables 2 and 3). This compound showed approximately 2 and 22 times greater selectivity for CCK_1 receptors relative to CCK_2 receptors than L-364,718 and loxiglumide, respectively. Pharmacological data showed that TP-680 is a selective and irreversible antagonist of CCK_1 receptors (Akiyama et al., 1996).

Other CCK_1 receptor antagonists have been developed, such as T-0632 (Tables 2 and 3), which is a novel nonpeptide and water-soluble compound that inhibits the specific binding of ^{125}I-CCK-8 to rat CCK_1 receptor in a concentration-dependent and competitive manner. The K_i value of T-0632 for the CCK_1 receptor, 0.24 nM, is 23,000-fold less than its K_i value (5,600 nM) for the CCK_2 receptor (Taniguchi et al., 1996).

TABLE 2
CCK_1 receptor antagonists



Devazepide (L-364,718), (3*S*)-(−)-*N*-(2,3 dihydro-1-methyl-2-oxo-5-phenyl-1 *H*-1,4-benzodiazepine-3-yl)-1*H*-indole-2-carboxamide; lorglumide, (±)-4-[(3,4-dichlorobenzoyl)amino]-5-(di-*n*-pentylamino)-5-oxopentanoic acid; loxiglumide, (±)-4-[(3,4-dichlorobenzoyl)amino]-5-(*N*-(3-methyoxypropyl)-*N*-pentylamino]-5-oxopentanoic acid; SR 27897, 1-[[2-(4-(chlorophenyl)thiazol-2-yl)aminocarbonyl]indolyl]acetic acid; IQM-95,333, (4α*S*,5*R*)-2-benzyl-5[*N*-(*tert*-butoxycarbonyl)-L-tryptophyl]amino-1,3-dioxoper-hydropyrido[1,2-*c*]pyrimidine; FK-480, (*S*)-*N*-[1-(2-fluorophenyl)-3,4,6,7-tetrahydro-4-oxo-pyrrolo[3,2,1-*jk*][1,4]benzodiazepin-3-yl]-1*H*-indole-2-carboxamide; 2-NAP, 2-naphthalenesulfonyl-L-aspartyl-(2-phenethyl)amide; T-0632, sodium (*S*)-3-[1-(2-fluorophenyl)-2,3-dihydro-3-[(3-isoquinolinyl-carbonyl)amino]-6-methoxy-2-oxo-1*H*-indole]propanoate; TP-680, (*R*)-1-[3-[(3-carboxypyridin-2-yl)thio]-2-[(indol-2-ylcarbonyl)amino]propionyl]-4-(diphenylmethyl)piperazine; PD-140,548, *N*-(α-methyl-*N*-[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]-L-tryptophyl]-D-3-(phenylmethyl)-β-alanine.

Interest in nonpeptide CCK receptor-selective ligands has directed efforts toward the incorporation of conformationally restricted structures as spacers between Trp and Phe residues in the sequence of the CCK_2 receptor endogenous ligand CCK-4 (Trp-Met-Asp-Phe-NH_2). Thus, recently, a new series of CCK-4 restricted analogs with a 3-oxoindolizidine ring were synthesized. The most remarkable results were obtained with IQM-95,333 (Tables 2 and 3), which displays a CCK_1 receptor affinity (K_i = 0.62 nM) similar to that of L-364,718, but with a much higher selectivity (K_i CCK_2/K_i CCK_1 > 8000; Martin-Martinez et al., 1997).

Another CCK_1 receptor antagonist, SR-27,897 (Tables 2 and 3), which is chemically unrelated to peptoids, benzodiazepines, or glutamic acid derivatives, has been developed. This compound was obtained by optimization of a lead compound discovered through the random screening of a large chemical library. SR-27,897 is a

TABLE 3
Affinities of CCK_1 receptor antagonists in brain and pancreas membranes

Antagonist	K_i		Selectivity CCK_2/CCK_1	Reference
	CCK_1	CCK_2		
	nM			
L-364,718	0.1	375	3,750	Evans et al. (1986)
SR-27897	0.2	160	800	Gully et al. (1993)
IQM-93,333	0.6	>5,000	>8,000	Martin-Martinez et al. (1997)
PD-140,548	2.8[a]	260[a]	93	Boden et al. (1993)
FK-480	0.4[a]	72[a]	180	Ito et al. (1994a)
2-NAP	250	70,000	300	Hull et al. (1993)
T-0632	0.24	5,600	23,000	Taniguchi et al. (1996)
TP-680	1.2	1,812	1,510	Akiyama et al. (1996)

[a] IC_{50} value.

highly potent (K_i = 0.2 nM) and selective (CCK_2/CCK_1 IC_{50} = 800) antagonist of CCK_1 receptors (Gully et al., 1993).

3. Agonists of CCK_1 Receptors. Only a few compounds have been reported to be CCK_1-selective agonists; most of them are tetrapeptides, hexapeptides, and benzodiazepine derivatives.

Two series of CCK analogs have been developed. One series, exemplified by A-71378 [des-NH_2-Tyr(SO_3H)-Nle-Gly-Trp-Nle-(NMe)Asp-Phe-NH_2], contains an (NMe)Asp residue that is critical for CCK_1 receptor selectivity (Holladay et al., 1992). The other series derived by replacement of the methionine residue of Boc-CCK-4 (Boc-Trp-Met-Asp-Phe-NH_2) with side chain-substituted Lys derivatives: Boc-Trp-Lys(X)-Asp-(NMe)Phe-NH_2, such as A-71623 (X = *o*-toluylaminocarbonyl [Tac]) and A-70874 (X = *p*-hydroxycinnamoyl [Hyc]; Lin et al., 1991; Tables 4 and 5). Exploration of this tetrapeptide series continued through the examination of the effects of *N*-methylation at the Asp residue. The results obtained showed that analogs containing either (NMe)Asp or (NMe)Asp-(NMe)Phe are highly potent (IC_{50} values in the nanomolar range) and selective CCK_1 receptor agonists (Holladay et al., 1992).

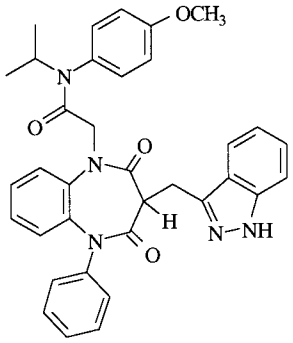
The sulfate ester of CCK-8 borne by the tyrosine residue is a critical determinant of the biological activity of this peptide. To increase the stability of this molecule, the sulfated tyrosine has been replaced by a synthetic amino acid (LD)-Phe(*p*-CH_2SO_3Na) in which the OSO_3H group was replaced by the nonhydrolyzable CH_2SO_3H group. The biological activity of the new derivative (LD)-Phe(*p*-CH_2SO_3Na)-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 displays high affinity for CCK_1 and CCK_2 receptors (nanomolar range; Marseigne et al., 1989).

In the hexapeptide series, it has also been reported that replacement of Asp-Tyr(SO_3H) of CCK-8 with Hpa(SO_3H) (Hpa is 4-hydroxyphenylacetyl) and *N*-methylation of Phe do not diminish the affinity for CCK_1 or CCK_2 receptors (Pierson et al., 1997). Inversion of the chirality of Asp7 in conjunction with *N*-methylation of Phe8 produces a compound [Hpa(SO_3H)-Met-Gly-Trp-Met-D-Asp-MePhe-NH_2] that exhibits high affinity and 2100-fold selectivity for CCK_1 receptors. Moreover, moving the *N*-methyl group from Phe to Asp decreased the affinity for CCK_2 receptors without affecting that for CCK_1 receptors, giving a compound Hpa(SO_3H)-Nle-Gly-Trp-Nle-MeAsp-Phe-NH_2 (ARL-15849; Tables 4 and 5) with a 6600-fold higher selectivity for the latter receptors (Pierson et al., 1997).

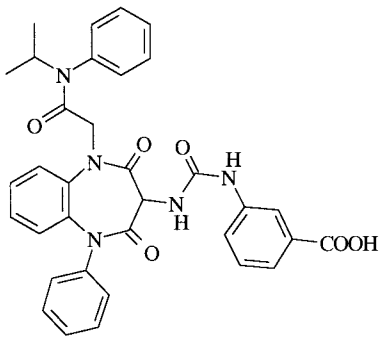
Recently, a series of 1,5-benzodiazepines acting as CCK_1 receptor agonists in vitro and in vivo were discovered. Potency within this series was modulated by substituents on the *N*1-anilinoacetamide moiety (Aquino et al., 1996), with substitution and/or replacement of the C3-position phenylurea moiety (GW5823, GW7854; Hirst et al., 1996; Willson et al., 1996; Henke et al., 1997; Tables 4 and 5).

TABLE 4
CCK_1 receptor agonists

des-NH_2-Tyr(SO_3H)-Nle-Gly-Trp-Nle-(NMe)Asp-Phe-NH_2	A-71378
Boc-Trp-Lys(*o*-tolylaminocarbonyl)-Asp-MePhe-NH_2	A-71623
Boc-Trp-Lys(*p*-hydroxycinnamoyl)-Asp-(NMe)Phe-NH_2	A-70874
4-hydroxyphenylacetyl(SO_3H)-Nle-Gly-Trp-Nle-(Me)Asp-Phe-NH_2	ARL-15849



GW-5823



GW-7854

GW-5823, 2-[3-(1*H*-indazol-3-ylmethyl)-2,4-dioxo-5-phenyl-2,3,4,5-terahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-isopropyl-*N*-(4-methoxyphenyl) acetamide; GW-7854, 3-[3-[1-[(isopropylphenylcarbamoyl)methyl]-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepin-3-yl]ureido] benzoic acid.

TABLE 5
Affinities of CCK_1 receptor agonists in brain and pancreas membranes

Agonist	K_i		Selectivity CCK_2/CCK_1	Reference
	CCK_1	CCK_2		
	nM			
A-71378	0.5[a]	570[a]	1140	Holladay et al. (1992)
A-71623	3.7[a]	4500[a]	1200	Lin et al. (1991)
A-70874	4.2[a]	710[a]	170	Lin et al. (1991)
ARL-15849	0.03	224	6590	Pierson et al. (1997)
GW-5823	22.9[a]	1000[a]	50	Henke et al. (1997)

[a] IC_{50} value.

B. CCK_2 (CCK-B) Receptors

1. CCK_2 Receptor Clones. Affinity crosslinking studies of the CCK_2 receptor using ^{125}I-[Leu or NLeu^{15}]-gastrin-2-17,disuccinimidyl suberate and either a 60 to 70% pure canine gastric parietal cell preparation or a solubilized porcine gastric mucosal extract identified two glycoproteins of 78 and 74 kDa, respectively (Svoboda et al., 1982; Baldwin et al., 1986; Chiba et al., 1988; Baldwin, 1993).

Using low-stringency hybridization methods, the CCK_2 receptor cDNA was cloned from a rat pancreatic acinar carcinoma cell line (AR4–2J) cDNA library known to express CCK_2/gastrin receptors. This cDNA was shown to be identical with the CCK_2 receptor cDNA cloned from a rat brain cDNA library (Wank et al., 1992b). At the same time, the gastrin receptor cDNA was also cloned from a canine parietal cell cDNA library using a COS cell plasmid expression approach (Kopin et al., 1992). The rat and canine CCK_2 receptors are 452 and 453 amino acids long, respectively, and share an 84% amino acid identity. This degree of homology is consistent with interspecies variations of the same receptor and has been considered as an early indication that the gastrin receptor is simply the CCK_2 expressed in the stomach (see below). Similar to the CCK_1 receptor, hydropathy analysis predicts seven TM domains as expected of a member of the GPCR superfamily (Dohlman et al., 1991). The sequence contains at least three consensus sites for *N*-linked glycosylation (Asn-X-Ser/Thr), consistent with the heavy and variable degree of glycosylation reported using ligand affinity crosslinking techniques (Baldwin et al., 1986; Chiba et al., 1988; Baldwin, 1993). Similar to the CCK_1 receptor, there are conserved cysteines in the first and second ECLs that may form a disulfide bridge required for stabilization of the tertiary structure (Silvente-Poirot et al., 1998), and a cysteine in the C terminus of the receptor may serve as a membrane-anchoring palmitoylation site (O'Dowd et al., 1988; Ovchinikov et al., 1988; Fig. 3).

To date, the CCK_2 receptor has been cloned through low-stringency hybridization of cDNA libraries from various sources: rat brain and stomach, the pancreatic tumoral cell line AR4–2J (Wank et al., 1992b), human brain (Pisegna et al., 1992; Ito et al., 1993; Lee et al., 1993; Denyer et al., 1994) and stomach (Pisegna et al., 1992), and guinea pig gallbladder and stomach (de Weerth et al., 1993b). In addition, CCK_2 receptor cloning has been achieved from gastric enterochromaffin and parietal cells and brain of *Mastomys natalensis* (Nakata et al., 1992), calf pancreas (Dufresne et al., 1996), and a rabbit genomic library (Blandizzi et al., 1994; Table 1). The CCK_2 receptor is highly conserved in humans, canine, guinea pig, calf, rabbit, *M. natalensis*, and rat, with an overall amino acid identity of 72% and pairwise amino acid sequence identities of 84 to 93%.

2. Gastrin Receptors Are CCK_2 Receptors. Gastrin receptors in the stomach and CCK_2 receptors in the brain were historically viewed as distinct types of CCK receptors on the basis of their different relative affinities for CCK and gastrin-like peptides (Menozzi et al., 1989). However, the canine parietal cell gastrin receptor expressed in COS cells exhibits the same relative affinities for CCK-8 and gastrin as those of native human and guinea pig CCK_2 receptors. The canine parietal gastrin receptor was also considered to be a distinct receptor because of a reversal in affinity for L-364,718 versus L-365,260 in comparison with CCK_2 receptors in the brain of other species (Lotti and Chang, 1989). The basis for this reversal has subsequently been ascribed to a species-specific change of a single nucleotide resulting in a single amino acid substitution (Leu355 in canine receptor versus Val319 in the human receptor) in TMVI (Beinborn et al., 1993). Similar to the human, guinea pig, and rat CCK_2 receptors (Pisegna et al., 1992; Wank et al., 1992b), cloning of the CCK_2 receptor from canine brain (Wank, 1995) resulted in a single cDNA identical to that for the canine parietal cell gastrin receptor (Kopin et al., 1992). Clearly, the identification of a single CCK_2 receptor-encoding gene through low- and high-stringency hybridization of cDNA and genomic libraries and Northern and Southern blot analyses in numerous species indicates that gastrin receptors do correspond to CCK_2 receptors located in the gastrointestinal tract and do not constitute a third type of CCK receptor (Wank, 1995).

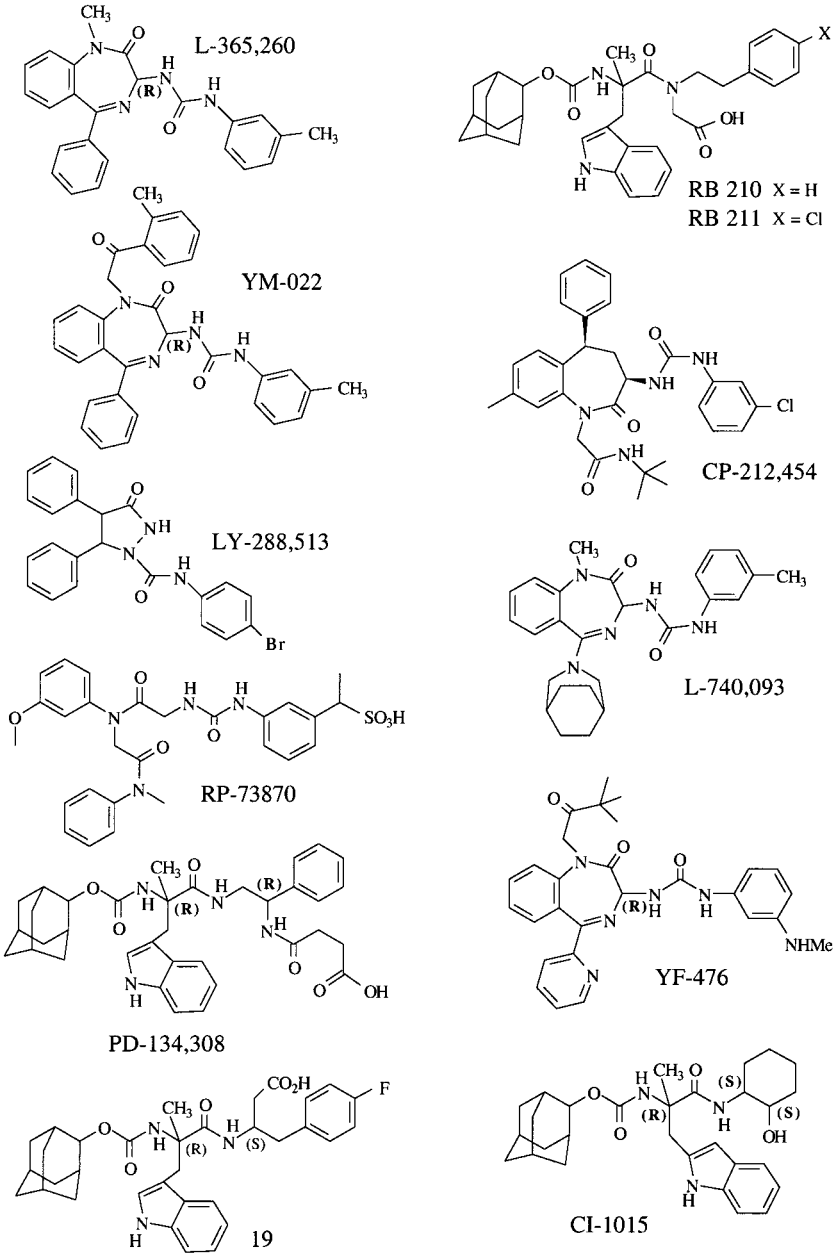
3. Antagonists of CCK_2 Receptors. Many attempts have been made to develop potent and specific nonpeptide antagonists of CCK_2/gastrin receptor. As a result, several new chemical entities appeared, exhibiting high selectivity for specific populations of CCK_2/gastrin receptors. The various compounds under development belong to the following main chemical classes: amino acid, benzodiazepine, dipeptoid, pyrazolidinone, and ureidoacetamides derivatives (for a review, see Makovec and D'Amato, 1997).

Efforts were notably devoted to the design of an optimized asperlicin structure. Because the asperlicin structure is composed of several heterocyclic domains, it was hypothesized that alternative substructures embedded within the molecular framework of this natural product may provide a rational starting point for the design of novel nonpeptide CCK receptor ligands. On this basis, scientists at Eli Lilly Corp. developed a series of quinazoline derivatives by using a bond disconnection approach (Yu et al., 1991). A combination of the key fragments of the Lilly and Merck series led to the development of novel nonpeptide CCK_2 receptor antagonists with substitution on the quinazolinone and phenyl rings. Binding data for this class of compounds suggest that the linker between these rings is a critical determinant for CCK_2 receptor-binding affinity. However, these new compounds have a low selectivity for

CCK_2 receptor (Padia et al., 1997). Indeed, the spatial arrangement of the two moieties appears to be critical for both potency and selectivity. The introduction of —NH— as a linker significantly enhanced CCK_2 receptor-binding affinity and selectivity, providing compounds with nanomolar binding affinity and good selectivity (K_i CCK_1/K_i CCK_2 > 500). Moreover, these compounds are active when administered per os (Padia et al., 1998).

On the other hand, the moderate affinity of L-364,718 for CCK_2 receptors suggested that the benzodiazepine nucleus might also hold a key to selective ligands for these receptors. The first compound of interest developed using this strategy was L-365,260 (Tables 6 and 7),

TABLE 6
CCK_2 receptor antagonists



L-365,260, (3*R*)-(+)-*N*-(2,3-dihydro-1-methyl-2-oxo-phenyl-1*H*-1,4-benzodiazepin-3-yl)-*N*′-(3-methylphenyl)urea; YM-022, (*R*)-1-[2,3-dihydro-1-(2′-methylphenacyl)-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl]-3-(3-methyl-phenyl)urea; LY-288,513, (4*S*,5*R*)-*N*-(4-bromophenyl)-3-oxo-4,5-diphenyl-1-pyrazolidine carboxamide; RP-73870, {{[*N*-(methoxy-3-phenyl)-*N*-(*N*-methyl-*N*-phenyl-carbamoylmethyl)-carbamoylmethyl]-3-ureido}-3-phenyl}-2-ethyl-sulfonate-(*RS*); PD-134,308, 4-{[2-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[tricyclo-[3.3.1.1^{3,7}]-dec-2-yloxy)-carbonyl]amino]propyl]amino]-1-phenyl-ethyl]amino)-4-oxo-[*R*-(*R**,*R**)]butanoic acid; RB 210, *N*-[*N*-[(2-adamantyloxy)carbonyl]-DL-α-methyltryptophanyl]-*N*-(2-phenylethyl)glycine; compound 19 (Augelli-Szafran et al., 1996), 3-[2-(adamantan-2-yloxycarbonylamino)-3-(1*H*-indol-3-yl)-2-methylpropronyl-amino]-4-(4-fluorophenyl)butyric acid; CP-212,454, *N*-*tert*-butyl-2-[3-(3-(3-chlorophenyl)ureido)-2-oxo-5-phenyl-8-methyl-2,3,4,5-tetrahydro-1*H*-1-benzazepin-1-yl]ethanoic acid amide; L-740,093, *N*-[(3*R*)-5-(3-azabicyclo[3.2.2]nonan-3-yl)-2,3-dihydro-1-methyl-2-oxo-1*H*-1,4-benzodiazepin-3-yl]-*N*′-(3-methylphenyl)urea; YF-476, (3*R*)-*N*-(1-(*tert*-butylcarbonylmethyl)-2,3-dihydro-2-oxo-5-(2-pyridyl)-1*H*-1,4-benzodiazepin-3-yl)-*N*′-(3-(methylamino)phenyl)urea; CI-1015, tricyclo[3.3.1.1^{3,7}]dec-2-yl[1*S*-[1α(*S**)2β]-[2-[(2-hydroxycyclohexyl)amino]-1-(1*H*-indol-3-ylmethyl)-1-methyl-2-oxoethyl]carbamate.

TABLE 7
Affinities of CCK_2 receptor antagonists in brain and pancreas membranes

Antagonist	K_i		Selectivity CCK_1/CCK_2	Reference
	CCK_1	CCK_2		
	nM			
L-365,260	800	7	115	Lotti and Chang (1989)
PD-134,308	1,440	0.9	1,600	Horwell et al. (1991)
LY-288,513	11,600	31	370	Howbert et al. (1992)
RP-73,870	1,634	0.5	3,300	Pendley et al. (1995)
YM-022	150	0.1	1,500	Nishida et al. (1994)
RB-210	1,518	14	110	Blommaert et al. (1993)
CP-212,454	180	0.5	360	Lowe et al. (1995)
L-740,093	1,600	0.1	16,000	Patel et al. (1994)
YF-476	113[a]	0.2[a]	565	Semple et al. (1997)
CI-1015	2,900	3	967	Trivedi et al. (1998)

[a] IC_{50} value.

which revealed to be the first potent and selective nonpeptide CCK_2 receptor antagonist (Bock et al., 1989). One factor that determined CCK receptor selectivity in this series was the C3-stereochemistry of the benzodiazepine ring system, with the (3*R*)-enantiomer generally providing CCK_2 receptor selectivity. Moreover, recent studies have shown that the C5-phenyl moiety of the core benzodiazepine structure could be replaced by C5-cycloalkyl groups, a modification that retained CCK_2 receptor affinity and selectivity. In particular, the C5-cyclohexyl analog displayed subnanomolar affinity for CCK_2 receptors (IC_{50} = 0.28 nM), with improved selectivity (K_i CCK_1/K_i CCK_2 = 6500) compared with L-365,260 (Chambers et al., 1993).

A major drawback associated with these early benzodiazepine-derived CCK_2 antagonists was their limited bioavailability and inactivity via the oral route of administration. The incorporation of a (*tert*-butylcarbonyl) methyl group at the 1-position (Semple et al., 1996a) or a 2-pyridyl group at the 5-position (Semple et al., 1996b) of the parent benzodiazepine structure provides a significant increase in absorption. Similar results have been achieved through the incorporation of an amine-based cationic solubilizing group within the benzodiazepine framework, with a cyclic amine to form an amidino functionality in the 5-position (L-740,093; Showell et al., 1994; Tables 6 and 7). Other attempts to improve aqueous solubility included the introduction of acidic groups (L-368,935 and L-369,466; Bock et al., 1994) or lipophilic surrogates (Chambers et al., 1995) into the 3-position of the aryl urea component of either the 1,4-benzodiazepin-2-one parent system or closely related structures (CP-212,454; Lowe et al., 1995; Tables 6 and 7). The opposite strategy has also been used with the introduction of basic amino substituents into the same region. YM022 is the optimal structure of this new series, with subnanomolar affinity for CCK_2 receptors (Nishida et al., 1994). Moreover, when these modifications are combined within the same molecule, the resulting improvements in the in vivo effects appear to be essentially additive, as shown by the compound YF476 (Tables 6 and 7), which has a good oral bioavailability in dogs (Semple et al., 1997).

Other nonpeptide CCK_2 receptor antagonists have been developed, derived through rational design from the CCK tetrapeptide (Hughes et al., 1990). This led to tryptophan dipeptoid derivatives such as PD-134,308 (CI-988; Tables 6 and 7) with nanomolar affinity for CCK_2 receptors (Horwell, 1991; Horwell et al., 1991). PD-134,308 exhibits a 1600-fold selectivity for CCK_2 over CCK_1 receptors. C-terminal modifications of this compound led to molecules with subnanomolar affinity for CCK_2 receptors. For example, further attempts to optimize the substitution on the phenyl ring led to a compound 19, which has an extraordinarily high affinity for the CCK_2 receptor (IC_{50} = 0.08 nM) and a high degree of selectivity (K_i CCK_1/K_i CCK_2 = 940; Augelli-Szafran et al., 1996). A direct comparison of the structure of the dipeptoid derivatives showed that the size of these molecules could be reduced to increase their lipophilicity. Such compounds have been synthesized, and some of them have been found to be potent and selective CCK_2 receptor antagonists. Moreover, as expected, one of them (RB 211) was shown to be more efficient in crossing the blood-brain barrier than the parent compounds (Blommaert et al., 1993) and devoid of the weak CCK_1 receptor agonist properties of dipeptoids (Höcker et al., 1993; Ding et al., 1995). On the other hand, to improve the properties of PD-134,308, numerous conformational restrictions were introduced in its structure. Unfortunately, neither N-terminal cyclization (Fincham et al., 1992b), macrocyclization (Didier et al., 1992; Bolton et al., 1993), nor rigidification of the amide bond (Fincham et al., 1992a) led to any positive result. Only a C-terminal cyclization of PD-134,308 derivatives, by means of a tetrahydronaphtyl group, has been reported to increase the affinity for CCK_2 receptors (Higginbottom et al., 1993). This approach has also been used for compounds such as RB 210 (Tables 6 and 7), in which C-terminal constraints can be easily introduced. Thus, the β-carbon of the phenethyl side chain of RB 210 was linked to the α-carbon bearing the carbonyl function, by means of a methylene bridge. This resulted in the formation of a proline ring (Bellier et al., 1997). The most potent compounds of this new series had similar affinities for CCK_2 receptors as RB 210. Structure-affinity relationships of this series indicated that lengthening of the distance between the amide nitrogen atom and the phenyl ring was of little importance, whereas the position of the carboxylate group could not be modified. Therefore, the pyrrolidine ring was replaced by piperidine to slightly modify the possible orientation of the aromatic moiety toward the carboxylate without violating any of the requirements previously established in both linear and constrained series for the recognition of CCK_2 receptors. However, the resulting compounds behave as moderately potent CCK_2 receptor antagonists (Bellier et al., 1998).

As previously mentioned, the clinical development of PD-134,308 (CI-988) was limited due to its poor bioavailability, which was attributed to poor absorption and efficient hepatic extraction. Scientists at Parke-Davis also envisaged that reducing the molecular weight of the parent compound would lead to better absorption. Thus, they synthesized a series of analogs in which the key α-methyltryptophan and adamantyloxycarbonyl moieties, required for receptor binding, were kept intact and the C terminus was extensively modified. These modifications led to compounds such as CI-1015 (Tables 6 and 7) for which the oral bioavailability in rat was improved nearly 10-fold and the blood-brain barrier permeability was also enhanced relative to CI-988 (Trivedi et al., 1998).

Two other series have been described, leading to the synthesis of derivatives that have both excellent selectivity and high affinity for CCK_2 receptors: the ureidoacetamide class of CCK_2 receptor antagonists (RP-73,870; Pendley et al., 1995) and the pyrazolidinones (LY-288,513; Howbert et al., 1992; Tables 6 and 7). Development of the latter series has been discontinued because of adverse effects in preclinical toxicological studies. The nonpeptide ureidoacetamides are potent and selective ligands with nanomolar or subnanomolar affinities for CCK_2 receptors and a 100- to 1000-fold selectivity for these receptors over CCK_1 receptors. Despite its relatively poor oral bioavailability, RP-73,870 was as potent as other antiulcer compounds after oral administration in a duodenal ulceration model (Pendley et al., 1995).

4. Agonists of CCK_2 Receptors. Different strategies have been followed to design potent and selective agonists of CCK_2 receptors. One of these was to protect CCK-8 [Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2] from degrading enzymes such as aminopeptidase A (Migaud et al., 1996) and a thiol/serine protease cleaving this peptide at the Met-Gly bond (Camus et al., 1989; Rose et al., 1996). The biologically active Boc[$Nle^{28,31}$]CCK27-33 (BDNL; Ruiz-Gayo et al., 1985) was used as the parent compound to design enzyme-resistant analogs. In this compound, the major sites of cleavage are at the Trp30/Nle31 and Nle28/Gly29 bonds. BDNL is potentially resistant to aminopeptidase cleavage due to its *tert*-butyloxycarbonyl N-terminal-protecting group (Ruiz-Gayo et al., 1985; Durieux et al., 1986a). Thus, several enzyme-resistant BDNL analogs containing either a retro-inversion of the Nle28-Gly amide bond, an (NMe)Nle31 residue, or a combination of these two modifications have been synthesized (Charpentier et al., 1988a). This led to BC 264 (Tables 8 and 9), a highly potent CCK_2 receptor agonist that exhibits about the same affinity (K_i = 0.1–0.5 nM) in all species (guinea pig, rat, mouse, monkey, humans) and was at that time the only systemically active CCK_2 receptor agonist (Charpentier et al., 1988a; Durieux et al., 1991). The peptidase-resistant bioactive analog [^{3}H]pBC264 was also developed (Durieux et al., 1989) by replacing the Boc group with a tritiated propionyl residue. The radioactivity present in the mouse brain 15 min after i.v. injection of the tritiated compound represented 1.6/10,000 of the total radioactivity injected. Moreover, as shown by HPLC, [^{3}H]pBC264 was very resistant to metabolism, because more than 85% of the radioactivity present in the brain corresponded to the intact molecule (Ruiz-Gayo et al., 1990). On the other hand, despite its intrinsic flexibility, CCK-8 was found through NMR to exist preferentially under a folded form in aqueous solution (Fournié-Zaluski et al., 1986) with a proximity between Asp1 and Gly4. This property was used to synthesize cyclic peptides through amide bond formation between Asp1 or between α- or β-carboxyl group of Glu1 and Lys4 side chains, such as BC 254 and BC 197 (Tables 8 and 9), which were found highly potent and selective CCK_2 receptor agonists (Charpentier et al., 1988b, 1989). Another nonsulfated CCK-8 analog, [*N*-methyl-$Nle^{28,31}$]CCK26-33 (SNF-8702; Tables 8 and 9), has also been described, which has about 4000-fold greater affinity for CCK_2 than for CCK_1 receptors (Knapp et al., 1990).

The role of the amino acid in position 31 of CCK-8 in the recognition of CCK_1 and CCK_2 receptors was investigated through the replacement of Met31 by amino acids with side chains of varying chemical nature. Thus, the introduction of a Phe residue in position 31 in Boc[$Nle^{28,31}$]CCK27-33 slightly modified the affinity for CCK_2 receptor (K_i = 3.7 nM) but led to a larger decrease (K_i = 220 nM) in the affinity for CCK_1 receptors. A similar discrimination was observed when the amino acid in position 31 is an alanine residue (Marseigne et al., 1988).

TABLE 8
CCK_2 receptor agonists

Boc-D.Asp-Tyr(SO_3H)-Nle-D.Lys-Trp-Nle-Asp-Phe-NH_2	BC 197
Boc-Tyr(SO_3H)-gNle-mGly-Trp-NMe(Nle)-Asp-Phe-NH_2	BC 264
HOOC-CH_2-CO-Trp-NMe(Nle)-Asp-Phe-NH_2	RB 400
Asp-Tyr(SO_3H)-(NMe)Nle-Gly-Trp-(NMe)Nle-Asp-Phe-NH_2	SNF-8702
Boc-γD-Glu-Tyr(SO_3H)-Nle-D.Lys-Trp-Nle-Asp-Phe-NH_2	BC 254

TABLE 9
Affinities of CCK_2 receptor agonists in brain and pancreas membranes

Agonist	K_i		Selectivity CCK_1/CCK_2	Reference
	CCK_1	CCK_2		
	nM			
BC 197	2900	150	20	Charpentier et al. (1989)
BC 264	78	0.1	780	Charpentier et al. (1988b)
RB 400	>3000	0.42	>7000	Million et al. (1997)
SNF-8702	3800	0.9	4000	Knapp et al. (1990)
BC 254	2500	0.56	4500	Charpentier et al. (1989)

Because nonpeptide ligands have historically offered greater opportunity for manipulation of both pharmacodynamic (selectivity and efficacy) and pharmacokinetic (oral bioavailability, duration) parameters, the development of nonpeptidic CCK_2 receptor selective agonists endowed with good stability and bioavailability should provide useful pharmacological tools and possibly therapeutic agents. To design such derivatives, the C-terminal tetrapeptide CCK-4 appeared to be a good molecule to start with, because of its significant CCK_2 receptor affinity and selectivity, although it has been shown to trigger panic attacks in humans (de Montigny, 1989; Bradwejn et al., 1991b). Several modifications were made to CCK-4, such as the N-terminal protection of the tetrapeptide in Boc-CCK_4 (Harhammer et al., 1991) or modifications of the different amino acids such as the replacement of Met by Nle or (NMe)Nle (Corringer et al., 1993). Recent NMR and molecular dynamics studies indicated that the CCK_2 receptor-selective CCK-4 analogs adopt an S-shaped conformation with a relatively well-defined orientation of the side chains (Goudreau et al., 1994). The same type of folded structures has been reported for several potent agonists derived from CCK-4 and containing a [*trans*-3-propyl-L-proline] (Nadzan et al., 1991), a diketopiperazine skeleton (Shiosaki et al., 1990), or a [(alkylthio)proline] residue (Kolodziej et al., 1995). With this template, other cyclic CCK-4 analogs have been synthesized in which the Trp-Met dipeptide was changed to a diketopiperazine moiety resulting from a cyclization between Nle and *N*-substituted (D)Trp residues and coupled with a small linker to Asp-Phe-NH_2 (Weng et al., 1996a). Moreover, the side chain of Nle in the compound Boc-Trp-(NMe)Nle-Asp-Phe-NH_2 together with the N terminus of Trp appeared to be good candidates for another possible cyclization. Thus, cyclic compounds were designed through molecular modeling to mimic the proposed biologically active conformation of these CCK-4 analogs. The goal of this study was to stabilize the bioactive conformation of CCK_2 receptor agonists to aid in the design of nonpeptide ligands. This led to the development of macrocyclic constrained CCK-4 analogs that are endowed with agonist properties and able to cross the blood-brain barrier (Blommaert et al., 1997).

Selective and peptidase-resistant CCK_2 receptor ligands that derive from Boc-[Nle^{31}]CCK30-33 through the incorporation of non-natural hydrophobic amino acids have also been developed (Weng et al., 1996b). Among these compounds, Boc-[Phg^{31},Nal^{33}]CCK30-33 proved to be a full agonist at rat hippocampal CCK_2 receptors. Moreover, it appeared that modifications of the hydrophobic and steric character of either the C- or N-terminal amino acid substituents of CCK-4 derivatives could affect the agonist or antagonist profile of these peptides. This was shown by the fact that the agonist Boc-[Phg^{31},Nal^{33}]CCK30-33 could be chemically converted to an antagonist through the addition of two alkyl groups on the terminal $CONH_2$ (Weng et al., 1996b).

Very recently, a new series of highly potent and selective CCK_2 receptor agonists were developed (Million et al., 1997). Boc-Trp-(NMe)Nle-Asp-Phe-NH_2, the C-terminal tetrapeptide of BC 264, was shown to have a high affinity and to behave as a specific agonist at CCK_2 receptors and to adopt the S-shaped preferential conformation. To determine the essential structural components of specific CCK_2 receptor agonists, a step-by-step lengthening of the C-terminal tetrapeptide of BC 264 was carried out. Various diacidic moieties, such as malonate or succinate residues, were coupled to the N-terminal portion of the tetrapeptide, leading to RB 400 [HOOC-CH_2-CO-Trp-(NMe)Nle-Asp-Phe-NH_2] and RB 403 (Tables 8 and 9). RB 400 was also derivatized under its benzylamide and methyl ester forms. Compounds that belong to the RB 400 series possess high affinities for the CCK_2 receptor, with a subnanomolar affinity (K_i = 0.42 nM) being obtained in case of RB 400 itself (Million et al., 1997).

III. Molecular Biology of CCK Receptors

A. CCK Receptor Gene Structure

The genes encoding the CCK_1 receptor (Miller et al., 1995; Wank, 1995; Inoue et al., 1997) and the CCK_2 receptor (Song et al., 1993) in humans are organized in a similar manner consisting of five exons and four introns. The receptor genes have homologous exon/intron splice sites with exon 1 coding for the extracellular N-terminal sequence, exon 2 coding for the sequence from the beginning of TMI to the first part of TMIII, exon 3 coding for the sequence from TMIII to the beginning of TMV, exon 4 coding for the sequence from TMV to the first fourth of the third intracellular loop, and exon 5 coding for the remainder of the receptor (Fig. 4). The genes for the rat (Takata et al., 1995) and mouse (Lacourse et al., 1997) CCK_1 receptors and rabbit (Blandizzi et al., 1994) and mouse (Nagata et al., 1996) CCK_2 receptors are organized similarly to those for humans. This high degree of conservation of the sequence and organization between CCK_1 and CCK_2 receptor genes and the fact that the brain and pancreas of the bullfrog *Rana catesbeiana* and *Xenopus laevis* express only one CCK receptor (Vigna et al., 1984, 1986) suggest that the

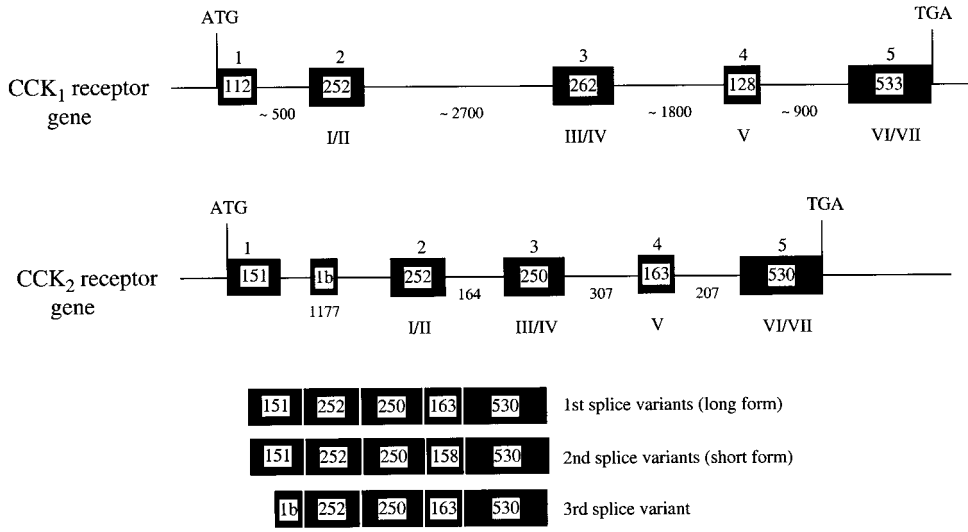


FIG. 4. Schematic representation of genes encoding human CCK_1 and CCK_2 receptors. Shown are position and size of the exons (shaded boxes) and introns (lines) comprising the genes for the CCK_1 and the CCK_2 receptors; smaller arabic numbers represent size of each exon and intron in base pairs. Roman numerals refer to putative transmembrane-spanning regions encoded within each exon. ATG and TGA, putative start and stop codons, respectively. CCK_2 receptor gene: the second splice variant (short form) differs only in the size of exon 4, in which a sequence is absent compared with long form, corresponding to a block of five amino acids within the third intracellular loop. The third splice variant encodes an N-terminally truncated receptor. The gene structure is similar, except that there is an alternative first exon (exon 1b) that makes up the 5′ untranslated region of this truncated receptor.

CCK_1 and CCK_2 receptor genes evolved sometime after amphibia from duplication of a common ancestral gene (as for the gene encoding the receptor ligands, CCK and gastrin). This concept is further supported by the cloning of a gene encoding a CCK receptor from a *X. laevis* brain cDNA library. This receptor is expressed in brain and stomach but is undetectable in pancreas. The deduced amino acid sequence from this gene has 55 and 56% amino acid identity with the human CCK_1 and CCK_2 receptors, respectively. This receptor expressed in COS-7 cells has a CCK_1 receptor type pharmacological profile (sulfated CCK > gastrin-17 > nonsulfated CCK-8 > CCK-4) like that of the native receptor in *X. laevis* brain and pancreas (Vigna et al., 1986; Schmitz et al., 1996) but with a relatively high affinity for sulfated gastrin, as expected for a CCK_2 receptor. Nevertheless, like typical CCK_1 receptors, the CCK receptor obtained from the *X. laevis* brain cDNA library has a higher affinity for L-364,718 than for L-365,260, and it is not recognized by CAM 1714 or CAM 1028 (Schmitz et al., 1996).

Alternative splicing of exon 4 of the human CCK_2 receptor gene results in two CCK_2 receptor transcripts that differ by a block of five amino acids within the third intracellular loop (Song et al., 1993; Fig. 4). The shorter transcript is largely predominant in stomach, although its relative distribution in individual cell types has not been examined. To date, the physiological relevance of the two isoforms of the human CCK_2 receptor is not known. A comparison of the shorter and longer isoforms revealed no significant differences in agonist affinity and signal transduction (Ito et al., 1993, 1994b; Wank et al., 1994b).

Another splice variant of the human CCK_2 receptor transcript, designated ΔCCK_2 receptor, which differs at the 5′ end from the CCK_2 receptor transcript described earlier, was discovered using a polymerase chain reaction-based cloning strategy (Miyake, 1995). ΔCCK_2 receptor encodes an N-terminally truncated receptor that starts with the methionine Met67 in TMI and is otherwise identical in the remaining sequence. The gene structure is similar to that previously reported for the human CCK_2 receptor (Song et al., 1993) except that the first intron was of ~10 kb (compared with 1.177 kb) and contained the sequence for the alternative first exon that makes up the 5′ untranslated region of ΔCCK_2 receptor (Fig. 4). The first methionine of exon 2, which is common to both CCK_2 and ΔCCK_2 receptors, serves as the translational initiation site for the ΔCCK_2 receptor. ΔCCK_2 receptor transiently expressed in COS-7 cells has a ~3-fold lower affinity for CCK-8 and a ~30-fold lower affinity for gastrin compared with the CCK_2 receptor, but its affinity for the antagonists L-365,260 and L-364,718 is unchanged. Both CCK_2 and ΔCCK_2 receptor transcripts have been detected in brain, stomach, and pancreas through the use of reverse transcription-polymerase chain reaction (Miyake, 1995). According to the guidelines defined by the IUPHAR committee, because these splice variants do not appear to be major variants, they are not indicated by subscript lowercase letters.

On the other hand, Jagerschmidt et al. (1994) isolated several CCK_2 receptor mRNA isoforms from rat brain tissue, including a truncated mRNA species. Unspliced precursor mRNA and the mature form were identified in the cerebral cortex, hypothalamus, and hippocampus in

apparently differing proportions according to the region examined, suggesting that the expression of the CCK_2 receptor could be modulated at a post-transcriptional level. Thus, although five precursor mRNAs were found in the cerebral cortex and the hypothalamus, only one fully processed messenger was detected in the hippocampus. In the case of the cerebellum, only a completely unspliced mRNA form was found, which is in agreement with previous studies showing that CCK_2 receptor-binding sites are not expressed in this structure in the rat (Pélaprat et al., 1987).

B. Chromosomal Localization of CCK Receptor Genes

The human CCK_1 receptor gene has been localized to chromosome 4 using a panel of human/hamster hybrid DNAs (Huppi et al., 1995). The mouse CCK_1 receptor gene has been mapped to a syntenic region on chromosome 5 using a wild $\times$ inbred backcross panel of mice [(BALB/cAN $\times$ Mus spretus) $F_1 \times$ BALB/cAN] (Huppi et al., 1995). This region of mouse chromosome 5 is syntenic with human chromosome 4p16.2-p15.1 (Huppi et al., 1995). The human CCK_1 receptor was further mapped to 4p15.1-p15.2 using fluorescence in situ hybridization and physically mapped between the markers AFMb355ya5 and AFMa283yh5 (Inoue et al., 1997). The rat CCK_1 receptor gene has been localized to a syntenic region on chromosome 14 by fluorescence in situ hybridization (Takiguchi et al., 1997).

The human CCK_2 receptor has been localized to chromosome 11 in humans and a syntenic region on chromosome 7 in the mouse using a panel of human/hamster hybrid DNAs (Huppi et al., 1995). Fluorescence in situ hybridization of human metaphase chromosomal spreads has further localized the human CCK_2 receptor gene to the distal short arm of chromosome 11 (11p15.4; Song et al., 1993; Zimonjic et al., 1994). The colocalization of the CCK_1 receptor gene with the dopamine D_5 receptor gene at 4p15.1-p15.3 (Sherrington et al., 1993) and of the CCK_2 receptor gene with the gene encoding the dopamine D_4 receptor at 11p15.4-p15.5 (Gelernter et al., 1992; Pisegna et al., 1992) is especially interesting in view of the coexistence of CCK and dopamine in midbrain neurons and the regulation of mesolimbic dopaminergic pathways by both CCK_1 and CCK_2 receptors (Crawley and Corwin, 1994).

C. Animal Models without Detectable Levels of CCK Receptors

An inbred strain of Long Evans rats, the Otsuka Long-Evans Tokushima Fatty rats, that is considered to be a model for late-onset non-insulin-dependent diabetes mellitus, was discovered to have no detectable levels of CCK_1 receptor gene expression. Subsequent cloning of their CCK_1 receptor gene revealed a deletion of 6847 bp encompassing the promoter region and first and second exons (Takiguchi et al., 1997). Although these rats are known to have polygenic abnormalities, the presence of several metabolic and behavioral abnormalities has been attributed to the loss of CCK_1 receptor expression.

Targeted disruption of the CCK_2 receptor gene has been achieved in mice (Nagata et al., 1996). Homozygous mutant mice were viable and fertile and appeared to be grossly normal into adulthood (Langhans et al., 1997). $CCK_2^{-/-}$ mutant mice have much fewer gastric parietal and ECL cells than so wild-type animals, which is in line with the growth-promoting effects of gastrin at the CCK_2 receptor previously seen in patients with hypergastrinemia due to the Zollinger-Ellison syndrome. Also, as expected, these mice were hypochlorhydric and hypergastrinemic (Nagata et al., 1996). Together, these results demonstrate the importance of the CCK_2 receptor in maintaining the normal cellular composition and function of the gastric mucosa.

Moreover, the physiological implication of CCK_2 receptor can now be further investigated in CCK_2 receptor-deficient mice obtained through gene targeting. The first experiments reported with this interesting model show a critical role of CCK_2 receptors in memory process. CCK_2 receptor-deficient mice have an impairment of performance in the memory task (Sebret et al., 1999; for more details, see *VIIB4. CCK and Memory Processes*).

IV. Receptor Structure/Function Studies

A. Signal Transduction

1. CCK_1 Receptors. The modulation of CCK_1 receptor affinity by guanine nucleotides in early studies suggested that they belong to the GPCR superfamily. This has been confirmed through the cloning of CCK_1 receptors (Wank et al., 1992a), which revealed their seven-transmembrane receptor structure.

In the pancreas, CCK is well known to be a major regulatory peptide that stimulates digestive enzyme secretion. The mode of action of CCK has been extensively explored. CCK-stimulated enzyme secretion is believed to be initiated by the binding of CCK to CCK_1 receptors localized on pancreatic acinar cells. Furthermore, it has been shown that the breakdown of phosphatidylinositol 4,5-biphosphate, which thereby produces both diacylglycerol and inositol trisphosphate (IP_3), is activated by CCK_1 receptor stimulation. Subsequent activation of Ca^{2+} phospholipid-dependent protein kinase by diacylglycerol and intracellular Ca^{2+} mobilization induced by IP_3 have been considered to act synergistically to cause digestive enzyme secretion (Pandol et al., 1985). The insensitivity of CCK_1 receptor inositol phosphate signaling to pertussis toxin suggests that its couples through the G_q family of G proteins (Pang and Sternweiss, 1990). Recently, a study using both phospholipase C (PLC) and G protein α-subunit-specific antibodies indicated that both G_q and $G_{11\alpha}$ are present in pancreas and that the CCK_1 receptor couples to G_q or G_{11} to activate PLC-β1 in pancreatic cell membranes (Piiper et al., 1997).

On the other hand, it has been demonstrated in rat pancreatic acini that the CCK_1 receptors are coupled to the phospholipase A_2 (PLA_2)/arachidonic acid pathways to mediate Ca^{2+} oscillations and amylase secretion (Yule et al., 1993; Yoshida et al., 1997). Nevertheless, other studies have shown that there are at least two pathways responsible for the increased production of arachidonic acid in response to CCK_1 receptor stimulation. One is the sequential effects of phospholipase C (PLC) and diglyceride lipase on phosphatidylinositol, and the other involves the action of the PLA_2 effect on phosphatidylcholine. Both pathways cause stimulation of amylase release (Pandol et al., 1991). In addition to the activation of the PLC and PLA_2 signal-transduction pathways, CCK_1 receptor stimulation can lead to an increase in the adenylyl cyclase signal-transduction cascade (Marino et al., 1993).

Thus, CCK_1 receptor is capable of coupling to both PLC and adenylyl cyclase at physiological concentrations in native cells. It is not clear whether this is a result of the independent coupling of CCK_1 receptor to G_s and G_q or simply the result of G protein $\beta\gamma$-subunit activation of an isotope of adenylyl cyclase. A study using a chimeric CCK receptor in which the first intracellular loops between CCK_1 and CCK_2 receptors were exchanged showed that Arg68 and Asn69 belonging to the loop of CCK_1 receptor are important for the stimulatory coupling of this receptor with adenylyl cyclase but are not involved in its coupling with G_q. These results support the idea that the CCK_1 receptor is directly coupled with both G_s and G_q (Wu et al., 1997).

Recent studies (for reviews, see Müller and Lohse, 1995; Daaka et al., 1997) have shown that some GPCRs use the same effectors as those of the tyrosine kinase receptor pathway [e.g., Shc (adapter protein)/growth factor receptor-bound protein 2/product of son of sevenless (SOS)], resulting in Ras and mitogen-activated protein kinase (MAPK) activation and leading to expression of transcriptional factors, such as c-*myc*, c-*jun*, and c-*fos*. It was recently shown that MAPKs and c-Jun NH_2-terminal kinases (JNKs, which phosphorylate serine residues of c-Jun) are rapidly activated by CCK-8 in rat pancreas both in vitro and in vivo (Dabrowski et al., 1996a,b; Tateishi et al., 1998). These results suggest that CCK might stimulate cell proliferation via its action at CCK_1 receptors. Moreover, the activation of both MAPKs and JNKs may be of importance in the early pathogenesis of acute pancreatitis (Dabrowski et al., 1996a). The mechanism by which the G_q protein-coupled CCK receptor activates Ras is not well understood. Results obtained by Dabrowski et al. (1996b) suggest that formation of Shc/growth factor receptor-bound protein 2/SOS complex via a PKC-dependent mechanism may provide the link between G_q protein-coupled CCK receptor stimulation and Ras activation.

A case report of a woman with gallstones and obesity was ascribed to abnormal processing of transcripts from a normal CCK_1 receptor gene that resulted in the predominance of mRNA with a 262-bp deletion corresponding to the third exon. Although this mutation could negatively affect expression or coupling to G proteins, neither in vivo nor in vitro data were obtained in support of such inferences. Unfortunately, other affected family members were not examined and expected splicing abnormalities in transcripts from other genes were not studied, so only an association could be established between the common phenotype of gallstones and obesity and the putative RNA processing abnormality in the affected patient (Miller et al., 1995).

2. CCK_2 Receptors. Molecular cloning of CCK_2 receptors has shown that this receptor is a member of the seven-transmembrane domain GPCR superfamily (Wank et al., 1992b). This confirmed previous results showing that nonhydrolyzable GTP analogs reduced the binding of selective CCK_2 receptor agonists, as expected of the coupling of these receptors with G proteins (Knapp et al., 1990; Durieux et al., 1992).

In contrast to CCK_1 receptors, the signal-transduction cascade for CCK_2 receptors has been rather poorly characterized, in large part because of the difficulty of working with isolated neurons or isolated gastric mucosal cells expressing CCK_2 receptors. Thus, for a long time, central CCK_2 receptors have not been proved to be linked to a well characterized second-messenger system in the brain, including the phosphoinositide system, although phosphoinositide metabolism was shown to be affected by CCK in neuroblastoma (Barrett et al., 1989) and in the embryonic pituitary cell line (Lo and Hughes, 1988). More recently, Zhang et al. (1992) showed that CCK-8 increased the turnover of phosphoinositides and IP_3 labeling in dissociated neonatal rat brain cells, in which both CCK_1 and CCK_2 receptors were expressed. One study of CCK_2 receptors, using synaptoneurosomes from guinea pig cortex, failed to provide support to their possible coupling with adenylyl cyclase or PLC, although Ca^{2+} release from intracellular stores, possibly via a G protein-independent mechanism, could be triggered by a CCK analog (Galas et al., 1992).

Expression of receptor cDNAs in a mammalian expression system allows for a readily available source of receptor for functional studies. In transfected cells (Cos, Chinese hamster ovary), it has been shown that like the CCK_1 receptor, the CCK_2 receptor couples to a pertussis toxin-insensitive G protein (Roche et al., 1990) that is probably related to the $G_{q/11}$ family, thereby causing activation of PLC (Tsunoda et al., 1988a,b, 1989; Delvalle et al., 1992). The region of the CCK_2 receptor interacting with G_q was determined in CCK_2 receptor with Lys333 Met, Lys334Thr, and Arg335Leu mutations transiently expressed in COS-7 cells and *X. laevis* oocytes. Indeed, these mutations resulted in the loss of G_q activation without affecting receptor affinity (Wang, 1997).

Site-directed mutagenic replacement of Asp100 in the rat CCK_2 receptor, a highly conserved residue in TMII of most GPCRs, results in a 50% reduction in CCK-8-stimulated phophoinositide turnover with no change in CCK-8 affinity and only a small (<6-fold) decrease in antagonist affinity (Jagerschmidt et al., 1995). These data led to the hypothesis that Asp100 points in the direction of the cluster of basic amino acids (Lys333/Lys334/Arg335), located in the third intracellular loop of the receptor at the bottom of the TMVI, that plays a critical role in CCK_2 receptor activation of G_q proteins (Wang, 1997).

Another residue, Phe347, which belongs to the TMVI domain, was identified as essential for the signal transduction process. Thus, the exchange of Phe347 for alanine disrupts the phosphatidylinositol-signaling pathway without affecting the binding of CCK receptor agonists (Jagerschmidt et al., 1998). This amino acid could be a residue implicated in transduction processes through its possible role in agonist-induced changes in receptor conformation and subsequent triggering of G protein activation. Indeed, the exchange of Phe347 for Ala could produce a conformational change in the sequence containing the basic triplet, located just beneath TMVI.

On the other hand, by analogy with CCK_1 receptors, it has been shown that CCK_2 receptors are coupled to a phospholipase pathway leading to the release of arachidonic acid via a PTX-sensitive G protein (Pommier et al., 1999) and to an MAPK pathway (Taniguchi et al., 1994).

B. Ligand-Receptor Interaction

1. Agonists. The examination of a 42-amino-acid N-terminal truncation of the human CCK_1 receptor and site-directed mutants in the region near the top of TMI suggested the interaction of amino acid residues Trp39 and Gln40 with CCK. Further binding data for the interaction between wild-type and Trp39Phe and Gln40Asn mutant CCK_1 receptors and a series of N-terminally modified CCK analogs that were applied to a model of the CCK_1 receptor (based on data from bacteriorhodopsin, rhodopsin, and the β-adrenergic receptors) suggested that the N-terminal moiety of CCK-8 interacts via hydrogen bonding with Trp39 and Gln40 (Kennedy et al., 1997). However, photoaffinity labeling with ^{125}I-desaminotyrosyl-Gly-[$Nle^{28,31}$,pNO_2-Phe^{33}]CCK-(26-33) of rat CCK_1 receptors overexpressed in Chinese hamster ovary cells demonstrated just the opposite result: the placement of Trp39 proximate to the C-terminal pNO_2-Phe33 residue of the probe (Ji et al., 1997). The interaction of CCK with the CCK_1 receptor was further modeled using separate single amino acid mutations, Lys105Val and Arg337Val, that resulted in a loss in CCK-8-stimulated calcium release. These data suggest that Lys105 and Arg337 in the CCK_1 receptor interact with Tyr(SO_3H) and Asp of CCK-8, respectively (Tsunoda et al., 1997).

A study of 58 chimeric receptors in which one to four divergent amino acids in the TM of the human CCK_2 receptor were replaced with the corresponding amino acids from the CCK_1 receptor identified only a single residue, Ser131, at the top of TMIII that confers a ~6-fold subtype selectivity for gastrin versus CCK-8 (Kopin et al., 1995). Chimeric and site-directed mutagenesis studies of the rat CCK_2 receptor containing CCK_1 receptor segmental substitutions suggested that a block of five amino acids (residues 204–208, including Cys205, which putatively forms a disulfide bridge with Cys127 at the top of TMIII) is important for gastrin selectivity (Silvente-Poirot and Wank, 1996) and that His207 is also important for CCK-8 affinity (Silvente-Poirot et al., 1998). Studies of human chimeric CCK_1/CCK_2 receptors made through exon shuffling of the respective receptor genes also demonstrated the importance of this area near the top of TMIII for conferring high gastrin affinity (Wu et al., 1997). Chimeric studies replacing the *X. laevis* CCK receptor with variable-length N-terminal segments of the human CCK_2 receptor revealed the need for multiple contact points in the N-terminal two-thirds (through TMV) of the CCK_2 receptor for conferring gastrin selectivity (Schmitz et al., 1996). Studies of Ala scanning mutagenesis in the N terminus near the top of TMI and the first ECL (ECL1) of the rat CCK_2 receptor identified one nonconserved (Arg57Ala) and four conserved amino acids (Asn115Ala, Leu116Ala, Phe120Ala, and Phe122Ala) that adversely affected CCK-8 affinity when mutated to Ala. Reciprocal mutations of these amino acids at equivalent positions in the rat CCK_1 receptor revealed only two mutations, Leu103Ala and Phe107Ala, that decreased CCK-8 affinity (Silvente-Poirot et al., 1998). These studies suggest that CCK peptide agonists interact with multiple amino acids in the extracellular domain of CCK receptors and that CCK_1 and CCK_2 receptors have distinct binding sites despite their shared high affinity for CCK-8. With the use of site-directed mutagenesis, the roles of three aromatic residues located in TMV (Phe227) and TMVI (Phe347 and Trp351) of the rat CCK_2 receptor were also evaluated in binding experiments. The results demonstrated that the highly conserved residues in GPCRs, Phe227 and Phe347, do not play an important role in the recognition of the agonists. In contrast, Trp351 appeared to be in the agonist-binding site of the receptor, where it probably interacts with the C-terminal sequence of CCK-8, as illustrated by the similar reduction in affinity for both CCK-8 and CCK-4 (Jagerschmidt et al., 1998).

2. Antagonists. Data from CCK receptor chimeric and site-directed mutagenesis studies suggest that the outer third of TMVI and TMVIII interacts with the benzodiazepine-based antagonists, L-364,718 and L-365,260. A survey of all TM amino acids of the human CCK_2 receptor in which one to four amino acids were replaced with the corresponding CCK_1 receptor amino acids identified two single-point mutations, Thr111Asn and His376Leu, that

cause a 23-fold decrease in L-365,260 affinity and a 63-fold increase in L-364,718 affinity, respectively (Kopin et al., 1995). The importance of the TMVII domain for antagonist affinity was confirmed by a rat CCK_2 receptor TMVII chimera with a 13-fold decrease in L-364,718 affinity (Mantamadiotis and Baldwin, 1994) that could be explained by the single-point mutation His381Leu (Jagerschmidt et al., 1996). The reversal of the relative affinity for L-364,718 and L-365,260 between canine gastrin receptor and both the rat and human CCK_2 receptors noted earlier has been explained by an interspecies variation of a single amino acid in TMVI (Leu355 in dog versus the corresponding Val349 in humans; Marino et al., 1993). The lack of effect of these TMVI and TMVII mutations on agonist affinity suggests that agonist- and antagonist-binding sites are, at best, only partially overlapping.

C. Receptor Regulation. GPCR function is significantly regulated by the mechanisms that determine receptor trafficking within the cell. The molecular and cellular mechanisms involved in regulation of translocation, sequestration, recycling, and degradation of GPCRs are not well understood, and the available data are largely controversial. Fusion of the C terminus of GPCR to the N terminus of the green fluorescent protein is a valuable tool in the study of receptor localization and trafficking. CCK_1-green fluorescent protein allowed for the direct observation of spontaneous and ligand-induced internalization of the receptor (Tarasova et al., 1997).

CCK_1 receptor internalization is independent of the state of phosphorylation and the presence of the C-terminal tail (Rao et al., 1997; Go et al., 1998). In contrast, internalization of the CCK_2 receptor is at least in part dependent on the phosphorylation of Ser/Thr residues in its C terminus (Pohl et al., 1997). In the phosphorylation-deficient CCK_1 receptor mutant with PKC consensus site mutations Ser260Ala and Ser264Ala, desensitization of the CCK-stimulated inositol 1,4,5-triphosphate response is delayed until the occurrence of receptor internalization (Rao et al., 1997). Desensitization of CCK_2 receptor stably expressed in Chinese hamster ovary cells does not require the C terminus and is independent of internalization, unlike the CCK_1 receptor (Choi et al., 1998).

V. Radioligands and Binding Assays: Heterogeneity of CCK_1 and CCK_2 Receptors

Initial studies describing the distribution and the binding characteristics of CCK_1 and CCK_2 receptors have used nonselective CCK receptor radioligands. Because CCK-8 is the physiological ligand of CCK receptors, it was first considered to be the most suitable probe for the characterization of CCK receptors in radioligand-binding studies. Preparation of stable, high-specific-activity radioiodinated CCK through conjugation to ^{125}I-Bolton Hunter reagent (^{125}I-BH) has been described using several CCK fragments, such as CCK-8 or CCK-33 (Sankaran et al., 1979; Lin and Miller, 1985). Specific binding sites for CCK have also been characterized using a ^{125}I-CCK-8 probe made resistant to degradation through reaction with the iodinated form of the imidoester, methyl-*p*-hydroxybenzimidate (Praissman et al., 1983). Characterization of CCK_1 and CCK_2 receptors was performed in the presence of selective nonradiolabeled ligands to saturate only one of the CCK receptors (Hill and Woodruff, 1990). Now, selective radioligands are available for the specific labeling of CCK_1 or CCK_2 receptors.

A. Radioligands at CCK_1 Receptors

[^{3}H]-(±)-L-364,718 is a potent and selective CCK_1 receptor antagonist that binds saturably and reversibly to rat pancreatic membranes. The radioligand recognizes a single class of binding sites with a high affinity (K_d = 0.23 nM), and the potency of various CCK receptor agonists and antagonists to inhibit its binding correlates with both their ability to inhibit ^{125}I-CCK-8-specific binding and the known pharmacological properties of these compounds in peripheral tissues (Chang et al., 1986). Nevertheless, in a more recent study, Talkad et al. (1994) showed that ^{125}I-CCK-8 binds to two different states of the CCK_1 receptor in rat pancreatic acini (a high-affinity state and a low-affinity state), whereas [^{3}H]L-374,718 binds to a low-affinity state and to a previously unrecognized very low-affinity state. Similar measurements using transfected COS cells also identified three different states of the CCK_1 receptor, suggesting that this feature is an intrinsic property of the CCK_1 receptor molecule itself (Huang et al., 1994)

The peptide antagonist of the CCK_1 receptor JMV-179 was modified at its N terminus through the incorporation of *p*-hydroxyphenylpropionate (BH reagent) and was subsequently radioiodinated (Silvente-Poirot et al., 1993b). The results obtained with this first antagonist radioligand, ^{125}I-BH-JMV-179, demonstrated that CCK_1 receptors exist under two interconvertible affinity states regulated by G proteins in rat pancreatic plasma membranes.

B. Radioligands at CCK_2 Receptors

Several peptide ligands have been used to characterize CCK_2-binding sites, such as [^{3}H]pentagastrin, [^{3}H]gastrin or ^{125}I-gastrin, and [^{3}H]CCK-4 (Gaudreau et al., 1985; Clark et al., 1986; Durieux et al., 1988).

The highly potent agonist [^{3}H]pBC264 (Durieux et al., 1989) has a subnanomolar affinity for CCK_2 receptors (K_d = 0.15–0.20 nM) in brain membranes from mouse, cat, rat, guinea pig, and humans (Durieux et al., 1992). [^{3}H]pBC264 binds to membranes in a time-dependent, reversible, and saturable manner. Moreover, even in the rat brain, a tissue with high levels of nonspecific binding and low density of CCK receptors (Williams et al., 1986), the specific binding of [^{3}H]pBC264 reached 80% of total

binding at a radioligand concentration close to the K_d value (Durieux et al., 1992). In guinea pig and mouse brain, specific [^{3}H]pBC264 binding was almost not affected by NaCl and/or guanyl-5′-yl-imidodiphosphate. In contrast, in rat brain, the affinity of [^{3}H]pBC264 was decreased and the maximal number of binding sites was increased by NaCl and the guanyl nucleotide, suggesting that a proportion of CCK_2 receptors are constitutively coupled to G proteins (Durieux et al., 1992).

The high selectivity of [^{3}H]SNF8702 also permits the characterization of CCK_2 receptors in brain tissues without interference from the population of CCK_1 receptors present (Knapp et al., 1990). The results obtained in guinea pig brain cortex demonstrated that [^{3}H]SNF8702 binds to a larger population of CCK_2 sites than [^{3}H]pBC264, which is not the case in mouse brain. These results could reflect the presence of several CCK-binding states with different sensitivities to ions and nucleotides. Thus, a part of the receptors labeled by [^{3}H]pBC264 in guinea pig brain may be insensitive to these reagents, unlike the additional sites bound by [^{3}H]SNF8702 (Knapp et al., 1990; Durieux et al., 1992).

Selective nonpeptide antagonist radioligands have been developed. [^{3}H]L-365,260 binds saturably and reversibly to brain membranes, and Scatchard analysis indicated a single class of high-affinity (K_d = 2 nM) binding sites (Chang et al., 1989). Recently, a new series of nonpeptide CCK_2 receptor antagonists has been described by Horwell et al. (1991). Some of these compounds have been radioiodinated ([^{125}I-PD-142,308; Horwell et al., 1995) or tritiated ([^{3}H]PD-140,376; Hill et al., 1993). The latter radioligand has advantages over the alternative radioligand [^{3}H]L-365,260 because it has a greater selectivity and affinity for the CCK_2 receptors and yields a higher ratio of specific to nonspecific binding in both cerebral cortex and gastric mucosa (Hunter et al., 1993). Interestingly, in addition to the high-affinity population of CCK_2 receptors, [^{3}H]PD-140,376 labeled a low-affinity state.

C. Heterogeneity of CCK_2 Receptor-Binding Sites

Binding studies using linear or cyclic CCK-8 analogs allowed the discovery of a heterogeneity of CCK_2-binding sites in guinea pig brain (Durieux et al., 1986b; Knapp et al., 1990; Rodriguez et al., 1990). Thus, CCK_2 receptors have been shown to exist in three different affinity states (Huang et al., 1994). This heterogeneity has been confirmed in saturation and competition binding studies. Thus, the Hill coefficient was in general significantly lower than unity in different tissues (Hunter et al., 1993; Huang et al., 1994; Harper et al., 1996).

The existence of CCK_2 receptor heterogeneity has also been proposed from experiments performed in the presence of guanosine-5′-(β,γ-imido)diphosphate or guanosine-5′-*O*-(3-thio)triphosphate. The results obtained clearly showed that these nonhydrolyzable GTP analogs reduced the binding of selective CCK_2 receptor ligands (Wennogle et al., 1988). However, different sensitivities to guanyl nucleotides were observed depending on the structures of the ligands used (Knapp et al., 1990; Durieux et al., 1992; Lallement et al., 1995; Suman-Chauhan et al., 1996).

Several authors have described CCK_2 receptor agonists apparently capable of discriminating two (Durieux et al., 1986b; Derrien et al., 1994b; Million et al., 1997) or even three (Huang et al., 1994) different affinity states. More recently, similar results have been obtained with antagonists (Hunter et al., 1993; Harper et al., 1996; Bellier et al., 1997).

Several hypotheses could be proposed to explain this apparent heterogeneity of CCK_2 receptor-binding sites. It is possible that the coupling of CCK_2 receptors to different G proteins (see *IVA2. CCK_2 Receptors*) induces different receptor conformation with different affinities for the ligands (for a review, see Kenakin, 1995). Another explanation would be that depending on the molecular interaction of a ligand with its binding site, preferential or differential coupling with a G protein can occur (Spengler et al., 1993).

VI. Distribution of CCK Receptors

A. Distribution in Central Nervous System

Specific CCK-binding sites were demonstrated in membranes from brain homogenates almost two decades ago (Hays et al., 1980; Innis and Snyder, 1980a,b; Saito et al., 1980; Praissman et al., 1983). Since then, numerous studies using autoradiography and, more recently, in situ hybridization and immunocytochemistry have investigated the regional distribution and specific cellular localization of CCK receptors throughout the neuraxis. Early studies used radioligands such as ^{125}I-CCK-33, ^{125}I-CCK-8, [^{3}H]pentagastrin, [^{3}H]CCK-8, [^{3}H]CCK-4 or [^{3}H]Boc[Nle^{28,31}]CCK27-33 (Gaudreau et al., 1983, 1985; Zarbin et al., 1983; Van Dijk et al., 1984; Dietl et al., 1987; Pélaprat et al., 1987; Durieux et al., 1988; Niehoff, 1989) that do not distinguish between the two CCK receptors. In general, these studies performed in several species (e.g., rat, guinea pig, monkey, humans) showed high densities of CCK-binding sites in several areas, including the cerebral cortex, striatum, olfactory bulb and tubercle, and certain amygdaloid nuclei. Moderate levels were observed in the hippocampus, claustrum, substantia nigra, superior colliculus, periaqueductal gray matter, and pontine nuclei. Low densities were reported in several thalamic and hypothalamic nuclei and in the spinal cord (Fig. 5).

Initial evidence for species differences in the distribution of CCK receptors was also provided by these studies. For example, in the cerebellum, high densities of CCK-binding sites were present in guinea pig, whereas only low levels were detected in rat (Zarbin et al., 1983; Gaudreau et al., 1985; Mantyh and Mantyh, 1985). CCK-binding sites have now been identified and visual-

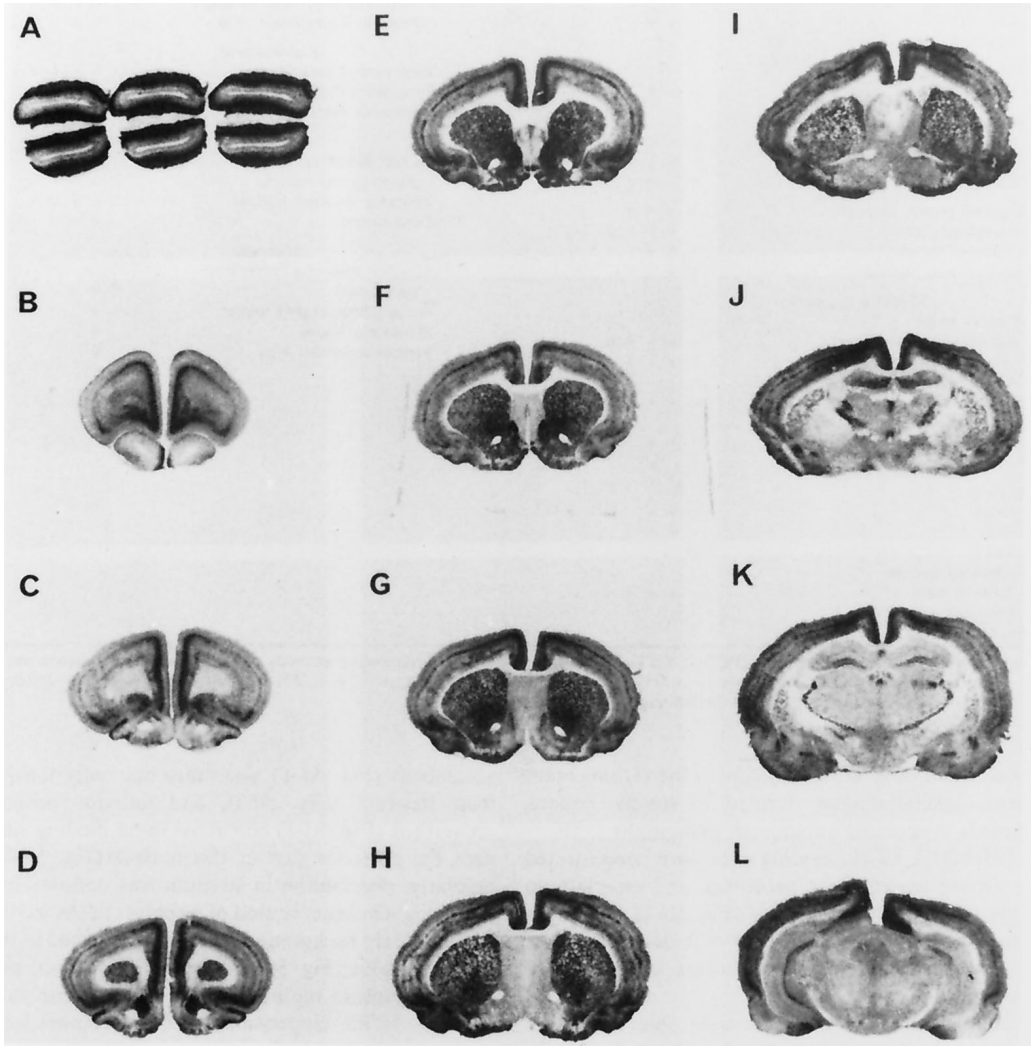


FIG. 5. Autoradiograms showing the distribution of [^{3}H]BDNL binding to CCK_1 and CCK_2 receptors in the rat forebrain and midbrain. Moderate to high densities of receptors are observed in the olfactory bulbs (A), the anterior olfactory nucleus (B), the neocortex, and especially in layer III of the medial frontal (B–C) and cingulate (E–I) cortices, the layer IV of frontal (B and C) and frontoparietal (D–J) cortices, the layers II–IV of retrosplenial cortex (L), the olfactory tubercle (E–I), the endopiriform nucleus (E–K), the nucleus accumbens (D–F), the striatum (D–I), and the hippocampus, where CCK receptors are more concentrated in the dentate gyrus and subiculum (K). [^{3}H]BDNL, Boc-Tyr(SO_3H)-[4^{3}H]Nle-Gly-Trp-[4^{3}H]Nle-Asp-Phe-NH_2.

ized in the nervous system of numerous species ranging from goldfish to humans (e.g., Dietl et al., 1987; Kritzer et al., 1988, 1990; Hyde and Peroutka, 1989; Miceli and Steiner, 1989; Hill et al., 1990; Ghilardi et al., 1992; Moons et al., 1992; Schiffmann et al., 1992; Kuehl-Kovarik et al., 1993; Madtes and King, 1994; Morency et al., 1994; Himick et al., 1996; Mercer et al., 1996; Oliver and Vigna, 1996). These studies showed both similarities and sometimes striking differences in the comparative distribution of CCK receptors from one species to another. More comprehensive analyses and discussion about CCK receptor distribution differences in several brain regions among multiple species can be found elsewhere (Gaudreau et al., 1985; Sekiguchi and Moroji, 1986; Williams et al., 1986; Dietl and Palacios, 1989).

With the advent of specific radioligands that could differentiate between the two types of CCK receptors, it has become apparent that CCK_1 and CCK_2 receptors exhibit a sometimes overlapping, yet distinct, distribution throughout the CNS. The vast majority of CCK receptors in the CNS are of the CCK_2 type, with CCK_1 receptors restricted to rather discrete regions. The precise anatomical localization of the two CCK receptor types, as detailed later, serves to provide morphological substrates for many of the diverse functions attributed to neural CCK, including involvement in feeding, satiety, cardiovascular regulation, anxiety, pain, analgesia, memory, neuroendocrine control, osmotic stress, dopamine-related behaviors, and neurodegenerative and neuropsychiatric disorders (see Crawley and Corwin, 1994).

1. CCK_1 Receptors. Radioligand studies, initially conducted in the rat, showed CCK_1 receptors to be mainly located in the interpeduncular nucleus, area postrema, and medial nucleus tractus solitarius, with additional areas of binding found in the habenular nuclei, dorsomedial nucleus of the hypothalamus, and central amygdala (Moran et al., 1986; Hill et al., 1987, 1988a; Moran and McHugh, 1988; Woodruff et al., 1991; Carlberg et

al., 1992; Zajac et al., 1996; Qian et al., 1997). Studies in primates have revealed dramatic species differences, demonstrating a much higher prevalence and broader distribution of CCK_1 receptors in the monkey and humans than that in rodents (Hill et al., 1988b, 1990; Graham et al., 1991). Thus, in the monkey, CCK_1 receptor-binding sites are located not only in the area postrema, nucleus, tractus solitarius, and hypothalamic dorsomedial nucleus, but also in the supraoptic nucleus, paraventricular nucleus, mammillary bodies, supramammillary region, infundibular region, dorsal motor nucleus of the vagus, and the neurohypophysis. In addition, the mesostriatal dopaminergic system exhibits CCK_1 receptor binding in both its origin (substantia nigra pars compacta and adjacent ventral tegmental area) and forebrain targets (caudate and putamen). CCK_1 receptors are also found in the dorsal horn of monkey and human spinal cord. Peripherally, the nodose ganglion and vagus nerve contain and transport CCK_1 receptors (Corp et al., 1993; Widdop et al., 1994).

As determined by in situ hybridization using a cRNA probe, CCK_1 receptor mRNA in the rat is distributed within most of the above areas exhibiting CCK_1 receptor-binding sites (Honda et al., 1993). Moreover, additional areas containing CCK_1 receptor mRNA were revealed. In the forebrain, moderate to light mRNA expression is localized in the olfactory bulb, anterior olfactory nuclei, olfactory tubercle, piriform cortex, neocortex, claustrum, taenia tecta, all principal cell layers of the hippocampal formation, medial nucleus of the amygdala, and nucleus of the lateral olfactory tract. Moderate expression is also present in the lateral septal nucleus, bed nucleus of the stria terminalis, preoptic nucleus, thalamic reticular nucleus, and several hypothalamic regions, including the arcuate nucleus and lateral and posterior hypothalamic areas. Limited labeling for CCK_1 mRNA has been observed in the brainstem, with expression found only in the dorsal motor nucleus of the vagus nerve and the interpeduncular, caudal linear raphe, and hypoglossal nuclei.

Finally, it should be noted that a recent report on the immunohistochemical distribution of the CCK_1 receptor in rat CNS, using a newly developed and partially characterized antiserum, described numerous brain regions displaying CCK_1 receptor-like immunoreactivity (Mercer and Beart, 1997). In addition to being present within most of the areas shown above to contain CCK_1 receptor-binding sites or mRNA, other regions with either perikaryal or axonal/dendritic immunolabeling included the nucleus accumbens, anteroventral thalamic nucleus, medial mammillary nucleus, superior colliculus, periaqueductal gray matter, nuclei raphe obscurus and dorsalis, and parabrachial, trigeminal, vestibular, and inferior olivary nuclei, as well as layers 2 to 6 of the spinal cord. Further studies are necessary to confirm these results.

2. CCK_2 Receptors. In the telencephalon, autoradiographic binding studies (Moran et al., 1986; Pélaprat et al., 1987; Durieux et al., 1988; Woodruff et al., 1991; Carlberg et al., 1992; Qian et al., 1997) showed that high densities of CCK_2 receptors are localized in the external plexiform layer of the main olfactory bulb, middle layers of the neocortex (with particularly high levels in the retrosplenial and cingulate cortices), piriform cortex, nucleus accumbens, and parasubiculum (Table 10). Moderate levels are found in the olfactory bulb glomerular layer, deep layers of neocortex, olfactory tubercle, islands of Calleja, fundus striata, ventral pallidum, caudate-putamen, hippocampus, dentate gyrus, presubiculum, and some amygdaloid nuclei. Only low densities are present in other telencephalic areas such as the taenia tecta, septum, bed nucleus of the stria terminalis, diagonal band of Broca, globus pallidus, superficial layers of neocortex, and most amygdaloid nuclei. In the diencephalon, moderate levels of CCK_2 receptors are distributed within several hypothalamic nuclei, including the suprachiasmatic, supraoptic and ventromedial nuclei, and within the thalamic reticular nucleus. Low binding densities are found in other diencephalic regions such as the medial preoptic, arcuate, and dorsomedial hypothalamic nuclei; paraventricular, mediodorsal and reuniens thalamic nuclei; and zona incerta and lateral habenular nucleus. In the mesencephalon, moderate densities of CCK_2 receptor binding are localized in the parabigeminal nucleus, substantia nigra, and superior colliculus, with low levels present in the inferior colliculus, parabrachial nucleus, dorsal raphe nucleus, and periaqueductal gray matter. Relatively few CCK_2 receptor-binding sites are found in the myelencephalon, with low to moderate levels distributed within the pontine and superior olivary nuclei, and nucleus tractus solitarius. As noted, CCK_2 receptor binding in the cerebellum is species dependent. Indeed, with autoradiographic studies, CCK_2 receptors have been detected in the guinea pig, human, and mouse cerebellum, but not in rat cerebellum (Sekiguchi and Moroji, 1986; Williams et al., 1986, Dietl et al., 1987; Jagerschmidt et al., 1994). Finally, low levels of binding are observed in the dorsal and ventral horns of the spinal cord. In the periphery, CCK_2 receptor-binding sites are located in the trigeminal and dorsal root ganglia (DRG; Ghilardi et al., 1992) and in the vagus nerve (Corp et al., 1993).

In situ hybridization studies using cRNA probes showed that the distribution of CCK_2 receptor mRNA (Honda et al., 1993) is in good agreement with that of CCK_2 receptor-binding sites (see also Shigeyoshi et al., 1994; Hansson et al., 1998). Although some discrepancies were observed, virtually all of the nuclei and regions described earlier were shown to exhibit hybridization for CCK_2 receptor mRNA, with particularly strong signals found in the neocortex, piriform cortex, anterior olfactory nuclei, and several amygdaloid nuclei. Some areas with moderate to weak expression included the olfactory bulb and tubercle, hippocampal formation, claustrum,

TABLE 10
Distribution of [^{3}H]CCK-4 binding to CCK receptors in the rat brain

Frontal cortex		Septal region	
Layers I–III	++	Septum	+
Layer III, medial part	++++	Bed nucleus of the stria terminalis	++
Layer IV	+++	Septohippocampal nucleus	++
Layer V	++	Hippocampus	
Layer VI	+++	Subiculum	+++
Frontoparietal motor cortex		CA1–CA3	+
Layers I–III	++	Dentate gyrus	+++
Layer IV	+++	Amygdala	
Layers V, VI	++	Lateral nucleus	++
Frontoparietal somatosensory cortex		Posteromedial nucleus	++
Layers I–III	++	Amygdalo-hippocampal area	++
Layer IV	+++	Hypothalamus	
Layers V, VI	++	Ventromedial nucleus	+++
Striate cortex		Paraventricular nucleus	+++
Layers I–V	+++	Supraoptic nucleus	+++
Temporal cortex (auditory area)		Thalamus	
Layers I–III	+++	Lateral habenula	+
Layer IV	++++	Paraventricular nucleus	++
Layers V, VI	+++	Reticular thalamic nucleus	+++
		Zona incerta	+
Cingulate cortex, layer III	++++	Midbrain	
Retrosplenial cortex	++++	Superior	++
Entorhinal cortex	+++	Substantia nigra	++
Endopyriform nucleus	++++	Periacqueductal gray matter	+
Olfactory-system			
Olfactory bulbs			
External plexiform layer	+++		
Glomerular layer	+++		
Anterior olfactory nucleus	++		
Olfactory tubercule	+++		
Primary olfactory cortex (superficial layer)	++++		
Basal ganglia			
Striatum			
Head	+++		
Body	+++		
Tail	+		
Nucleus accumbens			
Anterior part	++++		
Posterior part	+		
Globus pallidus	+		

++++, high level; +++, moderate level; ++, low level; +, very low level.

other amygdaloid nuclei, septum, nucleus accumbens, caudate-putamen, substantia nigra, thalamic reticular nucleus, paraventricular, supraoptic and ventromedial hypothalamic nuclei, interpeduncular nucleus, red nucleus, vestibular nuclei, dorsal column nuclei, reticular formation, and lateral cerebellar nucleus. Diffuse labeling was also reported throughout the spinal cord. In peripheral sensory ganglia, CCK_2 receptor mRNA has been localized to a small population of DRG neurons (Zhang et al., 1993).

3. Regulation of CCK Receptors. It has become apparent that expression of CCK receptor-binding sites and mRNAs in the nervous system is not static but rather is malleable on different kind of perturbations. This is particularly evident in the hypothalamus where the levels of binding sites and/or mRNA for CCK_2 and/or CCK_1 receptors have been shown to increase in response to various physiological or pharmacological stimuli such as osmotic stress, hypophysectomy, food and water deprivation, and chronic morphine treatment (Day et al., 1989; Meister et al., 1994; Hinks et al., 1995; O'Shea and Gundlach, 1995; Munro et al., 1998). In primary sensory neurons, the expression of CCK_2 receptor mRNA is dramatically up-regulated after peripheral axotomy from the normal low percentage of in situ hybridization-labeled cells to encompass about two-thirds of all DRG neurons across all size categories on peripheral axotomy (Zhang et al., 1993). In contrast, mild cortical infarction results in decreased levels of CCK_2 receptor mRNA and binding sites in the entire ipsilateral cerebral hemisphere (Van Bree et al., 1995). These data on CCK receptor alterations are in line with previous demonstrations of changes in CCK mRNA and peptide levels after certain perturbations, thereby providing further evi-

dence that neural CCK ligand-receptor systems are capable of plastic responses to various stimuli.

B. Distribution in Gastrointestinal and Other Systems

In the gastrointestinal tract and other peripheral systems, CCK_1 receptors are present in pancreatic acinar cells, chief cells and D cells of the gastric mucosa, smooth muscle cells of the gallbladder, pyloric sphincter, sphincter of Oddi, some gastrointestinal smooth muscle and enteric neuronal cells, and anterior pituitary corticotrophs (for reviews, see Jensen et al., 1994; Wank et al., 1994a; Wank, 1995). CCK_1 receptors can also be expressed in several tumors, including pancreatic adenocarcinomas, meningiomas, and some neuroblastomas (Reubi et al., 1997a; Weinberg et al., 1997), as well as in certain pancreatic carcinoma, neuroblastoma, and lung cancer cell lines (Logsdon, 1986; Klueppelberg et al., 1990; Sethi et al., 1993). Furthermore, CCK_1 receptor mRNA has been found in esophageal, gastric, and colon cancers (Clerc et al., 1997). On the other hand, peripheral CCK_2 receptors are located in smooth muscle cells throughout the gastrointestinal tract (including the gallbladder), parietal, enterochromaffin-like, D cells and chief cells of the gastric mucosa, myenteric plexus neurons, pancreatic acinar cells, monocytes, and T lymphocytes (Sacerdote et al., 1991; Jensen et al., 1994; Mantyh et al., 1994; Wank et al., 1994; Wank, 1995; Song et al., 1996; Tarasova et al., 1996; Helander et al., 1997; Reubi et al., 1997b). Tumors and tumor cell lines expressing CCK_2 receptors include medullary thyroid, gastric, colon, ovarian and small cell lung carcinomas, astrocytomas, and certain pancreatic and lung cancer cell lines (Sethi et al., 1993; Wank, 1995; Reubi and Waser, 1996; Clerc et al., 1997; Reubi et al., 1997a).

VII. Physiological Implications of CCK Receptors

A. Peripheral Functions

As described in detail in *VIB. Distribution in Gastrointestinal and Other Systems*, CCK_1 receptors in the periphery are primarily localized in the pancreas, gallbladder, pylorus, intestine, and vagus nerve (Sankaran et al., 1980; Smith et al., 1984; Moran et al., 1987, 1990; Szecowka et al., 1989; Hill et al., 1990; Wank et al., 1992a). In the pancreas, CCK acts at CCK_1 receptors on acinar cells to stimulate the secretion of the digestive enzyme pancreatic amylase (Liddle et al., 1984; Freidinger, 1989; Jensen et al., 1989). In the gallbladder, CCK acts at CCK_1 receptors to stimulate gallbladder contraction (Chang and Lotti, 1986; Gully et al., 1993). Commercial preparations of CCK are used clinically to evaluate gallbladder contraction in human gallbladder disease (Ondetti et al., 1970).

The role of peripheral CCK_1 receptors in the regulation of feeding behavior is an area of intense investigations. CCK_1 receptors appear to mediate the transmission of sensory information from the gut to the brain. Peripherally administered CCK inhibits food consumption, even after fasting, in many species, including humans (Gibbs et al., 1973; Pi-Sunyer et al., 1982; Stacher et al., 1982; for reviews, see Smith and Gibbs, 1992; Crawley and Corwin, 1994). Furthermore, CCK_1 receptor antagonists increase food consumption and postpone satiety in several species, supporting the idea that endogenous CCK participates in the physiological regulation of feeding behavior (Dourish et al., 1989; Wolkowitz et al., 1990; Corwin et al., 1991; Reidelberger et al., 1991; Moran et al., 1992, 1993; for a review, see Crawley and Corwin, 1994). The entry of food into the intestine triggers the release of endogenous CCK by the intestinal mucosa, thereby activating CCK_1 receptors in the periphery. In particular, CCK_1 receptors on the vagus nerve (Moran et al., 1987) appear to be critical for the satiety-inducing action of CCK. Thus, lesions of the vagus nerve completely block the CCK-induced satiety syndrome (Crawley et al., 1981; Smith et al., 1981; South and Ritter, 1988). These findings have led to the hypothesis that CCK released from the intestine after a meal activates CCK_1 receptors on the vagus nerve to transmit sensations of fullness to the brain, which subsequently terminates feeding behaviors and initiates the

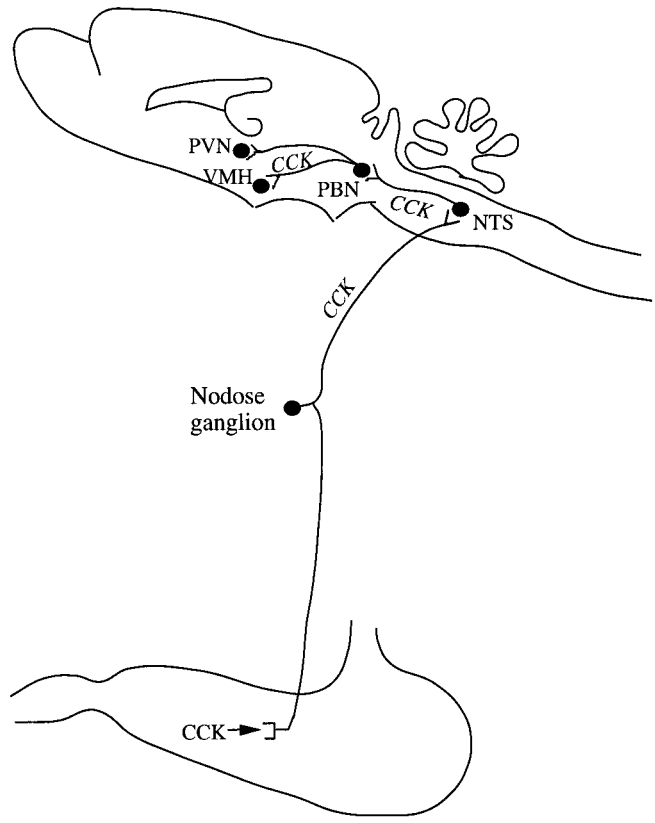


FIG. 6. Schematic representation of the mechanism of action of CCK in the regulation of feeding behavior. It is proposed that CCK from the intestine is delivered, after a meal, in the circulation to the stomach, where it acts directly on vagal afferents to transmit sensations of fullness to the brain. NTS, nucleus tractus solitarius; PVN, paraventricular nucleus; PBN, parabrachial nucleus; VMH, ventromedial nucleus of the hypothalamus (reproduced from Dockray, 1988).

sequence of behaviors associated with satiety (Smith and Gibbs, 1992; Fig. 6). CCK_1 receptor agonists have been proposed as anorectics for the treatment of obesity (Simmons et al., 1994; Wettstein et al., 1994). Conversely, CCK_1 receptor antagonists have been proposed for the treatment of anorexia disorders (Wolkowitz et al., 1990).

CCK_2 receptors in the periphery are primarily localized in the stomach (Kopin et al., 1992) and on the vagus nerve in some species (Mercer and Lawrence, 1992). As previously demonstrated, gastrin acts at CCK_2 receptors to stimulate gastric acid secretion (Schubert and Shamburek, 1990). Similarly, CCK stimulates gastric acid secretion (Sandvik and Waldum, 1991), and this effect can be blocked by CCK_2 receptor antagonists (Bado et al., 1991; Pendley et al., 1995). To further explore the peptidergic pharmacology of the pyloric sphincter, it is desirable to have a preparation that would allow the examination of contraction independent of basal motor activity and could exclude contribution from the enteric nervous system. Such a preparation of isolated antral cells has been obtained through enzymatic disaggregation of tissue strips from different species, as well as disaggregated isolated cell preparations from the pyloric sphincter. Results obtained from these assays show that pyloric smooth muscle contractions are stimulated by low doses of CCK and that gastric emptying induced by a lipid-enriched meal is inhibited by CCK_2 receptor antagonists (Debas et al., 1975; Lopez et al., 1991). The latter compounds have been proposed for the treatment of gastric ulcers (Pendley et al., 1995).

Another relatively simple functional assay for CCK receptors is the guinea pig ileum longitudinal muscle myenteric plexus, which contains both CCK_1 and CCK_2 receptors. It has been demonstrated that CCK-8 elicits contraction through both receptors. Moreover, it has been shown that activation of CCK_2 receptor released only acetylcholine, whereas activation of CCK_1 receptor is responsible for the release of both substance P and acetylcholine (Dal Forno et al., 1992; Corsi et al., 1994).

B. Central Functions

In line with its wide distribution in brain, CCK is involved in the modulation/control of multiple central functions. In particular, numerous experimental and clinical studies have clearly shown that CCK, through its action at CCK_1 and CCK_2 receptors, participates in the neurobiology of anxiety, depression, psychosis, cognition, and nociception.

1. CCK in Panic Attacks and Anxiety. The initial suggestion that the CCK system might be involved in anxiety came from experiments of Bradwejn and de Montigny (1984, 1985a,b) that showed that benzodiazepine receptor agonists could attenuate CCK-induced excitation of rat hippocampal neurons. Subsequent clinical studies demonstrated that bolus injections of the CCK_2 receptor agonist CCK-4 or pentagastrin provoke panic attacks in patients with panic disorders (Bradwejn et al., 1990, 1991b, 1992a,b). The induced symptoms are comparable to those produced by a standard panic-provoking agent (35% CO_2; Bradwejn and Koszycki, 1991) and can be attenuated by antipanic pharmacological agents such as antidepressants (Bradwejn and Koszycki, 1994; Shlik et al., 1997a; van Megen et al., 1997). CCK-4 also provokes panic attacks in healthy human subjects (de Montigny, 1989; Bradwejn et al., 1991a; McCann et al., 1994); however, sensitivity to the peptide is enhanced in panic disorder patients relative to healthy volunteers (Bradwejn et al., 1991b; van Megen et al., 1994), suggesting that endogenous CCK system may be altered in panic disorder and contributes to pathological anxiety. Recent investigations have revealed that the panicogenic effects of CCK_2 receptor agonists are not limited to panic disorder, because individuals with social phobia, generalized anxiety disorder, obsessive compulsive disorder, and premenstrual dysphoric disorder also exhibit an augmented behavioral response to these ligands (Le Melledo et al., 1995; De Leeuw et al., 1996; van Vliet et al., 1997; Brawman-Mintzer et al., 1997; Katzman et al., 1997). Although these data suggest that CCK sensitivity is not peculiar to panic disorder, the threshold of vulnerability to CCK_2 receptor agonists appears to be lower in panic disorder relative to other psychopathologies in which anxiety is a significant component (Katzman et al., 1997). In parallel, a number of investigators have reported that CCK peptides (Boc-CCK-4, BC 197) administered systemically or intracerebrally produce anxiogenic-like effects in different animal species, including mouse, rat, guinea pig, cat, and monkey (Blommaert et al., 1993; Harro et al., 1993; for a review, see Daugé and Roques, 1995). However, the anxiogenic effects of CCK peptides in animals have not been observed by all investigators, and the relevant negative findings should not be ignored (Shlik et al., 1997b). The conflicting data reported in the animal literature are attributable in part to the failure to address the various factors that potentially influence susceptibility to the anxiogenic effects of CCK (Bradwejn and Vasar, 1995). For instance, rats with low exploratory behavior (i.e., "anxious" rats) have been reported to exhibit a higher density of CCK receptor-binding sites in the frontal cortex and hippocampus relative to that in rats with high exploratory behavior (i.e., "nonanxious" rats; Harro et al., 1990; Koks et al., 1997). Thus, the effects of CCK compounds could vary considerably because of existing differences in the distribution and binding characteristics of CCK receptor types and/or affinity states among species. Recently, the effects of the selective CCK_2 receptor agonists BC 264 and BC 197 and of the nonselective CCK receptor agonist BDNL were investigated in rats subjected to the elevated plus-maze. Surprisingly, BDNL and BC 197 did induce anxiogenic-like effects, but BC 264 was devoid of any effect (Fig. 7). The behavioral responses to

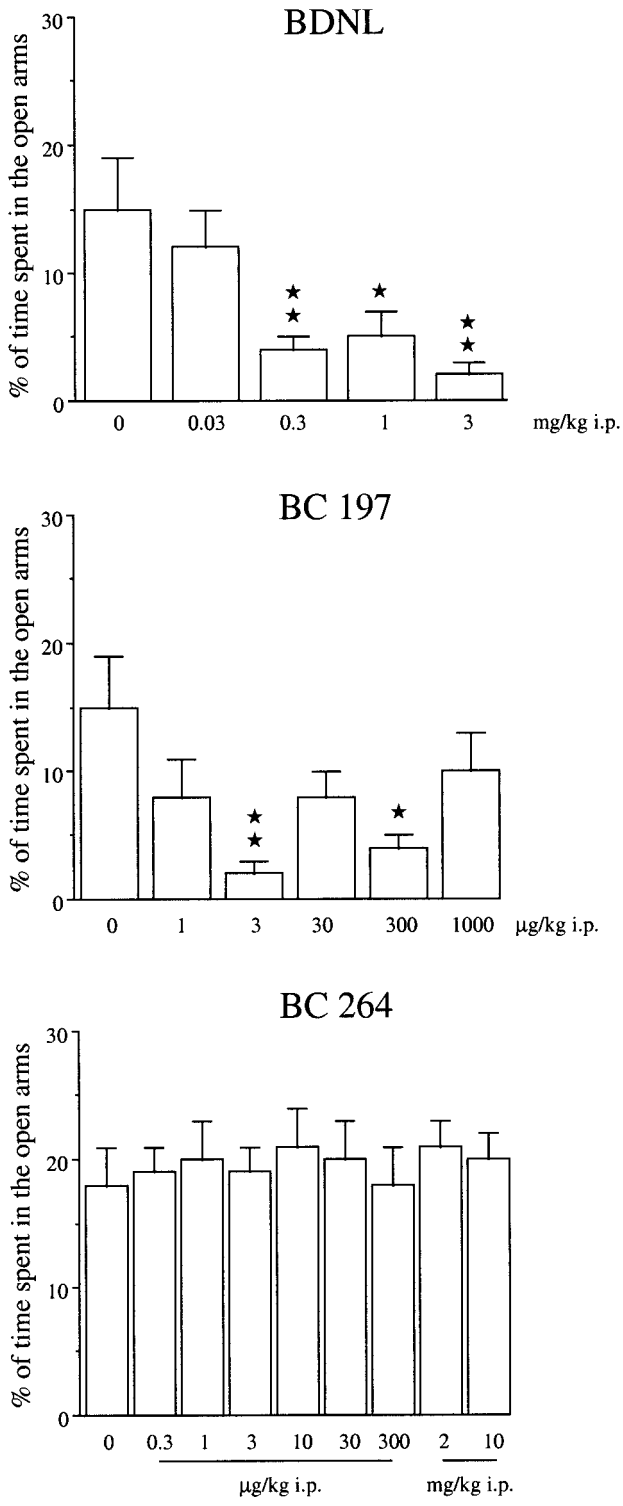


FIG. 7. Effects of i.p. injection of BDNL, BC 197, and BC 264 administered 30 min before the experiment in the elevated plus-maze. The behavioral responses of rats were measured in the elevated plus-maze for 5 min. They are expressed as the percentage ± S.E.M. of time spent in open arms. $^{*}P < .05$ and $^{**}P < .01$ compared with control group.

BDNL and BC 197 could be suppressed by CI-988, as expected from the involvement of CCK_2 receptors (Derrien et al., 1994b). On the other hand, Palmour et al. (1993) studied the anxiogenic effects of CCK receptor agonists in a nonhuman primate model. CCK-4 administered i.v. to African green monkeys has strong and dose-related effects on behaviors thought to reflect anxiety and panic. Interestingly, BC 264 also produces these behavioral responses, but the profile of behavior is somewhat different because at low doses, hypervigilance and stereotypy are prominent.

The behavioral effects of CCK_2 receptor agonists in humans are accompanied by marked biological alterations, including robust increases in heart rate, blood pressure, and minute ventilation (Bradwejn et al., 1992a, 1998), increased hypothalamic-pituitary-adrenal axis activity (de Montigny, 1989; Abelson et al., 1991; Kellner et al., 1997; Shlik et al., 1997a), and elevated blood levels of dopamine, epinephrine, norepinephrine, and neuropeptide Y (Boulenger et al., 1996). The extent to which the biological alterations due to CCK_2 receptor agonist administration are comparable to those underlying naturally occurring panic attacks remains to be determined. Functional imaging studies in healthy volunteers have shown that CCK-4-induced anxiety is associated with cerebral blood flow activation in the anterior cingulate gyrus, the claustrum-insular-amygdala region, and the cerebellar vermis (Benkelfat et al., 1995). Although these studies indicate that brain mechanisms are activated after CCK-4 administration, they do not elucidate the precise neuronal circuitry subserving CCK-4-induced panic. It has been proposed that brainstem nuclei, including nucleus tractus solitarius, medulla, and parabrachial nucleus, are important sites of action of exogenous CCK-4 (Shlik et al., 1997b). These structures contribute to the regulation of respiration and cardiopulmonary function and have close anatomical and functional links with the locus ceruleus, a brain region involved in the expression of fear and anxiety. Studies in animals have shown that CCK interacts with brainstem structures to modulate respiration, heart rate, and blood pressure (Denavit-Saubié et al., 1985), and it is likely that the prominent cardiorespiratory symptoms elicited by exogenous CCK-4 in humans result from direct or indirect stimulation of CCK receptors in brainstem nuclei. The emotional symptoms evoked by CCK-4 may rise from an action of this peptide on brainstem structures and a subsequent activation or inhibition of higher CNS regions mediated through neuronal projections.

The neurobiological mechanisms by which CCK_2 receptor agonists provoke panic and concomitant biological changes have been the subject of considerable research activity. Animal studies suggest that anxious behavior induced by various CCK fragments is associated with selective CCK_2 receptor stimulation (Harro et al., 1993). CCK_2 receptors also appear to participate in the expression of anxiety in humans after systemic administration of CCK-4 and pentagastrin. Thus, acute treatment with the selective CCK_2 receptor antagonist L-365,260 was reported to block CCK-4-induced panic

attacks in panic disorder patients (Bradwejn et al., 1994) and pentagastrin-induced panic symptoms in healthy volunteers (Lines et al., 1995). Although CCK_2 receptors appear to be the key component from which CCK-4 triggers panic symptoms, there is growing evidence that the peptide produces its effects through interactions with other neurotransmitter systems. Animal studies have demonstrated that serotonin, norepinephrine, dopamine, opioids, corticotropin-releasing factor, and the benzodiazepine/γ-aminobutyric acid complex play salient roles in the induction of anxiety with CCK (Crawley, 1995; Zacharko et al., 1995). Similarly, clinical studies have revealed important interactions between CCK and serotonin (Shlik et al., 1997a; van Megen et al., 1997), norepinephrine (Le Melledo et al., 1998), and the benzodiazepine/γ-aminobutyric acid complex (de Montigny, 1989) in the induction of panic-like behavioral and physiological symptoms.

Interestingly, single-strand conformational polymorphism analysis showed that a significant association exists between panic disorder and polymorphism of the CCK_2 receptor gene (Kennedy et al., 1999). The CA repeat polymorphism in the upstream promoter region appears to be different in patients versus control subjects, suggesting that CCK_2 receptor gene variations may be a relevant factor in the neurobiology of panic disorder. In addition, a polymorphism, also revealed by single-strand conformational polymorphism analysis, has been found in the promoter region of the gene encoding the CCK precursor (Wang et al., 1998).

Recent attempts to evaluate the therapeutic effects of CCK_2 receptor antagonists in panic disorder have produced disappointing results (Adams et al., 1995; Kramer et al., 1995), mainly because the two compounds available for human use, L-365,260 and CI-988, have unfavorable pharmacokinetic properties. Fortunately, several pharmaceutical compagnies have developed CCK_2 receptor antagonists with superior pharmacokinetic profiles. These compounds are currently under evaluation for their potential interest in the treatment of anxiety and other psychopathologies.

2. CCK and Schizophrenia. To date, modifications in functioning of the dopamine system are generally accepted as a key component in the hypothetical pathophysiological mechanisms of schizophrenia. The existence of interactions between dopaminergic and CCKergic systems has been demonstrated by a large body of electrophysiological, behavioral, and neurochemical data (for a review, see Crawley, 1991; Derrien et al., 1993a; Ladurelle et al., 1993). Moreover, dopamine has been shown to be colocalized with CCK in the posterior part of the nucleus accumbens (Hökfelt et al., 1980). This observation can have clinical relevance because the A-10 dopaminergic neurons that project to the nucleus accumbens, much more than the other dopaminergic systems, are probably concerned by the pathophysiological mechanisms of schizophrenia (Crawley and Corwin, 1994). Numerous experiments have shown that CCK modulates the release of dopamine and that dopaminergic agents modulate the release of CCK (Crawley and Corwin, 1994). The interactions between CCK and dopamine are complex and often bidirectional, with CCK potentiating or inhibiting the action of dopamine, depending on the brain region examined. Thus, local administration of the CCK_2 receptor agonists BC 264 or CCK-8 reduced dopamine release in the nucleus accumbens of microdialysed rats, whereas via the i.p. route, the former agonist produced a large increase in dopamine release in the same area (Ladurelle et al., 1993, 1997). One hypothesis to account for the i.p. effects of BC 264 could be that this agonist, acting on the CCK_2 receptors located in the dorsal subiculum/CA1 of the hippocampus, stimulates the glutamatergic projections to the anterior nucleus accumbens, resulting in dopamine release (Sebret et al., 1999).

The precise role of CCK in schizophrenia remains incompletely understood. The most prominent finding relevant to this disorder is a reduction in postmortem CCK mRNA levels in different brain areas (frontal, cerebral and entorhinal cortices, and subiculum) of schizophrenic patients (Virgo et al., 1995; Bachus et al., 1997). In addition, significant reductions in CCK-like immunoreactivity have been reported in several brain regions of schizophrenic patients (Ferrier et al., 1983, 1985; Carruthers et al., 1984), especially those with predominantly negative symptoms. On the other hand, a lower density of CCK receptor-binding sites has been found in the hippocampus and frontal cortex of schizophrenic patients compared with controls (Farmery et al., 1985). However, it should be noted that not all studies confirmed the decrease in CCK mRNA levels in schizophrenia. Indeed, in the postmortem study of Schalling et al. (1990), schizophrenic patients had even higher CCK mRNA levels in the ventral tegmental area and substantia nigra than control subjects. Such a finding should suggest that elevated CCK synthesis in regions rich in dopaminergic neurons may be associated with schizophrenia. Methodological problems, study groups of patients that were too small, and patient heterogeneity might have contributed to these inconsistent results. Nevertheless, on the whole, the available data suggest that schizophrenia may be associated with reduced CCK activity. This reduction may be attributed to either a decreased processing of preproCCK in neurons or a reduction in synaptic levels of CCK due to activations in catabolic or putative reuptake processes (Migaud et al., 1995) or some neurodegeneration of CCKergic neurons in schizophrenia.

The inference that schizophrenia may be associated with hypoactive CCKergic transmission along with reports that CCK analogs have neuroleptic-like activity in animal paradigms relevant to schizophrenia spurred a great deal of interest in the potential antipsychotic activity of CCK peptides. Several open studies reported

that administration of nonselective CCK receptor agonists (CCK-8; CCK-33, cerulein) improved psychotic symptoms in schizophrenic patients when added to ongoing neuroleptic treatment (for a review, see Montgomery and Green, 1988; Payeur et al., 1993). These findings were encouraging and suggested that CCK receptor agonists in combination with typical neuroleptics may be useful for the treatment of schizophrenia. However, subsequent placebo-controlled studies indicated that nonselective CCK receptor agonists or antagonists are ineffective in the treatment of schizophrenia (Innis et al., 1986; Whiteford et al., 1992). New generations of agonists and antagonists acting with selectivity at CCK_1 or CCK_2 receptors are available, and clinical trials with these new compounds, alone or in combination with dopaminergic agents, are eagerly expected.

3. CCK and Depression. One of the physiological actions of the neuropeptide CCK seems to involve modulation of the nigrostriatal and mesolimbic dopaminergic pathways. Taking into consideration that the mesolimbic dopaminergic pathways play a crucial role in motivation and rewarding processes, which are likely to be altered in depression (for a review, see Willner, 1990), a role of CCK in mood disorders cannot be excluded.

Several studies have shown that selective CCK_2 receptor agonists, such as BC 264 and BC 197, potentiate the decrease in motor activity in mice that have been subjected to electric footshocks the day before (conditioned motility suppression test used to study antidepressant drugs), whereas CCK_2 receptor antagonists, on their own, exert an opposite effect (Smadja et al., 1995). These results suggest that CCK_2 receptor antagonists have antidepressant-like properties in mice.

The involvement of CCK in behavioral responses associated with anticipatory stress has already been demonstrated, and the importance of external stimuli, such as a novel environment, in revealing the behavioral effects of CCK receptor agonists or antagonists has been emphasized in several studies (Crawley, 1984; Daugé et al., 1989; O'Neill et al., 1991; Lavigne et al., 1992). In the conditioned immobility test, anticipatory stress on the day of the test might increase the sensitivity of the CCK system, allowing the effects of CCK_2 receptor agonists and antagonists to be detected. The antidepressant-like effects observed with CCK_2 receptor antagonists could result from an increase in extracellular dopamine, because they were preventable by both D_1 and D_2 receptor antagonists in the forced-swim test (Hernando et al., 1994; Fig. 8). Taken together, these data suggest that depression is associated with a hyperactive CCK_2 receptor system and that CCK_2 receptor antagonists may be useful in the treatment of depressive syndromes (Daugé and Roques, 1995).

However, relatively little is known about the role of CCK in clinical depression. Several laboratories have demonstrated that patients with major depression display cerebrospinal fluid CCK concentrations comparable

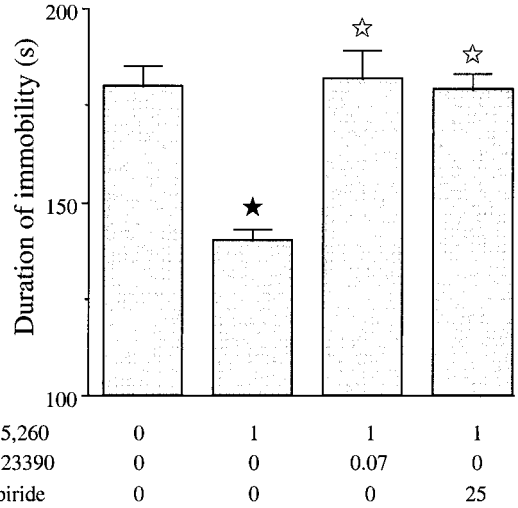


FIG. 8. Prevention of the effects of L-365,260 (1 mg/kg i.p.) by the selective dopamine D_1 receptor antagonist SCH-23390 (0.07 mg/kg s.c.) or the dopamine D_2 receptor antagonist sulpiride (25 mg/kg s.c.) in the forced-swim test in mice. $^{*}P < .05$ compared with the control group; $^{\circ}P < .05$ compared with the same dose of L-365,260 without antagonists (Newmann-Keuls test).

to those of control subjects (Gerner and Yamada, 1982; Geracioti et al., 1993). However, there is some evidence that an increase in cerebrospinal fluid CCK levels can occur in particularly severe depression (Löfberg et al., 1998). On the other hand, postmortem studies have revealed that compared with healthy controls and patients with schizophrenia, suicide victims have elevated prepro-CCK mRNA levels and an increased density of CCK-containing neurons in the dorsolateral prefrontal cortex and a higher density of CCK receptors in the frontal cortex (Ferrier et al., 1985).

4. CCK and Memory Processes. There is increasing preclinical evidence that the CCK system may play a role in memory processes. The presence of CCK is conspicuous in brain regions suspected to underlie memory functions, including the hippocampal formation, amygdaloid nuclei, and cerebral cortex. It has been suggested that CCK_1 and CCK_2 receptors have different roles in learning and memory functions (Harro and Oreland, 1993). In particular, a balance between CCK_1 receptor-mediated facilitatory effects and CCK_2 receptor-mediated inhibitory effects on memory retention has been postulated (Lemaire et al., 1992, 1994). However, there are conflicting reports on the effects of CCK_2 receptor agonists in animal models of memory. For instance, although some groups have reported that selective CCK_2 receptor agonists (e.g., CCK-4, BC 264) impair memory (Katsuura and Itoh, 1986; Daugé et al., 1992; Lemaire et al., 1992; Derrien et al., 1994a), others have found that these peptides enhance memory (Gerhardt et al., 1994). Treatment with BC 264 has also been described to elicit prominent hypervigilance in monkeys and to increase behavioral arousal in rats (Daugé and Roques, 1995). The latter findings suggest a possible role for CCK_2

receptor in attentional activation that can facilitate learning.

To date, only a few studies have been devoted to the effects of CCK receptor agonists on human memory. In one study, the administration of the nonselective CCK receptor agonist ceruletide had no demonstrable effect on recent or remote memory, although at higher doses it produced mild sedation. On the other hand, electrophysiological investigations of event-related brain potentials showed that ceruletide improved selective attention in healthy volunteers (Schreiber et al., 1995). Ceruletide has also been reported to improve cognitive processing in young, but not in elderly, healthy subjects (Dodt et al., 1996). Recently, Shlik et al. (1998) found that the continuous administration of the selective CCK_2 receptor agonist, CCK-4, had no effect on psychomotor performance, although it produced impairments in cognitive tests of free recall and recognition. The results of this study suggest that CCK-4 may exert a negative influence on memory consolidation and retrieval.

Factors that potentially contribute to discrepant findings include differences in experimental paradigms, dosage, and mode of drug administration. Another possible explanation of the discrepant findings on the role of CCK receptors in memory function might be due to the heterogeneity of CCK receptors (discussed earlier). In the two-trial memory task based on exploration of novelty, it has been shown that BC 264 enhanced spatial working memory, supporting the cognitive-enhancing properties of this agonist, whereas BC 197 was found to induce an amnesic effect (Fig. 9), a result in good agreement with the memory deficit obtained with CCK-4 (for a review, see Daugé and Léna, 1998). Interestingly, similar observations were made with a propionyl analog of BC 264, pBC 264, in both young and aged rats (Taghzouti et al., 1999). Thus, the latter CCK_2 receptor agonist enhanced consolidation and retrieval processes in young and aged rats but did not affect acquisition. Moreover, it has been shown through microdialysis that BC 264, injected i.p. at pharmacologically active doses, increased the extracellular levels of dopamine and its metabolites (dihydroxyphenyl acetic acid and homovanillic acid) in the anterior part of the nucleus accumbens (Ladurelle et al., 1997). Thus, it could be hypothesized that activation of dopaminergic transmission in the nucleus accumbens, which has been involved in some components of memory processes (Taghzouti et al., 1985; Ploeger et al., 1994; Floresco et al., 1996), could be the mechanism by which BC 264 produces its effect on attention and/or memory. On the other hand, the effects due to BC 197 might be nonspecific. Indeed, BC 197 can exert anxiogenic-like effects (Derrien et al., 1994b), and the response observed after peripheral administration of this CCK_2 receptor agonist in the two-trial memory task could reflect more such effects than a true disruption of memory processes (review in Daugé and Léna, 1998).

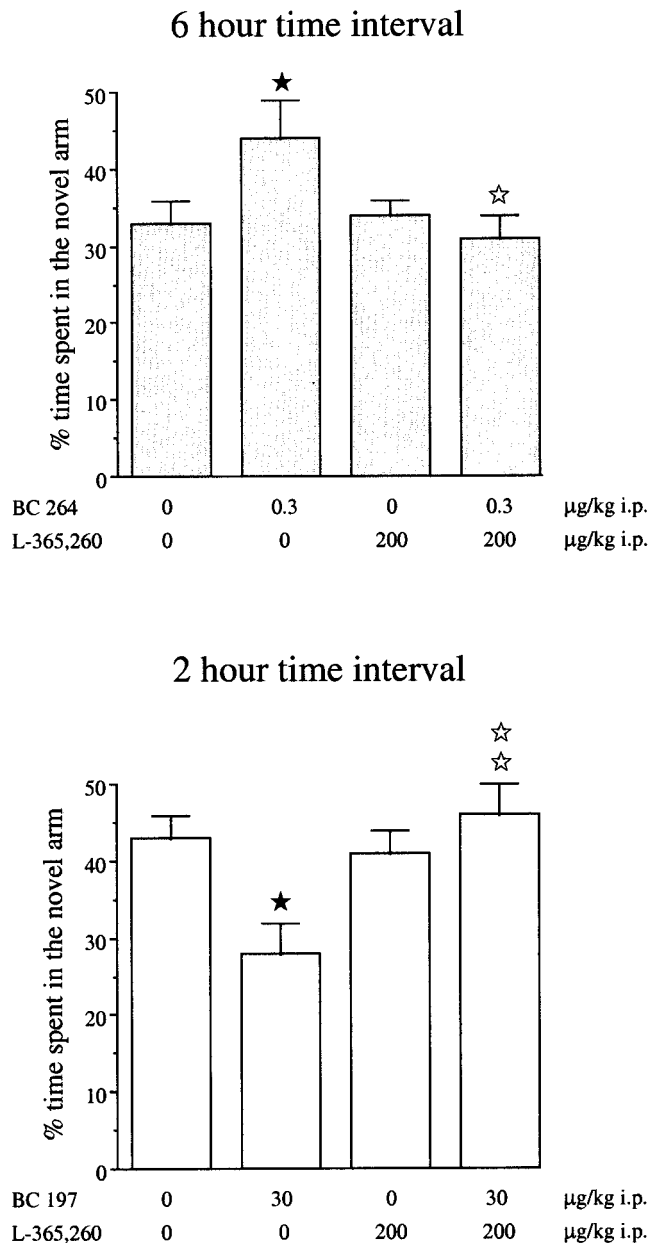


FIG. 9. Effects of the selective CCK_2 receptor agonists BC 264 and BC 197 on working memory in a two-trial task in the Y maze. In the first trial (acquisition phase), one arm of the maze was closed and the rats were allowed to visit the other two arms for 3 min. During the second trial (retrieval phase), rats had free access to the three arms for 3 min. When the two trials were separated by a 2-h time interval, recognition memory allowed the control rats to spend more time in the novel arm. When the two trials were separated by a 6-h time interval, recognition memory was lost, and the control rats spent approximately the same time in the three arms of the maze. BC 264 or BC 197 was injected i.p. 30 min before the second trial (restitution phase). The CCK_2 receptor antagonist L365,260 was injected i.p. 60 min before the experiment. The results are expressed as mean ± S.E. of the percentage of time spent in the novel arm. $^{*}P < .05$ compared with control; $^{*}P < .05$ and $^{**}P < .01$ compared with CCK_2 receptor agonist alone (Duncan test).

These results provide further evidence of the heterogeneity of CCK_2 receptors and show that their stimulation in rats, depending on the agonists used, can mediate

distinct behavioral responses. On the other hand, the modulation of memory processes by BC 264 or analogs could offer a new perspective in the treatment of attention/memory disorders associated with ageing or neurodegenerative diseases.

5. Interactions between CCK and Enkephalin Systems.

a. In the Control of Pain. Anatomical studies have shown that the distribution of CCK-8 and CCK receptors parallels that of endogenous opioids and opioid receptors in the pain-processing regions in both the brain and the spinal cord (Gall et al., 1987; Pohl et al., 1990). This overlapping distribution triggered numerous investigations on the role of CCK in nociception. Thus, several groups described a naloxone-reversible antinociceptive effect of CCK-8 or its analogs in relevant antinociceptive tests, such as the hot-plate, writhing, and tail-flick tests (for a review, see Baber et al., 1989). However, it has also been reported that CCK-8 has antiopioid properties. Thus, Faris et al. (1983) found that CCK reduced the antinociceptive effects produced by the release of endogenous opioids but did not modify opioid-independent analgesia induced by hind paw foot shock. In addition, numerous studies have shown that peripherally administered CCK receptor antagonists potentiate opioid antinociceptive responses, confirming the existence of a functional antagonism by endogenous CCK and opioid systems (for a review, see Roques and Noble, 1996). It has been hypothesized that CCK down-regulates opioid effects through activation of CCK_2 receptors. This hypothesis is supported by the data obtained with selective CCK_2 receptor antagonists. Indeed, these ligands strongly potentiate (+200–800%) the antinociceptive effects of endogenous enkephalins in rodents treated with RB 101, a mixed inhibitor of enkephalin-metabolizing enzymes (Fournié-Zaluski et al., 1992; Valverde et al., 1994). Interestingly, the combination of opioids with selective CCK_2 receptor antagonists enhanced the antiallodynic effects of morphine, suppressed the development of autotomy behavior in a model of neuropathic pain in rat, and efficiently relieved the allodynia-like symptoms in spinally injured rats (review in Roques and Noble, 1996).

The occurrence of functional interactions between the CCK and enkephalin systems in the control of pain has been suggested (Noble et al., 1993; Fig. 10). Schematically, the potentiation of the effects of exogenous or endogenous opioids by BDNL, a nonselective CCK_1/CCK_2 receptor agonist (Ruiz-Gayo et al., 1985), could be related to an increase in the release of enkephalins due to CCK_1 receptor activation (like that occurring by combined treatment with CCK-8 and a cocktail of peptidase inhibitors, Hendrie et al., 1989) and/or a direct improvement in the efficacy of transduction processes of the OP_3 (μ) opioid receptors, which might be allosterically evoked by CCK_1 receptor occupation (Magnuson et al., 1990). On the other hand, CCK_2 receptor activation could in turn negatively modulate the opioidergic system; this explains why the selective CCK_2 receptor agonist BC 264 produced a decrease in the lick latency in the hot-plate test in mice (Derrien et al., 1993b). If stimulation of CCK receptors is capable of modulating the opioid system, this sytem can in turn regulate the release of CCK peptides. Thus, the stimulation of OP_3 (μ) opioid receptors has an inhibitory influence on the K^+-evoked release of CCK-like material at spinal and supraspinal levels (Rattray and De Belleroche, 1987; Rodriguez and Sacristan, 1989; Benoliel et al., 1991, 1992). On the other hand, in vitro studies have shown that OP_1 (δ) opioid receptor agonists enhance the K^+-evoked release of CCK-like material from slices of rat substantia nigra and spinal cord (Benoliel et al., 1991, 1992). Also, the in vivo binding of the CCK_2 receptor selective agonist [^{3}H]pBC 264 in the mouse brain was found to be reduced by the administration of RB 101, a mixed inhibitor of enkephalin-degrading peptidases or BUBU [Tyr-D-Ser(*O-tert*-butyl)-Gly-Phe-Leu-Thr(*O-tert*-butyl)], an OP_1 (δ) receptor-selective agonist, supporting the idea that endogenous enkephalins increase the extracellular levels of CCK (competing with [^{3}H]pBC 264 at CCK_2 receptors) through the activation of OP_1 (δ) opioid receptors (Ruiz-Gayo et al., 1992).

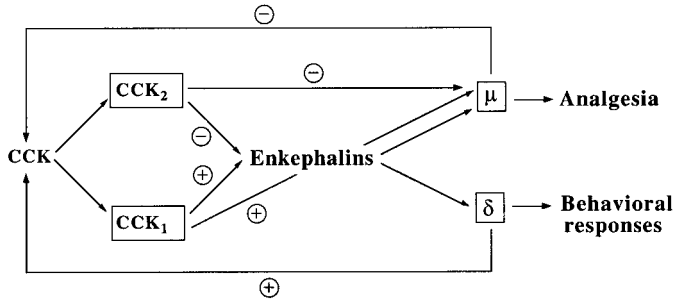


FIG. 10. Hypothetical model of the supraspinal interactions between CCK, via CCK_1 and CCK_2 receptors, and the opioid system via δ (OP_1)-opioid and μ (OP_3)-opioid receptors. CCK receptor agonists, endogenous or exogenous, stimulate CCK_2 and/or CCK_1 receptors, which can modulate the opioidergic (enkephalinergic) systems either directly (via the binding of opioid agonists or via C-fiber evoked activity) or indirectly (via the release of endogenous enkephalins). In addition, activation of μ (OP_3)-opioid receptors, which leads to antinociceptive responses, can negatively modulate the release of endogenous CCK, whereas δ (OP_1)-opioid receptor activation may enhance it.

b. In Behavioral Responses. In most behavioral studies, CCK has been found to behave as an antiopioid peptide (Noble et al., 1993; for a review, see Roques and Noble, 1996). A dysfunction in the balance between the two peptidergic systems involved in reward in the case of opioids and in attention and anxiety in the case of CCK could participate in the neurobiological mechanisms underlying vulnerability in drug addiction. Furthermore, it has been suggested that endogenous opioid peptides, especially enkephalins, might be involved in the cause of depression (for a review, see Roques et al., 1993) and that CCK-mediated processes might possibly counteract the antidepressant-like effects of opioids. In line with

these hypotheses, increasing the levels of endogenous enkephalins by RB 101 was shown to induce antidepressant-like effects in relevant paradigms, such as the forced swimming, conditioned suppression of motility, and learned helplessness tests (for a review, see Roques and Noble, 1996). In all these models of depression, rodents treated with RB 101 react to an adverse situation in the same way as after the administration of "classic" antidepressants, such as imipramine, desipramine, and amitriptyline.

Behavioral studies showed that blockade of CCK_1 and CCK_2 receptors produces opposite effects on the opioid-induced reduction of conditioned suppression motility due to endogenous enkephalins protected from peptidase inactivation by RB 101. Thus, the antidepressant-like effects of RB 101 were suppresed by the CCK_1 receptor antagonist L-364,718 and enhanced by the CCK_2 receptor antagonist L-365,260 (Smadja et al., 1995; Fig. 11). Given the reliable and strong facilitatory effects of CCK_2 receptor antagonists on the behavioral responses to RB 101, it was of interest to investigate the regions involved in the endogenous interactions between CCK and opioid systems. Because the mesolimbic system is known to be widely involved in the control of motivational and affective responses, two me-

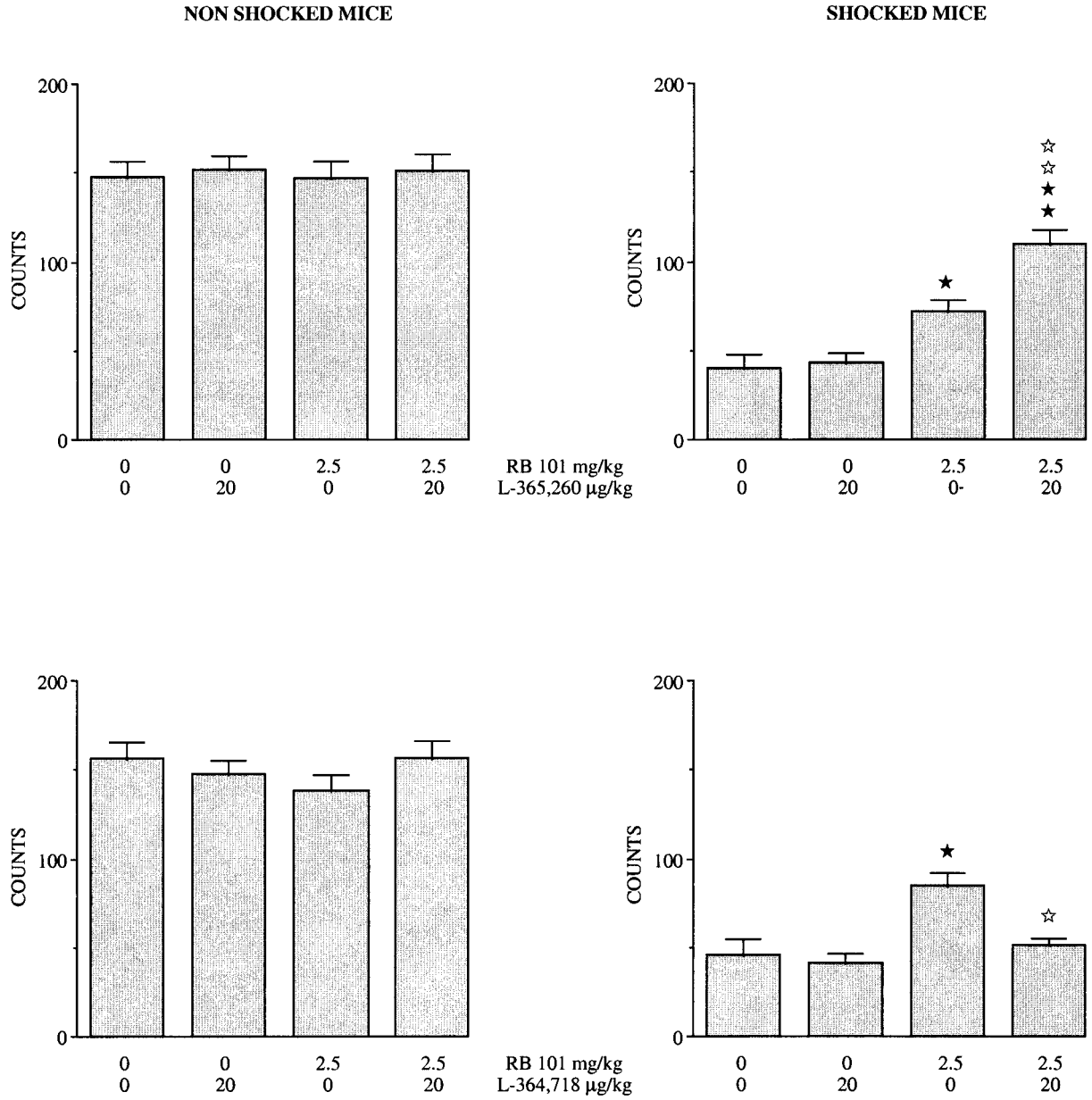


FIG. 11. Conditioned suppression of motility test in mice. Effects of the CCK_1 and CCK_2 receptor antagonists L-364,718 and L-365,260, respectively, on the antidepressant-like effects induced by i.v. injected RB 101. Mice were placed in a transparent rectangular cage with a metallic grid floor. Animal displacements were measured by drawing squares on the floor for counting. On the first day, the mouse was left in the test cage for 6 min and received electric footshocks. On the second day, the mouse was placed in the same cage without receiving electric footshocks, and motility changes were tested by counting the number of squares crossed, plus the number of rearings in 6 min. The mice belonging to the control group were handled in the same way as those in the conditioned suppression group except that they did not receive electric footshocks on the first day. $^{**}P < .01$ compared with control group; $^{*}P < .05$ and $^{**}P < .01$ compared with the same dose of RB 101 without antagonist.

solimbic structures were studied: the anterior nucleus accumbens and the central amygdala. Moreover, the nucleus accumbens has been implicated in the interaction between CCK and opioid systems in the control of other pharmacological responses (Kiraly and Van Ree, 1987; Mueller and Whiteside, 1990). The results obtained showed that the antidepressant-like effects of RB 101 were potentiated by microinjection of the CCK_2 receptor antagonist PD-134,308 in the anterior nucleus accumbens and the central amygdala, but not in the caudate nucleus, suggesting that the mesolimbic system plays an important role in the interaction between CCK and opioid systems in the control of these behavioral responses (Smadja et al., 1997).

On the other hand, the main challenge in the management of opioid addiction is to develop pharmacotherapy to minimize the short-term withdrawal syndrome and protracted opioid abstinence syndrome. Indeed, in the first days after the cessation of prolonged drug use, addicted subjects present an acute withdrawal syndrome, which consists of agitation, hyperalgesia, tachycardia, hypertension, diarrhea, vomiting, and subjective changes. Furthermore, a depression-like syndrome may persist for months or longer after the last dose of opiate. Relevant investigations have shown that during the acute morphine-withdrawal syndrome, there is an increased release of opioid peptides and that protection of these peptides by mixed enkephalin-degrading enzyme inhibitors reduces the opioid withdrawal syndrome (review in Roques et al., 1993). The recent demonstration that activation of CCK_2 receptors could negatively modulate the opioid system (see earlier) suggests that in contrast, the selective blockade of these receptors should increase the ability of mixed inhibitors to decrease the withdrawal signs. Indeed, this has recently been confirmed using RB 101 in association with the CCK_2 receptor antagonist PD-134,308 (Maldonado et al., 1995). Moreover, the protracted abstinence syndrome could be improved due the antidepressant-like properties of mixed inhibitors administered alone or in combination with the selective CCK_2 receptor antagonists. Thus, the possibility of relapse, the most important problem in the management of opioid addiction, should be minimized.

Interestingly, all of these behavioral studies showed that CCK_2 receptor antagonists do not apparently potentiate the subjective effects of opioids (for a review, see Roques and Noble, 1996). This finding should have important clinical implications in the management of pain, taking into account the strong antinociceptive responses to opioids in association with the CCK_2 receptor antagonists.

VIII. Conclusion

Since the original characterization of CCK by Ivy and Oldberg in 1928, followed by the isolation and sequencing of this hormone (Jorpes and Mutt, 1966), and its detection in the CNS (Vanderhaeghen et al., 1975), considerable advances have been made in the knowledge of the roles of this neuropeptide. The actions of CCK and related peptides have been extended to include endocrine secretion; motility and growth in the gastrointestinal system; and regulation of satiety, anxiety, pain, and dopamine-mediated behavior in the central and peripheral nervous systems. These actions are mediated by at least two distinct receptors, which have been pharmacologically characterized. The existence of these CCK receptors (CCK_1 and CCK_2) has subsequently been confirmed by their molecular cloning. Nevertheless, the large variety of functions mediated by CCK receptors, as well as pharmacological studies, suggests that some heterogeneity exists in CCK_1 and CCK_2 receptors. However, such a heterogeneity has not been confirmed in molecular biology studies, which have so far identified only two members of the CCK receptor family. The physiological and pathophysiological implications of these receptors can now be further investigated in CCK_2 receptor-deficient mice obtained through gene targeting (Nagata et al., 1996) and in Otsuka Long-Evans Tokushima Fatty rats, which have no functional CCK_1 receptors (Kobayashi et al., 1996). Several potential clinical applications concern the treatment of brain disorders and/or pain with CCK_2 receptor agonists or antagonists and of diseases involving food consumption with CCK_1 receptor ligands.

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