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CHOLECYSTOKININ ANTAGONISTS

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INTRODUCTION

Cholecystokinin (CCK) is a major intestinal hormone with an important role in regulating the control of pancreatic secretion and bile ejection. CCK is also one of the most widely distributed of brain neuropeptides. Its presence in the brain was first conclusively demonstrated in 1976 (1). Gastrin and CCK-8 have identical -COOH terminal penta-peptide sequences. Most gastrinlike activity in the brain is present as CCK-8, which exists in sulphated (CCK-S) and desulphated forms. CCK-containing neurones are widely distributed in brain. In some neurones CCK-8 coexists with other neurotransmitters.

Over the past decade major advances have occurred in our understanding of CCK receptors. As will be discussed later, there are at least 2 types of CCK receptor designated CCK-A and CCK-B. The gastrin receptor is similar to the CCK-B receptor and, to date, no compounds have been developed that will clearly distinguish between these two receptors.

Early in the 1980s the presence of putative CCK receptors was demonstrated in the pancreas and brain of the rat (2, 3) and derivatives of cyclic nucleotides were shown to antagonize the actions of CCK in the guinea pig pancreatic acini and ileum (4, 5). Although an important physiological role for CCK receptors in the periphery was recognized, the function of CCK in the brain was not understood.

In recent years specific and highly potent CCK antagonists have been developed including some that are highly selective for CCK receptor subtypes

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and have good brain penetrability. The availability of these compounds has prompted investigations into the functional role of CCK in brain and has opened up new possibilities for the treatment of CNS disorders.

CCK ANALOGUE BINDING AND CCK RECEPTOR SUBTYPES

High affinity CCK binding sites were initially demonstrated on isolated rat pancreatic acini (6) and rat cerebral cortex (3). Distinct differences in the specificity of brain and peripheral binding sites for various CCK-related peptides were immediately evident (2, 3, 7). First, the minimum sequence required for high affinity binding to brain sites differs from that in the periphery. In the brain, C-terminal tri- and tetra-peptides of CCK (8–10) appeared to bind to a single class of receptor to which CCK-8 also binds (11, 12), making the presence of separate high affinity binding sites for CCK-4 unlikely. However, at peripheral binding sites CCK-8 appears to be the minimal sequence for high affinity binding. This may be related to the sensitivity of the peripheral receptor to the presence and exact position of the sulphate moiety (13). This is apparently not the case for the central sites, hence CCK-4, gastrin, and desulphated-CCK bind to these sites, albeit with some loss of potency when compared with CCK-8 (9, 14), whereas these compounds have up to a 600-fold decrease in affinity for the peripheral site when compared with CCK-8 or CCK-33. Sulphation of CCK at position 7 from the COOH terminal is essential for high affinity binding at peripheral sites (13, 15) but not at central sites. This indicates structural differences between the two receptor types, possibly reflecting the evolutionary pressures for peripheral receptors to distinguish CCK from gastrin. It was later shown that the order of potencies of CCK-7 and CCK-8 analogues with substitutions at positions 3 or 4 differ markedly between pancreatic and brain receptors (16). In addition, the development of cyclic CCK-related peptides highly selective for central sites (17–20) provided further useful tools for distinguishing receptor types. The use of CCK fragments combined with autoradiography provided the first evidence for the presence in the brain of two CCK receptor types, "A" (alimentary) and "B" (brain) (21). The evidence from binding studies for CCK-A and CCK-B receptors has been supported in functional experiments, for example, amylase release from pancreatic acinic (22) and excitatory responses in rat hippocampal neurones (23). Extensive evidence now indicates that the original classification of peripheral CCK receptors as CCK-A type and brain receptors as CCK-B is an oversimplification. Yoder & Moody produced evidence for B type receptors in the periphery on small cell lung cancer cells (24), although, as previously mentioned, CCK-B receptors resemble gastrin receptors. Differences in peripheral recep-

tors have been reported from studies on pepsinogen secretion from chief cells from guinea-pig stomach. Here a class of receptors was designated as "C" receptors, differing from CCK-A receptors in the ability of gastrin I to stimulate secretion with equal potency to CCK-8. "G" receptors were similar to gastrin receptors but again displayed equal affinity for gastrin and CCK-8. The C receptors, like CCK-A receptors, were blocked by antagonists like L-364,718 (devazepide) and proglumide (25).

The distribution of CCK-A and CCK-B receptors in brain has been analyzed in a series of studies using binding and autoradiography techniques (26–29) in conjunction with highly selective CCK-A and CCK-B antagonists (30, 31). Autoradiography has revealed CCK-A receptors in area postrema, nucleus tractus solitarius, and interpeduncular nucleus of the rat (30, 31). There is, however, a substantial species variation in receptor specificity and localization (26). Accordingly, in the primate, CCK-A receptors occurred in substantia nigra (Figure 1), mammillary body, and substantia gelatinosa of the spinal cord, whereas in rat these regions contained CCK-B receptors (30). In the rat, the CCK-A receptors in the interpeduncular nucleus were presynaptic since they were depleted following a lesion in the habenula (31). Nevertheless, the majority of brain CCK receptors as revealed by autoradiography are CCK-B, with a high density of sites in ventromedial hypothalamus, cortex, hippocampus, and areas of the limbic system and basal ganglia (30, 31).

A further way to examine potential receptor heterogeneity has been to determine the molecular mass following solubilization and either photo-affinity labeling or chemical cross-linking. A variety of photo-affinity labels revealed some differences in the minimum molecular weights of receptors from differing peripheral sources. Thus, in the pancreas the complex was determined as a 76 kd subunit linked via a disulphide bond to a 40 kd subunit (32, 33). In gastric smooth muscle tumors (34) the major subunit was determined as 80 kd and in gallbladder as 70–85 kd (35). Both studies reported minor bands on the polyacrylamide gels corresponding to a mol wt of 100–120 kd. In contrast, the only subunit identified in solubilized cerebral cortex had a mol wt of 55 kd (36). This emphasizes the structural and functional differences in pancreas and brain CCK receptors. These studies all used probes based on long lengths of CCK (e.g. CCK-33 CCK-39) that can give rise to potential degradation artifacts common in this type of approach. Subsequent studies based on the shorter CCK-8 molecule (37–42) revealed a receptor complex of minimum molecular mass 85–95 kd for the pancreatic subunit (43) and 70–85 kd for the bovine gallbladder (44). All minor bands of 40 to 50, 92, 100, or 200 kd, previously reported, appeared to be dependent on the cross-linker type and its concentration (44). This difference in mol wt for the pancreatic and gallbladder receptor complexes might represent molecular heterogeneity of the peripheral CCK receptor. Further, the applica-

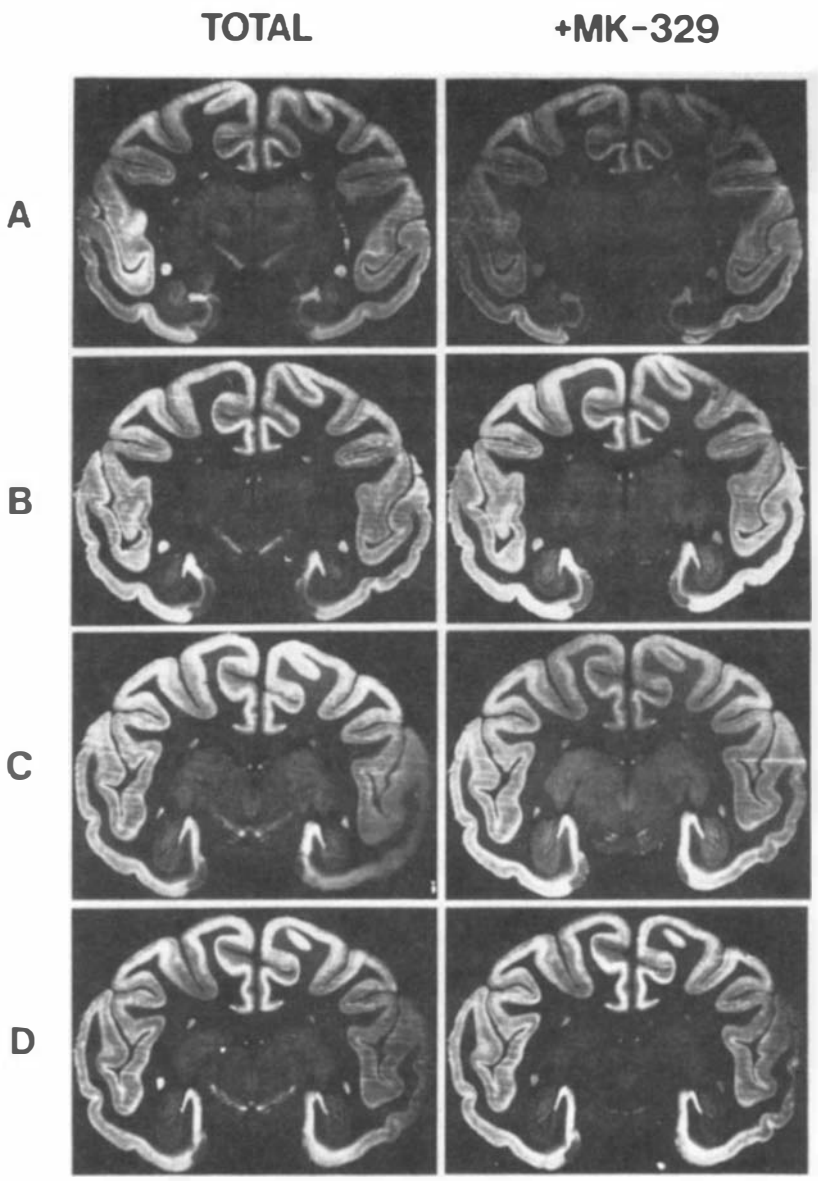

Figure 1 Autoradiographs of 1251-Bolton Hunter CCK binding to progressively caudal sections through the substantia nigra in cynomolgus monkey brain. CCK-A receptors defined with the CCK-A antagonist MK-329 (devazepide) were present throughout the rostrocaudal axis of the substantia nigra and were concentrated in the zona compacta (snc) rather than the zona reticulata (snr). Taken from ref. 30.

tion of such probes to central receptors might help to confirm the existence of differences in the molecular structures of CCK-A and CCK-B type CCK receptors.

The autoradiographic studies in the brain have proved a useful method of locating CCK receptor subtypes. However, additional approaches are required to demonstrate possible functional relevances of the receptors. In this respect the use of electrophysiological techniques has been particularly valuable and has revealed the presence of CCK receptors not readily detectable by autoradiography. Using slice preparations maintained in artificial CSF and recording from single neurones, excitatory responses to CCK can be demonstrated in the ventromedial nucleus of the hypothalamus where CCK has potent excitatory actions mediated by CCK-B receptors (45). However, in the dorsal raphe nucleus a completely different pattern of pharmacological specificity is observed. The 5-hydroxytryptamine (5-HT) containing neurones in the dorsal raphe nucleus can be subdivided into at least three different populations of cells. In one group the neurones are potently excited by CCK but are unaffected or only rarely affected by bombesin (46, 47). The CCK response appears to be mediated by CCK-A receptors (46, 47). About 40% of 5-HT-sensitive neurons in the dorsal raphe that are unaffected by CCK are potently excited by bombesin; the third group of 5-HT-sensitive cells in the dorsal raphe nucleus are unaffected by CCK or bombesin (46, 47).

Data summarizing the sites where CCK receptors have been located, the type of receptor thought to be present, and the possible activity mediated by CCK at this site are given in Table 1.

CCK RECEPTOR ANTAGONISTS

CCK receptor antagonists can be grouped into five broad categories: (*a*) derivatives of cyclic nucleotides; (*b*) derivatives of amino acids; (*c*) partial sequences and derivatives of the C-terminal sequence heptapeptides of CCK; (*d*) benzodiazepine derivatives and (*e*) nonpeptide "peptoids", based on fragments in the CCK molecule.

Derivatives of Cyclic Nucleotides

Dibutyryl cyclic guanosine monophosphate (Bt_2cGMP) (Figure 2) was the first competitive antagonist of CCK-mediated actions to be discovered (4). It was found to cause reversible and selective inhibition of CCK-stimulated amylase secretion from rat pancreatic cells. Subsequently, it was found to block the actions of CCK at many peripheral sites, for example it antagonized the action of CCK on the ileum myenteric plexus of the guinea-pig (5), inhibited the CCK-stimulated release of insulin from rat pancreas (48), and prevented CCK-evoked contraction of guinea-pig gallbladder (49). However, Bt_2cGMP failed to act as a specific inhibitor of CCK binding in mouse

Table 1

Site of CCK receptor	Type of receptor	Species examined	Action of CCK: Well-established	Action of CCK: Unknown or speculative	References
Pancreatic acinic	A	Rat, guinea pig, dog	Amylase secretion		2, 3, 22, 186
Pancreatic islet cells	A	Rat	Stimulation of insulin release		8, 48
Gastric mucosa (fundic glands)	A	Guinea pig	Control of pepsinogen secretion, and contraction of longitudinal muscle		180, 183, 189
	B		Contraction of neurally mediated circular muscle		
Gallbladder (smooth muscle)	A	Guinea pig, cow, human	Contraction of longitudinal muscle		35, 49, 189, 194, 205
	B		Contraction of neurally mediated circular muscle		
Pyloric sphincter + ileum + colon	A	Cow, guinea pig	Control of gastric emptying and intestinal mobility		187, 189, 201
	B		Contraction of neurally mediated circular muscle		
Gastric smooth muscle cells	A	Human	Inhibition of gastric emptying		34, 203
Gastric smooth muscle tumor cells	A			Unknown	
Stomach chief	C + G	Guinea pig	Pepsinogen secretion		25, 206

Retina	unclassified	Toad		Unknown	198
Caudate nucleus	B	Rat		Regulation of motor function	179, 182
Cerebral cortex	B	Mouse, rat, guinea pig, human, hamster		Modulation of dopaminergic systems	26, 29, 63, 82, 181, 182, 188, 197
Dentate gyrus	B	Rat, hamster, macaque		Unknown	8, 63, 196, 199
Olfactory bulb	B	Rat, hamster, human		Control of food intake and satiety	8, 29, 63, 181, 182, 188
Claustrum	B	Rat		Unknown	63
Nucleus accumbens	B	Rat, human not in mouse	Mediation of turning behavior and enhanced dopamine efflux		8, 27, 63, 133, 134, 184, 188, 207
Substantia nigra	A	Monkey		Hypoexploration.	
and striatum	A	Rat		Increased firing rate of dopamine neurons	8, 30
	B	Cow		Potentiation of the inhibitory effect of apomorphine and suppression of dopamine release	184, 190, 191, 195, 207
Amygdala	B	Rat, macaque, hamster, human, not in mouse or guinea pig		Facilitation of memory	27, 29, 63, 181, 188, 191, 200
Hippocampus	B	Rat, macaque, hamster, human		Facilitation of memory	8, 23, 29, 181, 182, 188, 200
Caudate nucleus	B	Rat, human		Unknown	182, 188

Table 1 (*continued*)

Site of CCK receptor	Type of receptor	Species examined	Action of CCK: Well-established	Action of CCK: Unknown or speculative	References
Cerebellum	B	Mouse, guinea pig, human, not rat		Unknown	26, 27, 188
Area postrema and nucleus tractus solitarius	A	Rat, human		Unknown	21
Medial temporal lobe	B	Macaque		Modulation of cortical afferents	191
Interpeduncular nucleus	A	Rat, mouse, not guinea pig		Unknown	138
Spinal cord (dorsal horn)	A	Monkey, human		Mediation of pain and morphine analgesia	
Dorsal raphe	A	Rat		Stimulation of cell firing	132
CHP212 Neuroblastoma cells	A	Human		Unknown	137
Vagus nerve complex	A	Rat, hamster, human		Satiety effects	29, 188, 192, 204
Hypothalamus	B	Rat, human, hamster, not mouse or guinea pig	Regulation of oxytocin vasopressin release		29, 202
	A	In magnocellular cell groups		Unknown	
Thalamic reticular nucleus	B	Rat		Modulation of sensory transmission	63, 193

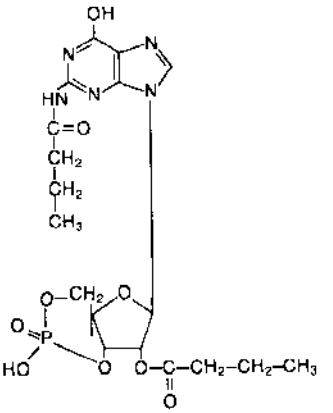

Figure 2 Structure of dibutyrl cyclic GMP.

cerebral cortex (7), nor did it inhibit the CCK-evoked release of acetylcholine from guinea-pig gallbladder (49), a process believed to be neurally mediated.

Further studies have shown that although unsubstituted cyclic nucleotides do not act as CCK antagonists, the butyryl derivatives of cGMP and cAMP do show antagonist activity, although Bt_2cGMP is the most potent. In addition, the 8-bromo derivatives of cGMP and cAMP are equipotent with Bt_2cGMP (50, 51). The potency with which these cyclic nucleotides inhibited CCK-stimulated calcium efflux and enzyme secretion correlates closely with the potency with which the nucleotide inhibits ^{125}I-CCK binding, suggesting that the compounds are acting at the recognition site of the CCK receptor.

Amino Acid Derivatives

During the 1970s, some amino acid derivatives were found to possess anti-gastrin activity (52, 53). The chemical similarities between gastrin and CCK made it probable that such amino acid derivatives would show CCK antagonist properties, and this was indeed the case (54). Proglumide (D,L-4-benzamido-N,N-di-n-propylglutaramic acid) and benzotript (N-p-chlorobenzoyl-L-tryptophan) have subsequently been shown to be competitive, CCK-specific, antagonists for numerous peripheral sites. For example, both cause a rightward shift in the dose-response curve for CCK-stimulated amylase secretion (54, 55), antagonize the synergistic effect of CCK and glucose on insulin release (56, 57), and block the antagonism of the CCK-induced contraction of the smooth muscle in the gallbladder, stomach, and ileum (57). These antagonists were found to be significantly more potent than Bt_2cGMP, and to have the added advantage of being active after oral administration (54, 57). Numerous studies followed in which proglumide was used to determine the physiological role of CCK in mediating various behaviors.

Interpretation of some of these results is difficult and discrepancies exist between the doses required to block behavioral events and the potency of proglumide at CCK receptors. Many of these studies were concerned with satiety. Proglumide was found to reverse the CCK-induced reduction of food intake in rats (58–60). This effect was observed when CCK was administered either peripherally or centrally, specifically in the area postrema of the brain (61, 62). Although the distribution of CCK receptors in the brain might point to a CNS role for CCK in the control of feeding, it is not clear to what extent the effect of the peptide on satiety is due to a peripheral mechanism (63). Proglumide and benzotript have been used with limited success to analyze other behavioral and functional actions of CCK (64–67). The results from these studies need to be re-evaluated in the light of the development of selective CCK-B antagonists and caution should be exercised in interpreting results obtained using antagonists with relatively low selectivity between CCK-A and CCK-B receptor subtypes in experiments intended to define the receptor mediating the behavioral actions of CCK. This problem is compounded by the finding that the original classification of CCK receptors into "peripheral" and "central" subtypes no longer holds.

To determine the structural requirements for the interaction of the amino acid derivative class of antagonists with the CCK receptors, derivatives of benzotript and proglumide were tested. For derivatives of tryptophan it appeared that potency was increased with increased hydrophobicity of the N-acyl moiety (68), and of the various compounds tested N-carbo-benzoxy-tryphophan (CBZ-tryptophan) was the most potent. Further testing of CBZ-amino acids (69) established the additional importance of other structural features, with aromatic amino acids being more potent than aliphatic ones of comparable hydrophobicity.

The synthesis and evaluation of new glutaramic acid derivatives produced CCK antagonists that not only displayed potencies hundreds of times greater than proglumide (70, 71), but also had the added advantage, unlike the CBZ-amino acids, that in the rat, mouse, and guinea pig no agonist activity was observed (72).

Analogues of proglumide showed varying degrees of selectivity for CCK-A receptors and suggested possible sub-types of the peripheral CCK receptor. Thus, in a study of proglumide derivatives that differed in the length of the di-n-alkyl group and in the substituents in the benzene ring, some derivatives had higher affinity for the pancreatic CCK receptor than for the CCK receptor mediating gallbladder contraction, and vice versa.

With CR 1409 or lorglumide (DL-4-(3,4-dichlorobenzoylamino)-5-(di-n-pentylamino)-5-oxo-pentanoic acid), there was a 20–26-fold increase in potency for blocking CCK-stimulated gallbladder contraction, but only a twofold increase for blocking CCK-stimulated pancreatic amylase secretion (73–77).

Two compounds of this group are of particular interest. CR 1409 and CR 1505 or loxiglumide (D,L-4-(3,4-dichlorobenzoylamino)-5-(N-3-methoxypropylpentylamino)-5-oxo-pentanoic acid are both potent and specific competitive antagonists for CCK-A receptors, and both are active when administered orally (76). These compounds have proved active at peripheral sites in blocking the contractile or secretory effects of CCK on tissues such as the ileum, gallbladder, and pancreas (77–82), and the trophic effects of CCK but not bombesin in the pancreas (83). They also block the effect of CCK on satiety and the effect of CCK-4 and CCK-8S administered intracerebroventricularly on the abdominal irritant-induced stretch assay, although the interpretation of these results is tenuous (84–86). Variations in relative potency have been observed in different tissues and species. Thus, CR 1409 is 7000 times more potent than proglumide in displacing binding to CCK receptors on pancreatic acini and 1000 times more potent for pancreatic secretion and growth (83). In another study, CR 1409 was much more effective in its ability to block CCK-induced pancreatic growth in hamsters than in rats (87). A more recent addition to this series of compounds, CR 1392 (D,L-4-(3,4-dimethylbenzoylamino)-5-(di-n-pentylamino)-5-oxo-pentanoic acid) has demonstrated good antagonistic potential in a study of exocrine pancreatic secretion, and appears to be less toxic (88) although it is two-threefold less potent than CR 1409. This compound may be useful in studying the in vivo physiological effects of CCK, and may have therapeutic potential.

Peptides

The first CCK-related peptide found to act as a CCK receptor antagonist was CCK-27-32-NH_2 (89), which antagonizes CCK-induced pancreatic enzyme secretion. In further studies, a new class of CCK receptor antagonists was created, based on COOH-terminal fragments of CCK (90), with a potency 30 times that of Bt_2cGMP. Research on pancreatic acini tissue has identified some important structural characteristics necessary for antagonist activity (89, 91, 92). In one study, the C-terminal amide (91) was shown to be an important determinant of affinity for the receptor. The C-terminal phenylalanine was claimed to be essential for intrinsic activity but not for binding (89). However, in a series of CCK 26–32 fragments including acetylated analogues, the N-terminal acetyl group appeared to have little or no effect on the affinity of the peptide for the receptor (91). The L-tryptophan residue is important also for binding to both central and peripheral CCK receptors and the significance of the correct stereochemistry is emphasized by the finding that the replacement of the L-tryptophan residue by D-tryptophan in C-terminal octa- and hepta-peptide analogues of CCK results in peptides with CCK-antagonist properties (92).

Peptide CCK antagonists have been used in functional studies, for example the CCK analog CCK 27–33 antagonized a CCK-mediated depression of

synaptic transmission in hippocampal slice preparations, whereas proglumide appears to have no effect (93). In another study, a synthetic peptide derivative of CCK-7, t-butyloxycarbonyl-Tyr (SO_3-)-Met-Gly-D-Trp-Nle-Asp2-phenylethyl ester inhibited binding of labeled CCK-9 to both pancreatic acini and cerebral cortical membranes (94) in addition to blocking agonist-stimulated amylase secretion.

Since 1980, two classes of CCK binding sites are thought to be present on pancreatic acini. Scatchard analysis following binding studies using radioiodinated CCK indicated the existence of a very high affinity site and a lower affinity site (6). Recently, a COOH-terminal heptapeptide of CCK,Boc-Tyr(SO_3)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester (CCK-JMV-180) was found to distinguish between these high and low affinity sites (95). CCK-8-stimulated amylase secretion from pancreatic acinii gives a bell-shaped dose-response curve. The upstroke of the curve corresponds to the occupation of high affinity stimulatory CCK receptors (Kd 70 pM). This secretory effect of CCK is mimicked by CCK-JMV-180. The downstroke of the dose-response curve reflects the occupation of low affinity, inhibitory, CCK receptors (Kd 10 nM). CCK-JMV-180 did not mimic the inhibition of amylase secretion at these receptors but rather reversed the effects of the natural peptide. This was interpreted as the compound having agonist activity at the high affinity pancreatic CCK receptors and competitive antagonist activity at the low affinity ones. Hence CCK-JMV-180 may provide a useful tool for analyzing the functions of these receptor types. A recent study suggested that the occupation of the low affinity CCK receptor sites is responsible for calcium mobilization (96), thought to play an important role in stimulus-secretion coupling in pancreatic acini. Likewise, the decapeptide CCK analog, caerulein, is thought to induce pancreatitis in rats by interacting with low affinity receptors (97) and inhibiting pancreatic digestive enzyme secretion. CCK-JMV-180 was reported to protect against the caerulin-induced pancreatitis (97).

Although CCK-JMV 180 may provide further insights into the functional significance of high and low affinity CCK receptors in the periphery, the lack of oral availability of it and other peptide CCK antagonists severely restricts their potential therapeutic use.

Benzodiazepine Derivatives

In a study involving iontophoretic application of CCK onto hippocampal neurones, it was reported that the benzodiazepines flurazepam, diazepam, and lorazepam, either acutely or chronically, could block some actions of CCK (98, 99), although some of the interpretations in these reports are open to question. In a more quantitative study on peripheral CCK receptors, the benzodiazepines chlordiazepoxide, medazepam, and diazepam were shown to

antagonize the contractile response to CCK in isolated strips from guinea-pig gallbladder (100, 101), although the potency of the compounds on CCK responses was not very high. That the effects of the benzodiazepines referred to above might be unrelated to their effects on benzodiazepine receptors was indicated by the finding that tifluadom, a benzodiazepine derivative that is a kappa opiate agonist but with very little effect on classical benzodiazepine receptors, is a potent CCK-A antagonist (102).

Lorazepam and chlordiazepoxide also inhibited nerve-mediated responses of ileal longitudinal muscle to CCK (103). However, benzodiazepines were weak in displacing CCK-binding in mouse brain (IC_{50} about 10 μM) (104).

The discovery during a natural product screening program (105) of a new, naturally occurring benzodiazepine, asperlicin, isolated from the fungus *Aspergillus alliaceus*, represented a major advance in the development of CCK receptor antagonists. Asperlicin proved to have a 300 to 400 times greater affinity for pancreatic, ileal, and gallbladder CCK receptors than proglumide, and is thus selective for CCK-A receptors as opposed to CCK-B or gastrin receptors. In further studies, four additional nonpeptide antagonists of CCK from the same fungal source (106, 107) were isolated and their structure determined. One compound, asperlicin B, was seven times more potent than asperlicin in binding studies using rat pancreatic membranes (106). Asperlicin had long-lasting CCK antagonist activity in vivo. However, low water solubility and poor oral bioavailability severely limited its use as a tool for pharmacological and physiological investigations. Thus, better antagonists were sought from this benzodiazepine class of CCK receptor antagonists (108). Analogues of asperlicin were synthesized that showed marked improvements in potency and water solubility (109). Then, using the known structures of 1,4-benzodiazepines and asperlicin, design and subsequent synthesis of the 3-substituted 1,4-benzodiazepin-2-amines followed (110). These proved to be potent, orally available CCK-A antagonists. Of particular interest was the compound 3S(−)-N-(2,3-dihydo-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3-yl)-1H-indole-2-carboxamide, now known as L-364,718, MK-329 or devazepide. This compound is one of a class CCK-A receptor antagonists with nanomolar affinity and selectivity for peripheral receptors, long-lasting efficacy in vitro and in vivo, and with oral bioavailability (111). Recent work on the synthesis and biological evaluation of 3-substituted benzolactams produced orally active, nanomolar potency antagonists for the pancreatic CCK receptor. Thus, the benzodiazepine part-structure is not essential for CCK antagonism. Molecular modeling studies suggest that the necessary elements could be the benzodiazepine and benzolactam core conformations, the 3-substituents, and a hydrophobic substituent at N-1 of the benzolactams or at C-5 of the benzodiazepines. These could all be similarly orientated so as to interact similarly with the CCK receptor (112).

Devazepide possesses potent CCK-A blocking activity in different tissues: pancreatic amylase secretion is antagonized (113–115), with a potency 600-fold greater than CR 1409 and 2,000,000-fold more than proglumide. Also antagonized is pancreatic protein, enzyme and pancreatic polypeptide release (116, 117), CCK-induced evaluation of plasma insulin levels in fed mice (118) and the camostate- and caerulein-induced growth of rat pancreas (119, 120). CCK-induced contraction of the colon and gallbladder is blocked by devazepide (121, 122), as is gastric emptying (123, 124). Furthermore, L-364,718 can readily penetrate the blood-brain barrier (125), although its central actions have not been well studied. Reportedly, devazepide can antagonize the CCK-8-induced effects on open field behavior (126) and impair CCK-8-induced memory processes (127, 128). Devazepide has been claimed to be a selective antagonist of the effects of CCK-8 on food intake (129, 130); the necessary dose of L-364,718 to block the anorexic effects of centrally administered caeruleins was at least two orders of magnitude higher than those required to antagonize peripheral CCK effects (131). Although devazepide shows selectivity for CCK-A versus CCK-B receptors it is nevertheless a potent CCK-B antagonist and care should be taken when attempting to distinguish between effects mediated by CCK-A or CCK-B receptors with this compound.

Nevertheless, because of its potency as a CCK-A antagonist, devazepide has proved to be a useful tool to characterize receptor subtypes involved in certain CCK-induced activities. It is now known, through the use of devazepide, that CCK-A receptors mediate the CCK-induced excitation of neurones in the rat dorsal raphe nucleus (46, 47, 132) and the CCK-related facilitation of dopamine efflux from the posterior region of the nucleus accumbens (133). This latter effect may be related to the finding that devazepide blocked the CCK-induced increased emotional state following direct injection into the posterio-median part of the nucleus accumbens (134).

Devazepide failed to bind significantly to solubilized CCK binding sites from the pig cerebral cortex, helping to establish that these solubilized receptor sites are of the CCK-B type with a similar pharmacological profile to that observed in membranes (135). Devazepide was a key tool in the autoradiographical demonstration of the presence of CCK-A receptors in various brain regions (30, 31). The use of radiolabeled L-364,718 has supported previous work showing the presence of CCK-A receptors in the area postrema and nucleus tractus solitarius (136), and also, surprisingly, identified CCK-A receptors on CHP212 neuroblastoma cells, in contrast to the CCK-B receptors widely distributed in cells of the CNS (137).

Further examples of species variations in the distribution of CCK-A receptors within the CNS have been demonstrated. The use of L-365,031 (1-methyl-3-(bromobenzoyl)amino-5-phenyl-3H-1,4-benzodiazepine-2-ore), an

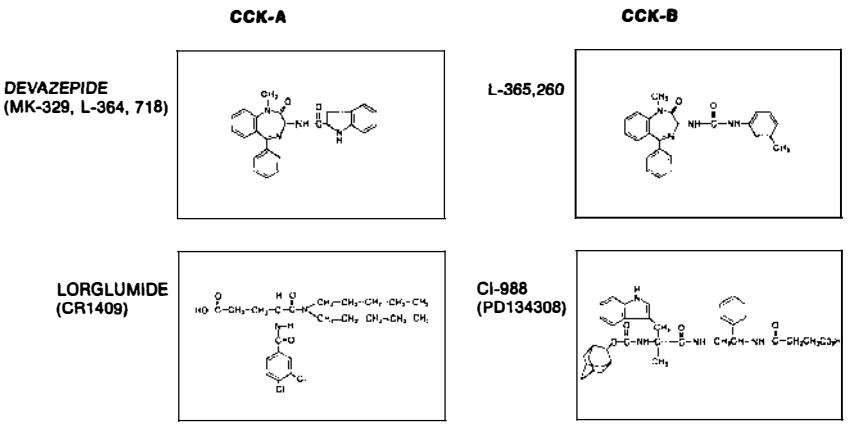

Figure 3 Structures of the CCK-A antagonists lorglumide and devazepide and of the CCK-B antagonists L365,260 and CI-988.

analogue of L-364,718 with similar selectivity for CCK-A receptors, revealed significant species differences in the distribution of CCK-A receptor sites within the CNS. A high density of CCK-A receptors is found in the region of the interpeduncular nucleus in the rat and mouse but not in the guinea pig (138). Thus, substantial differences appear even in closely related species. Devazepide has also been used to demonstrate the presence of CCK-A receptors in the dorsal horn (substantia gelatinosa) of the spinal cord, and it dose-dependently inhibited ^{125}I-CCK binding at low concentrations in the monkey and human, but not in the rat spinal cord (139), again demonstrating species-specific receptor distribution patterns.

During the development of L-364,718 it was observed that some analogues lost their selectivity for CCK-A. This led to the design of L-365,260 or (3R(+)-N-2,3-dihydo-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine)-3-yl)-N^1-(3-methylphenyl)urea) (134). The structure of L-365,260 and other CCK antagonists are shown in Figure 3. L-365,260 interacts stereoselectively and competitively with guinea-pig stomach gastrin and brain CCK-B receptors and in the original publications its quoted affinities for both were over two orders of magnitude higher than its affinity for peripheral pancreatic CCK-A receptors (141). It shows a similar high affinity for CCK-B receptors in rats, mice, and humans, although this affinity is lower in the dog. Additionally, [^{3}H]L-365,260 binds specifically to guinea-pig gastric glands (142). Whereas L-364,718 was reported to have a 125-fold higher affinity for pancreatic CCK-A receptors than for gastrin receptors, L-365,260 showed a 80-fold higher affinity for gastrin/CCK receptors than for pancreatic CCK-A

Table 2 Inhibition of ^{125}I-CCK binding to CCK receptors[1]

Compound	IC_{50}(nM) Mouse cortex CCK-B	Rat pancreas CCK-A	Pancreas/Cortex ratio
Agonist			
CCK-8S	330.3	0.1	0.3
Pentagastrin	0.8	600	750
CCK-8US	2.6	59	23
CCK-4	2.6	5330	2050
Antagonist			
CI-988	1.7	2717	1598
L-365,260	5.2	240	46
L-364,718	31	0.2	0.006
CR 1409	500	7.1	0.01

[a] Taken from Ref. 45

receptors (143). In our own studies, we found L-365,260 to be less selective for CCK-B receptors than originally reported, with a ratio of affinities for CCK-A versus CCK-B receptors of 46 (Table 2).

Recently, both L-364,718 and L-365,260 were used to investigate whether the satiety response to CCK is mediated by CCK-A or CCK-B receptors. L-365,260 was reported to be 100 times more potent than devazepide in increasing feeding frequency and preventing satiety in partially satiated rats (144). The conclusion from this study was that endogenous CCK causes satiety by interaction with CCK-B receptors in the brain, and contrasts with previous results that implicated CCK-A receptors in this response. However, these results must be interpreted with caution. A significant effect of L-365,260 was reported with doses as low as 100 pg/kg. L-365,260 has very low water solubility and in this study the compound was administered in a suspension in a carboxymethylcellulose vehicle (144). Clearly it is difficult to accurately inject such low doses from a suspension. Certainly there is a large discrepancy in the reported potency of L-365,260 in the satiety test (144) and its potency at other CNS effects believed to be mediated by CCK-B receptors, such as the elevated plus maze (see section on anxiolytic effects below) where the threshold effective dose of L-365,260 is nearly 1,000,000 times higher.

In a study from Japan, anthramycin, a benzodiazepine derivative produced by some streptomyces microorganisms, was reported to be a potent antagonist of CCK in mouse CNS (145). Anthramycin reversed CCK-8-induced antinociception and satiety, and was shown to displace [^{125}I]CCK-8 binding in various brain regions, but especially in the cortex. Further investigations into this compound are required to elucidate its pharmacological potential.

PEPTOIDS

Recently, we have reported on CI-988 (previously known as PD 134308) and PD 135158 (Figure 3), members of a new class of potent nonpeptide CCK antagonists of a novel chemical structure (45). These compounds are extremely potent displacers of binding from CCK-B receptors with IC50 values in the low nanomolar range (Table 2). In binding assays, using mouse cortex membranes as a source of CCK-B and rat pancreas as a source of CCK-A receptors, CI-988 and PD 135158 showed 1600- and 400-fold selectivity for the CCK-B receptors, respectively. This represents a unique selectivity compared with the other nonpeptide CCK-B receptor antagonist available (L-365,260), where in the same assays the selectivity ratio was 46 (45). The selectivity of these new ligands extended to other receptor systems and the compound were relatively inactive in displacing binding from muscarinic, histamine, GABA, 5-HT, or dopamine receptors. The functional potency of the compounds was also demonstrated in slices of rat brain, containing the ventromedial nucleus of the hypothalamus, where the compounds were potent antagonists of the stimulatory effects of CCK on cell firing. CI-988 and PD 135158 are also potent antagonists of the gastric secretory action of pentagastrin in the Gosh & Schild test (unpublished observation), providing additional evidence for the similarity between CCK-B receptors and gastrin receptors. CI-988 and PD 135158 have provided us with greatly improved research tools for use in mapping the distribution of CCK receptor subtypes (Figure 1) and for investigating the physiological role of CCK-B receptors.

As previously mentioned, functional CCK-A and CCK-B receptors can be demonstrated in brain using electrophysiological techniques. In single neurones in slices of the ventromedial hypothalamus CCK has a potent excitatory action; the receptors mediating this response can be classified as CCK-B and in this preparation CI-988 and PD-135158 were potent antagonists of CCK-induced increased firing with a Ke for CI-988 of 7.9nM, a result in good agreement with the Ki values obtained from binding data (45). In contrast, in slices of the rat dorsal raphe nucleus, CCK potently stimulates a subpopulation of the 5-HT-sensitive neurones and this response is mediated by CCK-A receptors (45) and is essentially unaffected by the two peptoid CCK-B antagonists.

OTHER COMPARISONS OF POTENCIES OF CCK ANTAGONISTS

Numerous studies have compared the potencies of various CCK-A antagonists on various in vitro and in vivo CCK-mediated activities and some of these have already been referred to. For accurate comparisons of potency all

Table 3 In vivo and in vitro potency of compounds at peripheral CCK-A receptors

Antagonist	IC_{50}[a] μmol/kg	In vivo secretion	In vitro secretion	In vitro binding
Proglumide	740	1	1	1
Asperlicin	11	67	300	600
CR1392	9	82	800	1,000
CR1505	6	123	900	1,000
CR1409	3	250	2,500	4,000
L-364,718	0.025	30,000	1,000,000	3,000,000

[a] IC_{50}, mean inhibitory concentration. Data obtained from Ref. 142.

assessments must be carried out under conditions as similar as possible and several papers have addressed this question in some detail. The first direct comparison of the in vivo potencies of various CCK antagonists was using a simple mouse assay, based upon visual determination of gastric emptying of a charcoal meal. This produced a rank order of potency of L-364,718 > asperlicin > proglumide (146). A more recent study compared the potencies of various antagonists in guinea-pig pancreas and gallbladder tissue slices. Binding studies produced a rank order of potency in gallbladder sections as L-364,718 > CR 1409 > asperlicin = CBZ-CCK-(27-32)NH_2 > Bt_2cGMP (147). Similar potencies were found in pancreas sections, and the potencies required to inhibit CCK-stimulated contraction or amylase release correlated closely with their ability to inhibit ^{125}I-CCK-8 binding in gallbladder and pancreas sections or acini, respectively. These studies provided no evidence of CCK-A receptor subtypes as measured by CCK antagonist affinity. Another study examined CCK antagonist potency in rat pancreatic secretion in vitro and in vivo (148). The rank order of potency of compounds to antagonize caerulein-stimulated amylase secretion in vivo agreed with their relative potencies in vitro, and with their affinity to bind to peripheral CCK-A receptors in vitro (see Table 3). However, the antagonists CR 1409 and L-364,718 were in relation to proglumide, 10–33 times less potent in vivo than in vitro.

The specificity of proglumide, CR 1409 and L-364,718 on CCK-8, carbachol- and glucose-stimulated insulin and glucagon secretion was compared in the mouse (149). Only L-364,718 antagonized CCK-8-stimulated secretion and not that caused by carbachol and glucose, indicating a greater specificity of the antagonist for this system. Structural comparisons between CCK receptor antagonist classes may provide new directives in the design of new, improved CCK antagonists. Recent efforts have been directed toward establishing links between the proglumaide and benzodiazepine classes (CR

1409 and L-364,718), with the result that hybrid CCK antagonists have been synthesized that show improved activity over existing proglumide-derived antagonists (150). Of particular interest has been the compound A-65,186, which possesses good potency and selectivity for CCK-A receptors. This type of molecular modeling approach may give rise to a greater variety of CCK-A antagonists in the future.

In terms of CCK-B antagonists, the most potent compounds yet described are PD 135302 and CI-988 (Table 1). These compounds have the added advantages of high receptor selectivity, good water solubility, and high potency in vivo.

USES AND APPLICATIONS OF CCK RECEPTOR ANTAGONISTS

CCK Physiology and Pharmacology

As described, CCK antagonists have been widely used to investigate the physiological roles for CCK and to identify the receptor subtypes involved in its actions. As better and more diverse antagonists have been developed, their use has broadened our understanding of CCK pharmacology and physiology.

Considerable interest has focused on the interaction of CCK and CCK antagonists with analgesics of the morphine type. Experiments revealed the reversal of opiate analgesia and morphine tolerance first by proglumide (151) and benzotript (152), and later by benzodiazepines (153) and L-364,718 (154). The initial studies using proglumide and benzotript supported the previous theory that CCK acts as an opiate antagonist, since morphine analgesia is potentiated by these antagonists (155). It was concluded that the endogenous release of CCK in response to opiate administration may act to return the organism to the basal level of pain sensitivity. It was later found that L-364,718 enhanced morphine analgesia in rats, and that the doses required were sufficiently high to block CCK-B receptors, indicating possible mediation of CCK involvement in antinociception by CCK-B type receptors (156). The earlier work also showed that proglumide can reduce and prevent the development of morphine tolerance (157). However, recent work using L-364,718 showed that, although still preventing tolerance development, this compound did not prevent the onset of opioid dependence (158), indicating that separate mechanisms mediate morphine analgesia and dependence. It was not clear whether the effects of L-364,718 (devazepide) were mediated by CCK-A or CCK-B receptors. The new generation CCK-B antagonist CI-988 has been shown recently to markedly potentiate the effects of morphine in the rat hot-plate test and on the excitability of a spinal nociception flexor reflex (159).

THERAPEUTIC POTENTIAL

Obviously in addition to providing a better understanding of the role of CCK within an organism, CCK antagonists may possess great therapeutic potential in humans. The recent improvements in potency, specificity, oral-bioavailability, and low toxicity of new CCK antagonists has increased hopes of producing therapeutically useful compounds. Past reviews have speculated on what these possible therapeutic uses could be (160, 161). Here we look briefly at past and present hopes regarding the therapeutic potential of CCK antagonists, referring where possible to recent relevant studies.

Periphery

PANCREATIC DISORDERS The inhibitory effect of CCK antagonists on pancreatic amylase secretion, coupled with observations that caerulein can induce a form of pancreatitis, suggests that they may have therapeutic potential for treatment of pancreatitis. Proglumide, CR 1409, CR 1505, and CR 1392 are demonstrably effective in reducing elevated serum amylase, pancreatic weight, and the histological alterations in the caerulein and the sodium taurocholate models of pancreatitis, even when administered after the pancreatitis has been induced (76, 162, 163). L-364,718 did not offer protective effects in these two pancreatitis models (164, 165) but improved biochemical, morphological, and mortality indexes in two surgical models of pancreatitis (166), where CCK potentiated the severity of the pancreatitis produced. So, despite some discrepancies in their effects, CCK antagonists are causing increasing optimism in this field.

Additionally, the ability of CCK to stimulate pancreatic growth and of CCK antagonists to antagonize this, has caused speculation about their potential value in the treatment of pancreatic cancer. In fact, recent work has demonstrated that CCK is involved in camostate-induced growth of acidophilic putative preneoplastic foci in rat pancreas and that CR 1409 inhibits its growth (167).

BILIARY DISORDERS CCK is believed to be a major regulator of gallbladder contraction, with CCK antagonists reducing contractions. Biliary colic is thought to result from intense contractions of the gallbladder when a stone obstructs the outlet and causes recurrent abdominal pain. Recently, loxiglumide was given to six human patients with biliary colic due to gallstones, and an immediate response was observed in all cases. All patients were pain-free in 20 minutes with no observed side effects (168). Thus, the use of loxiglumide or other CCK-A antagonists in the treatment of biliary colic looks promising.

GASTRIC DISORDERS Since CCK antagonists inhibit the CCK-induced delay in gastric emptying, they may be of therapeutic use in disorders involving problems with gastric emptying, and could also reduce the development of satiety by this mechanism and hence enhance appetite.

The role of CCK as a regulator of intestinal motility has led to suggestions that CCK antagonists may be of therapeutic use in the treatment of irritable bowel syndrome. This has not yet been demonstrated and it is not clear whether CCK-A or CCK-B antagonists would be preferable.

GASTRIN The CCK-B receptor is similar if not identical to the gastrin receptor. Currently available CCK-B antagonists have additional gastrin-blocking actions and as gastrin antagonists may be of value in the control of gastric acid secretion and in the treatment of gastrin-dependent proliferative disorders and gastrin-dependent tumors.

SATIETY As described earlier, there have been reports that CCK antagonists can reverse the satiety effects of systemic infusions of CCK-8, leading to increased food intake and hence improved appetite. Such observations suggest a possible role for CCK antagonists in the treatment of some anorectic conditions and possible appetite suppressant properties for agonists. However, postprandial plasma levels of CCK are apparently not sufficient to produce satiety (169), and the question of whether CCK antagonists could reduce satiety in disease states is still speculative.

POTENTIATION OF OPIATE/MORPHINE ANALGESIA Since CCK antagonists cause potentiation of opiate analgesia, while at the same time protecting against the development of narcotic tolerance, they may be clinically useful in the management of chronic pain, possibly by reducing the opiate dose required. In addition, this potentiation is achieved without the potentiation of other conditions such as respiratory depression and constipation (161). However, a recent study showed that whereas acute pretreatment with proglumide or lorglumide enhances spinal morphine analgesia, chronic treatment diminishes spinal morphine analgesia (170).

Central Nervous System Disorders

ANTIPSYCHOTIC The coexistence of dopamine and CCK in the midbrain has led to speculation that CCK antagonists may be therapeutically relevant for the treatment of schizophrenia. Clinically, neuroleptics and dopamine receptor blockers are effective in the treatment of schizophrenia and agonists or indirect agonists such as amphetamine produce or exacerbate the psychotic

symptoms. CCK and dopamine are colocalized in certain midbrain regions, those in the VTA projecting mainly to the nucleus accumbens, suggesting that CCK receptors might have some role in psychotic disorders. This notion is supported by findings that chronic treatment with amphetamine significantly reduced the Bmax for [^{3}H] CCK binding in the cingulate cortex (171). A study showing that intravenous administration of the antagonist CR-1409 reversed the depolarization inactivation of dopamine cells in the A9 and A10 regions induced by chronic haloperidol treatment (172) led to a suggestion that CCK agonists might be of use as antipsychotics. However, much more work is required on the possible interactions between dopamine and CCK. Whether either agonists or antagonists at CCK-A or CCK-B receptors would be of any value as antipsychotics remains an open question that can be addressed using the new CCK antagonists. It is of interest that CCK-A receptors are present in the primate substantia nigra and VTA (30).

ANXIOLYTIC The development of the new and highly selective CCK-B antagonists previously referred to has provided us with powerful new research tools to explore the functional role of CCK in brain. Accordingly, we used these compounds to investigate the possibility that CCK might be involved in the regulation of anxiety. If so, CCK receptor agonists and antagonists would have effects in animal models of anxiety. One suitable model for testing anxiolytic drugs is the rat elevated X-maze (173). In this model untreated rats spend most of the test period in the two enclosed arms of the maze. Anxiolytics such as benzodiazepines cause an increase in the time spent in the open arm of the maze and conversely anxiogenic-treatment increases the time spent in the closed arm. The rat social interaction test can also be used to detect anxiolytic activity; in this test, social interaction of a pair of rats is suppressed by an aversive environment and this suppressed behavior is restored by anxiolytic drugs (174). The mouse black-white box is also sensitive to all types of anxiolytics (175); in this mouse test, suppression of exploration of the white compartment of the black-white box is restored by anxiolytics.

The CCK-B antagonists CI-988 and PD 135158 were found to be remarkably effective and potent as anxiolytics in the above tests. In the two rat tests for anxiolytics, CI-988 and PD 135158 were potent anxiolytic agents with maximal anxiolytic activity being produced at doses of less than 0.1mg/kg subcutaneously or intraperitoneally (45). Anxiolytic activity was maintained over a wide dose range and even at doses as high as 30 mg/kg there was no sign of sedation. The compounds were similarly potent as anxiolytics when used in the mouse black-white test (45, 176) and in the marmoset-human threat test of anxiety (45). Both compounds are active orally or subcutaneously.

Our results lead to the conclusion that brain CCK-B receptors are involved in anxiety in the mouse black-white box and in the rat elevated X-maze. The two nonpeptide antagonists CI-988 and PD 135158 are the most potent and selective CCK-B antagonists yet described and are potent anxiolytics in the test mentioned above. Furthermore, in preliminary experiments in our own studies the CCK-A antagonist devazepide also showed anxiolytic-like activity in the mouse and rat tests for anxiety, although because of the relative nonselectivity of the compound this was perhaps mediated by CCK-B receptors (45, 176). We have also demonstrated anxiolytic activity with the benzodiazepine CCK-B antagonist L-365,260 in the rat and mouse tests, although it is less active than PD 135158 or CI-988 (45, 176).

Based on these results, the role of CCK in anxiety was raised. We used the rat elevated X-maze for more detailed investigation of the possible anxiogenic actions of CCK agonists following intracerebral injection in rats. Caerulein (a mixed CCK-A and CCK-B agonist) or pentagastrin (a CCK-B selective agonist), at low doses (1 and 0.3 nmol; minimum effective dose respectively), significantly reduced the time rats spent in the open arm of the maze, suggesting an anxiogenic action. The anxiogenic effect of pentagastrin was antagonized by systemic administration of CI-988 (176, 177). Similar results were obtained in mice (G. N. W., J. H., & L. Singh, unpublished observations).

The results of our experiments with the new and selective CCK-B antagonists argue for an important role of central CCK-B receptors in anxiety. Important to the argument is the observation that the CCK-B antagonists themselves are anxiolytic, presumably by blockade of the endogenous peptide action. As previously mentioned, CCK-A receptors are now known to be present in discrete brain regions and the possible role of these receptors in anxiety and other behavioral states requires further investigation (178).

These observations form the first clear indication of a major physiological role in the CNS for CCK and related peptides. Interestingly, the CCK-B antagonists have several major advantages over the widely used benzodiazepine class of drugs. The sedation, common with benzodiazepine administration, was absent even at high doses of the drugs (45). Further, rebound anxiety upon withdrawal following chronic administration that occurs with benzodiazepines was absent following CCK-B antagonist administration (45).

DRUG ABUSE As previously mentioned, CI-988 (PD 134308) produced no signs of tolerance following a chronic dosing regime nor did it produce a withdrawal anxiogenesis. In fact, the CCK-B antagonists suppressed the benzodiazepine-induced rebound anxiety (45). These data suggest that in addition to an anxiolytic action the compounds themselves may have a role

in treatment of benzodiazepine dependence. Indeed CCK-B antagonists might well find a broader use in the treatment of withdrawal from drugs of abuse such as cocaine, alcohol, and nicotine. Much more work on these areas is required.

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Literature Cited

1. Dockray, G. J. R. 1976. Immunochemical evidence of cholecystokinin-like peptides in brain. *Nature* 264: 568–70
2. Innis, R. B., Snyder, S. H. 1980. Distinct cholecystokinin receptors in brain and pancreas. *Proc. Natl. Acad. Sci. USA* 77:6917–21
3. Hays, S. E., Beinfeld, M. C., Jensen, R. T., Goodwin, F. K., Paul, S. M. 1980. Demonstration of a putative receptor site for cholecystokinin in rat brain. *Neuropeptides* 1:53–62
4. Peikin, S. R., Costenbader, C. L., Gardner, J. D. 1979. Actions of derivatives of cyclic nucleotides on dispersed acini from guinea pig pancreas. *J. Biol. Chem.* 254(12):5321–27
5. Hutchinson, J. B., Dockray, G. J. 1980. Inhibition of the action of cholecystokinin octapeptide on the guinea pig ileum myenteric plexus by dibutyryl cyclic guanosine monophosphate. *Brain Res.* 202:501–5
6. Sankaran, H., Goldfine, I. D., Deveney, C. W., Wong, K-Y., Williams, J. A. 1980. Binding of cholecystokinin to high affinity receptors on isolated rat pancreatic acini. *J. Biol. Chem.* 255: 1849–53
7. Saito, A., Goldfine, I. D., Williams, J. A. 1981. Characterization of receptors for cholecystokinin and related peptides in mouse cerebral cortex. *J. Neurochem.* 37:483–90
8. van Dijk, A., Richards, J. G., Trzeciak, A., Gillessen, D., Möhler, H. 1984. Cholecystokinin receptors: Biochemical demonstration and autoradiographical localization in rat brain and pancreas using [^{3}H] cholecystokinin$_8$ as radioligand. *J. Neurosci.* 1021–33
9. Knight, M., Tamminga, C. A., Steardo, L., Beck, M. E., Barone, P., Chase, T. N. 1984. Cholecystokinin-octapeptide fragments: binding to brain cholecystokinin receptors. *Eur. J. Pharmacol.* 105:49–55
10. Horwell, D. C., Beeby, A., Clark, C. R., Hughes, J. 1987. Synthesis and binding affinities of cholecystokinin (30–33) as probes for central nervous system cholecystokinin receptors. *J. Med. Chem.* 30:729–32
11. Clark, C. R., Daum, P., Hughes, J. 1986. A study of the cerebral cortex cholecystokinin receptor using two radiolabelled probes: Evidence for a common CCK 8 and CCK 4 cholecystokinin receptor binding site. *J. Neurochem.* 46:1094–101
12. Durieux, C., Pelaprat, D., Charpentier, B., Morgat, J-L., Roques, B. P. 1988. Characterization of [^{3}H] CCK$_4$ binding sites in mouse and rat brain. *Neuropeptides* 12:141–48
13. Huang, S. C., Yu, D-H., Wank, S. A., Mantey, S., Gardener, J. D., Jensen, R. T. 1989. Importance of sulfation of gastrin or cholecystokinin (CCK) on affinity for gastrin and CCK receptors. *Peptides* 10:785–89
14. Lin, C. H., Miller, T. 1985. Characterization of cholecystokinin receptor sites in guinea-pig cortical membranes using [^{125}I]Bolton Hunter-cholecystokinin octapeptide. *J. Pharmacol. Exp. Ther.* 232:775–80
15. Kamiya, H-O. 1988. Evidence for cholecystokinin receptor and intracellular signal transduction in relation to amylase secretion. *Adv. Exp. Med. Biol.* 236:95–109
16. Takeda, Y., Hoshino, M., Yanaihara, N., Yanaihara, C., Isobe, J., et al. 1989. Comparison of CCK-8 receptors

in the pancreas and brain of rats using CCK-8 analogues. *Jpn. J. Pharmacol.* 49:471–81

17. Charpentier, B., Pelaprat, D., Durieux, C., Dor, A., Reibaud, M., et al. 1988. Cyclic cholecystokinin analogues with high selectivity for central receptors. *Proc. Natl. Acad. Sci. USA* 85:1968–72
18. Roy, P., Charpentier, B., Durieux, C., Dor, A., Roques, B. P. 1989. Conformational behaviour of cyclic CCK-related peptides determined by 400-MH_z ^{1}H-NMR: Relationships with affinity and selectivity for brain receptors. *Biopolymers* 28:69–79
19. Böhme, G. A., Durieux, C., Stutzmann, J-M., Charpentier, B., Roques, B. P., et al. 1989. Electrophysiological studies with new CCK analogs: Correlation with binding affinity on B-type receptors. *Peptides* 10:407–14
20. Charpentier, B., Dor, A., Roy, P., England, P., Pham, H., et al. 1989. Synthesis and binding affinities of cyclic and related linear analogues of CCK_8 selective for central receptors. *J. Med. Chem.* 32:1184–90
21. Moran, T. H., Robinson, P. H., Goldrich, M. S., McHugh, P. R. 1986. Two brain cholecystokinin receptors: implications for behavioral actions. *Brain Res.* 362:175–79
22. Fourmy, D., Pradayrol, L., Vaysse, N., Susini, C., Ribet, A. 1984. ^{125}I-(Thr_{34},Nle_{37})-CCK_{31-39} A non oxidizable tracer for the characterization of CCK receptor on pancreatic acini and radio-immunoassay of C-terminal CCK peptides. *J. Immunoass.* 5:99–120
23. Böhme, G. A., Stutzmann, J-M., Blanchard, J-C. 1988. Excitatory effects of cholecystokinin in rat hippocampus: pharmacological response compatible with 'central'- or B-type CCK receptors. *Brain Res.* 451:309–18
24. Yoder, D. G., Moody, T. W., 1987. High affinity binding of cholecystokinin to small cell lung cancer cells. *Peptides* 8:103–7
25. Cherner, J. A., Sutliff, V. E., Grybowski, D. M., Jensen, R. T., Gardner, J. D. 1988. Functionally distinct receptors for cholecystokinin and gastrin on dispersed chief cells from guinea pig stomach. *Am. J. Physiol.* 254:G151–55
26. Williams, J. A., Gryson, K. A., McChesney, D. J. 1986. Brain CCK receptors: species differences in regional distribution and selectivity. *Peptides* 7: 292–96
27. Sekiguchi, R., Moroji, T. 1986. A comparative study on characterization and distribution of cholecystokinin binding sites among the rat, mouse and guinea pig brain. *Brain Res.* 399:271–81
28. Niehoff, D. L. 1989. Quantitative autoradiographic localization of cholecystokinin receptors in rat and guinea pig brain using ^{125}I-Bolton-Hunter-CCK8. *Peptides* 10:265–74
29. Miceli, M. O., Steiner, M. 1989. Novel localizations of central- and peripheral-type cholecystokinin binding sites in Syrian hamster brain as determined by autoradiography. *Eur. J. Pharmacol.* 169:215–24
30. Hill, D. R., Shaw, T. M., Graham, W., Woodruff, G. N. 1990. Autoradiographical detection of cholecystokinin-A receptors in primate brain using 125I-Bolton Hunter CCK-8 and 3H-MK-329. *J. Neurosci.* 10:1070–81
31. Hill, D. R., Shaw, T. M., Dourish, C. T., Woodruff, G. N. 1990. CCK-A receptors in the rat interpeduncular nucleus: evidence for a presynaptic location. *Brain Res.* 454:101–5
32. Sakamoto, C., Goldfine, I. D., Williams, J. A. 1983. Characterization of cholecystokinin receptor subunits on pancreatic plasma membranes. *J. Biol. Chem.* 20:12707–11
33. Zahidi, A., Fourmy, D., Darbon, J-M., Pradayrol, L., Scemama, J-L., et al. 1986. Molecular properties of solubilized CCK receptor from guinea-pig pancreas. *Regul. Pept.* 15:25–36
34. Miller, L. J. 1984. Characterization of cholecystokinin receptors on human gastric smooth muscle tumors. *Am. J. Physiol.* 247:G402–10
35. Shaw, M. J., Hadac, E. M., Miller, L. J. 1987. Preparation of enriched plasma membranes from bovine gallbladder muscularis for characterization of cholecystokinin receptors. *J. Biol. Chem.* 262:14313–18
36. Sakamoto, C., Williams, J. A., Goldfine, I. D. 1984. Brain CCK receptors are structurally distinct from pancreas CCK receptors. *Biochem. Biophys. Res. Commun.* 124:497–502
37. Pearson, R. K., Miller, L. J. 1987. Affinity labeling of a novel cholecystokinin-binding protein in rat pancreatic plasmalemma using new short probes for the receptor. *J. Biol. Chem.* 262:869–76
38. Pearson, R. K., Powers, S. P., Hadac, E. M., Gaisano, H., Miller, L. J. 1987. Establishment of a new short, protcase-resistant, affinity labeling reagent for the cholecystokinin receptor. *Biochem. Biophys. Res. Commun.* 147:346–53
39. Klueppelberg, U. G., Gaisano, H. Y., Powers, S. P., Miller, L. J. 1989. Use

of a nitrotryptophan-containing peptide for photoaffinity labeling the pancreatic cholecystokinin receptor. *Biochemistry* 28:3463–68

40. Powers, S. P., Fourmy, D., Gaisano, H., Miller, L. J. 1988. Intrinsic photoaffinity labeling probes for cholecystokinin (CCK)-gastrin family receptors. *J. Biol. Chem.* 263:5295–300
41. Szecowka, J., Hallden, G., Goldfine, I. D., Williams, J.A. 1989. Purification of the pancreatic cholecystokinin receptor. *Regul. Pept.* 24:215–24
42. Duong, L. T., Hadac, E. M., Miller, L. J., Vlasuk, G. P. 1989. Purification and characterization of the rat pancreatic cholecystokinin receptor. *J. Biol. Chem.* 264:17990–96
43. Miller, L. J., Powers, S. P., 1988. Biochemical characterization of the pancreatic cholecystokinin receptor: A possible marker of cell differentiation and development. *Scand. J. Gastroenterol.* 23 (Suppl. 151):104–7
44. Schjoldager, B., Powers, S. P., Miller, L. J. 1988. Affinity labeling the bovine gallbladder cholecystokinin receptor using a battery of probes. *Am. J. Physiol.* 255:G579–86
45. Hughes, J., Boden, P., Costall, B., Domeney, A., Kelly, E., et al. 1990. Development of a class of selective cholecstokinin type B receptor antagonists having potent anxiolytic activity. *Proc. Natl. Acad. Sci. USA* 87: 6728–32
46. Pinnock, R. D., Woodruff, G. N., Boden, P. R. 1990. Pharmacology of a cholecystokinin receptor on serotonin neurones in the dorsal raphe of the rat brain. *Br. J. Pharmacol.* In press
47. Pinnock, R. D., Woodruff, G. N. 1990. Bombesin, neuromedin B and gastrin-releasing peptide excite a subpopulation of 5-hydroxytryptamine neurones in the dorsal raphe nucleus. *J. Neurosci.* In press
48. Otsuki, M., Okabayashi, Y., Ohki, A., Suehiro, I., Oka, T., et al. 1986. Dibutyryl guanosine 3',5'-monophosphate inhibits cholecystokinin potentiation of insulin release in the isolated perfused rat pancreas. *Endocrinology* 119:244–49
49. Takahashi, T., Yamamura, T., Kusunoki, M., Kantoh, M., Ishikawa, Y., et al. 1987. Differences between muscular receptors and neural receptors for cholecystokinin-octapeptide in the guinea-pig gallbladder. *Eur. J. Pharmacol.* 136: 255–58
50. Barlas, N., Jensen, R. T., Beinfeld, M. C., Gardner, J. D. 1982. Cyclic nucleotide antagonists of cholecystokinin: structural requirements for interaction with the cholecystokinin receptor. *Am. J. Physiol.* 242:G161–67
51. Rogers, J., Hughes, R. G., Matthews, E. K. 1988. Cyclic GMP inhibits protein kinase C-mediated secretion in rat pancreatic acini. *J. Biol. Chem.* 263: 3713–19
52. Vidal y Plana, R. R., Cifarelli, A., Bizzarri, D. 1980. Effects of antigastrin drugs on the interaction of ^{125}I-human gastrin with rat gastric mucosa membranes. *Hepato-Gastroenterol.* 27:41–47
53. Rovati, A. L. 1976. Inhibition of gastric secretion by anti-gastrinic and H_2-blocking agents. *Scand. J. Gastroenterol.* 11(Suppl. 42):113
54. Hahne, W. F., Jensen, R. T., Lemp, G. F., Gardner, J. D. 1981. Proglumide and benzotript: Members of a different class of cholecystokinin receptor antagonists. *Proc. Natl. Acad. Sci. USA* 78:6304–8
55. Fried, M., Beglinger, C., Koehler, E., Whitehouse, I., Varga, L., Gyr, K. 1984. Effect of proglumide, a cholecystokinin receptor antagonist, on caerulein-stimulated pancreatic enzyme secretion and pancreatic polypeptide release in the dog. *Regul. Pept.* 8:117–22
56. Verspohl, E. J., Wunderle, G., Ammon, H. P. T., Williams, J. A., Goldfine, I. D. 1986. Proglumide (gastrin and cholecystokinin receptor antagonist) inhibits insulin secretion *in vitro*. *Arch. Pharmacol.* 332:284–87
57. Verspohl, E. J., Wunderle, G., Ammon, H. P. T. 1988. Proglumide antagonizes cholecystokinin effects on plasma glucose and insulin in rats *in vivo*. *Eur. J. Pharmacol.* 152:121–28
58. Shillabeer, G., Davison, J. S. 1984. The cholecystokinin antagonist, proglumide, increases food intake in the rat. *Regul. Pept.* 8:171–76
59. McLaughlin, C. L., Peikin, S. R., Baile, C. A. 1983. Feeding behavior response of Zucker rats to proglumide, a CCK receptor antagonist. *Pharmacol. Biochem. Behav.* 18:879–83
60. Collins, S., Walker, D., Forsyth, P., Belbeck, L. 1983. The effects of proglumide on cholecystokinin-, bombesin-, and glucagon-induced satiety in the rat. *Life Sci.* 32:2223–29
61. Willis, G. L., Hansky, J., Smith, G. C. 1986. Central and peripheral proglumide administration and cholecystokinin-induced satiety. *Regul. Pept.* 15:87–98
62. Inui, A., Inoue, T., Sakatani, N., Oya, M., Morioka, H., et al. 1987. Proglumide has access to brain and an-

tagonizes the central satiety effect of cholecystokinin octapeptide in the dog. *Brain Res.* 417:355–59

63. Gaudreau, P., Quiron, R., St-Pierre, S., Pert, C. B. 1983. Characterization and visualization of cholecystokinin receptors in rat brain using [3H] pentagastrin. *Peptides* 4:755–62
64. Crawley, J. N., Stivers, J. A., Hommer, D. W., Skirboll, L. R., Paul, S. M. 1986. Antagonists of central and peripheral behavioral actions of cholecystokinin octapeptide. *J. Pharmacol. Exp. Ther.* 236:320–30
65. Katsuura, G., Hsiao, S., Itoh, S. 1984. Blocking of cholecystokinin octapeptide behavioral effects by proglumide. *Peptides* 5:529–34
66. Hsiao, S., Katsuura, G., Itoh, S. 1984. Cholecystokinin tetrapeptide, proglumide and open-field behavior in rats. *Life Sci.* 34:2165–68
67. Jaffe, D. B., Aitken, P. G., Nadler, J. V. 1987. The effects of cholecystokinin and cholecystokinin antagonists on synaptic function in the CA1 region of the rat hippocampal slice. *Brain Res.* 415:197–203
68. Jensen, R. T., Jones, S. W., Gardner, J. D. 1983. Structure function studies of N-acyl derivatives of tryptophan that function as specific cholecystokinin receptor antagonists. *Biochem. Biophys. Acta* 761:269–77
69. Maton, P. N., Sutliff, V. E., Jensen, R. T., Gardner, J. D. 1985. Carbobenzoxy amino acids: structural requirements for cholecystokinin receptor antagonist activity. *Am. J. Physiol.* 248:G479–84
70. Makovec, F., Chiste, R., Bani, M., Pacini, M. A., Setnikar, I., et al. 1985. New glutaramic acid derivatives with potent competitive and specific cholecystokinin-antagonistic activity. *Arzneim.-Forsch./Drug Res.* 35(II): 1048–51
71. Makovec, F., Chiste, R., Bani, M., Revel, L., Setnikar, I. et al. 1986. New glutamic and aspartic derivatives with potent CCK-antagonistic activity. *Eur. J. Med. Chem. Chim. Ther.* 21:9–20
72. Jensen, R. T., Murphy, R. B., Trampota, M., Schneider, L. H., Jones, S. W., et al. 1985. Proglumide analogues: potent cholecystokinin receptor antagonists. *Am. J. Physiol.* 249:G214–20
73. Jensen, R. T., Zhou, Z-C., Murphy, R. B., Jones, S. W., Setnikar, I., et al. 1986. Structural features of various proglumide-related cholecystokinin receptor antagonists. *Am. J. Physiol.* 251: G839–46
74. Makovec, F., Bani, M., Chiste, R., Revel, L., Rovati, L. C. et al. 1986. Differentiation of central and peripheral cholecystokinin receptors by new glutaramic acid derivatives and cholecystokinin-antagonistic activity. *Arzneim-Forsch/Drug Res.* 36(I):98–102
75. Niederau, C., Niederau, M., Williams, J. A., Grendell, J. H. 1986. New proglumide-analogue CCK receptor antagonists: very potent and selective for peripheral tissues. *Am. J. Physiol.* 251:G856–60
76. Rovati, L. C., Bani, M., Makovec, F., Revel, L., Setnikar, I. 1987. Lorglumide and loxiglumide: Two potent and specific antagonists of peripheral CCK. In *Gastrin and Cholecystokinin. Chemistry, Physiology and Pharmacology,* ed J-P Bali, J Martinez, pp. 45–48. Amsterdam: Elsevier
77. Barthó, L., Holzer, P., Lembeck, F., Lippe, I. T., Setnikar, I. 1987. Evaluation of a new and potent cholecystokinin antagonist on motor responses of the guinea-pig intestine. *Br. J. Pharmacol.* 90:753–61
78. Setnikar, I., Bani, M., Cereda, R., Chiste, R., Makovec, F., et al. 1987. Pharmacological characterisation of a new potent and specific nonpolypeptidic cholecystokinin antagonist. *Arzneim.-Forsch./Drug Res.* 37(I):703–7
79. Makovec, F., Bani, M., Cereda, R., Chiste, R., Pacini, M. A., et al. 1987. Antispasmodic activity on the gallbladder of the mouse of CR 1409 (Lorglumide) a potent antagonist of peripheral CCK. *Pharmacol. Res. Commun.* 19: 41–51
80. Setnikar, I., Bani, M., Cereda, R., Chiste, R., Makovec, F., et al. 1987. Anticholecystokinin activities of loxiglumide. *Arzneim.-Forsch./Drug Res.* 37:(II):1168–71
81. Miyasaka, Y., Nakamura, R., Funakoshi, A., Kitani, K. 1988. Inhibitory effect of CR-1409, a competitive inhibitor of cholecystokinin, on pancreatic exocrine secretion in the conscious rat. *Tohoku J. Exp. Med.* 155:165–72
82. Hildebrand, P., Begliner, C., Köhler, E., Setnikar, I., Gyr, K. 1987. Biological effects of a proglumide derivative as cholecystokinin antagonist in conscious dogs. *Regul. Pept.* 18:213–20
83. Damge, C., Hajri, A., Aprahamian, M. 1987. Effect of CR 1409, a CCK-receptor antagonist, on the trophic action of CCK, caerulein, bombesin and GRP in the rat pancreas. See Ref. 76, pp. 85–88
84. Makovec, F., Bani, M., Chiste, R., Revel, L., Rovati, L. C., et al. 1986.

Different peripheral and central antagonistic activity of new glutaramic acid derivatives on satiety induced by cholecystokinin in rats. *Regul. Pept.* 16:281–90

85. Schneider, L. H., Murphy, R. B., Smith, G. P. 1988. Two proglumide analogues are equipotent antagonists of the inhibition of food intake by CCK-8. *Peptides* 9(Suppl. 1):207–14
86. Ambrose, F. G., Barbaz, B. S., Autry, W. L., Browne, R. G., Liebman, J. M. 1989. Unsulfated CCK-8 not blocked by proglumide or CR 1409 in the mouse abdominal irritant-induced stretching assay: Possible central site of action. *Peptides* 10:31–34
87. Douglas, B. R., Woutersen, R. A., Jansen, J. B. M. J., Rovati, L. C., Lamers, C. B. H. W. 1989. Study into the role of cholecystokinin in bombesin-stimulated pancreatic growth in rats and hamster. *Eur. J. Pharmacol.* 161:209–14
88. Otsuki, M., Fujii, M., Nakamura, T., Okabayashi, Y., Tani, S., et al. 1989. Effects of a new proglumide analogue CR 1392 on pancreatic exocrine secretion in the rat. *Digestion* 42:61–69
89. Spanarkel, M., Martinez, J., Briet, C., Jensen, R. T., Gardner, J. D. 1983. Cholecystokinin-27-32-amide, a member of a new class of cholecystokinin receptor antagonists. *J. Biol. Chem.* 258:6746-49
90. Jensen, R. T., Jones, S. W., Gardner, J. D. 1983. COOH-terminal fragments of cholecystokinin, a new class of cholecystokinin receptor antagonists. *Biochem. Biophys. Acta* 757:250–58
91. Gardener, J. D., Knight, M., Sutliff, V. E., Tamminga, C. A., Jensen, R. T. 1984. Derivatives of CCK-(26-32) as cholecystokinin receptor antagonists in guinea pig pancreatic acini. *Am. J. Physiol.* 246:G292–95
92. Fulcrand, P., Rodriguez, M., Galas, M-C., Lignon, M-F., Laur, J., et al. 1988. 2-Phenylethyl ester and 2-phenylethyl amide derivative analogues of the C-terminal hepta- and octapeptide of cholecystokinin. *Int. J. Pept. Prot. Res.* 32:384–95
93. MacVicar, B. A., Kerrin, J. P., Davison, J. S. 1987. Inhibition of synaptic transmission in the hippocampus by cholecystokinin (CCK) and its antagonism by a CCK analog (CCK_{27-33}). *Brain Res.* 406:130–35
94. Lignon, M-F., Galas, M-C., Rodriguez, M., Laur, J., Aumelas, A., et al. 1987. A synthetic peptide derivative that is a cholecystokinin receptor antagonist. *J. Biol. Chem.* 262:7226–31
95. Stark, H. A., Sharp, C. M., Sutliff, V. E., Martinez, J., Jensen, R. T., et al. 1989. CCK-JMV-180: a peptide that distinguishes high-affinity cholecystokinin receptors from low-affinity cholecystokinin receptors. *Biochem. Biophys. Acta* 1010:145–50
96. Sato, S., Stark, H. A., Martinez, J., Beaven, M. A., Jensen, R. T., et al. 1989. Receptor occupation, calcium mobilization, and amylase release in pancreatic acini: effect of CCK-JMV-180. *Am. J. Physiol.* 257:G202–9
97. Saluja, A. K., Saluja, M., Printz, H., Zavertnik, A., Sengupta, A., et al. 1989. Experimental pancreatitis is mediated by low-affinity cholecystokinin receptors that inhibit digestive enzyme secretion. *Proc. Natl. Acad. Sci. USA* 86:8968–71
98. Bradwejn, J., de Montigny, C. 1984. Benzodiazepines antagonize cholecystokinin-induced activation of rat hippocampal neurones. *Nature* 312:363–64
99. Bouthillier, A., de Montigny, C. 1988. Long-term benzodiazepine treatment reduces neuronal responsiveness to cholecystokinin: an electrophysiological study in the rat. *Eur. J. Pharmacol.* 151:135–38
100. Kubota, K., Sugaya, K., Sunagane, N., Matsuda, I., Uruno, T. 1985. Cholecystokinin antagonism by benzodiazepines in the contractile response of the isolated guinea-pig gallbladder. *Eur. J. Pharmacol.* 110:225–31
101. Kubota, K., Sugaya, K., Fujii, F., Itonaga, M., Sunagane, N. 1985. Inhibition of cholecystokinin response in the gallbladder by dibenamine and its protection by benzodiazepines. *Jpn. J. Pharmacol.* 39:274–76
102. Chang, R. S. L., Lotti, V. J., Chen, T. B., Keegan, M. E. 1986. Tifluadom, a κ-opiate agonist, acts as a peripheral cholecystokinin receptor antagonist. *Neurosci. Lett.* 72:211–14
103. Meldrum, L. A., Bojarski, J. C., Calam, J. 1986. Effects of benzodiazepines on responses of guinea-pig ileum and gall-bladder and rat pancreatic acini to cholecystokinin. *Eur. J. Pharmacol.* 123:427–32
104. Sugaya, K., Matsuda, I., Uruno, T., Kubota, K. 1986. Studies on the CCK antagonism by benzodiazepines (IX). Displacement of CCK by benzodiazepines in the binding in mouse brain CCK receptor. *Jpn J. Pharmacol.* 40:114P
105. Chang, R. S. L., Lotti, V. J., Monaghan, R. L., Birnbaum, J., Stapley, E. O., et al. 1985. A potent non-

peptide cholecystokinin antagonist selective for peripheral tissues isolated from *Aspergillus alliaceus*. *Science* 230:177–79

106. Goetz, M. A., Monaghan, R. L., Chang, R. S. L., Ondeyka, J., Chen, T. B., et al. 1988. Novel cholecystokinin antagonists from *Aspergillus alliaceus*. *J. Antibiotics* 41:875–77
107. Liesch, J. M., Hensens, O. D., Zink, D. L., Goetz, M. A. 1988. Novel cholecystokinin antagonists from *Aspergillus alliaceus*. *J. Antibiotics* 41:878–81
108. Evans, B. E., Bock, M. G., Rittle, K. E., DiPardo, R. M., Whitter, W. L., et al. 1986. Design of potent, orally effective, nonpeptidal antagonists of the peptide hormone cholecystokinin. *Proc. Natl. Acad. Sci. USA* 83:4918–22
109. Bock, M. G., DiPardo, R. M., Rittle, K. E., Evans, B. E., Freidinger, R. M., et al. 1986. Cholecystokinin antagonists. Synthesis of asperlicin analogues with improved potency and water solubility. *J. Med. Chem.* 29: 1941–45
110. Bock, M. G., DiPardo, R. M., Evans, B. E., Rittle, K. E., Freidinger, R. M., et al. 1988. Cholecystokinin antagonists. Synthesis and biological evaluation of 3-substituted 1,4-benzodiazepine-2-amines. *J. Med. Chem.* 31:264–68
111. Chang, R. S. L., Lotti, V. J. 1986. Biochemical and pharmacological characterization of an extremely potent and selective nonpeptide cholecystokinin antagonist. *Proc. Natl. Acad. Sci. USA* 83:4923–26
112. Parsons, W. H., Patchett, A. A., Holloway, M. K., Smith, G. M., Davidson, J. L., et al. 1989. Cholecystokinin antagonist. Synthesis and biological evaluation of 3-substituted benzolactams. *J. Med. Chem.* 32:1681–85
113. Hosotani, R., Chowdhury, P., McKay, D., Rayford, P. L. 1988. Effect of L-364,718, a new CCK antagonist, on amylase secretion in isolated rat pancreatic acini. *Pancreas* 3:95–98
114. Anderson, L., Dockray, G. J. 1988. The cholecystokinin antagonist L-364,718 inhibits the action of cholecystokinin but not bombesin on rat pancreatic secretion in vivo. *Eur. J. Pharmacol.* 146:307–11
115. Louie, D. S., Liang, J. P., Owyang, C. 1988. Characterization of a new CCK antagonist, L-364,718: in vitro and in vivo studies. *Am. J. Physiol.* 255:G261–66
116. Hosotani, R., Chowdhury, P., McKay, D., Rayford, P. L. 1987. L-364,718, a new CCK antagonist, inhibits biological actions of CCK in conscious dogs. *Peptides* 8:1061–64
117. Hosotani, R., Chowdhury, P., Rayford, P. L. 1989. L-364,718, a new CCK antagonist, inhibits postprandial pancreatic secretion and PP release in dogs. *Dig. Dis. Sci.* 34:462–67
118. Reagan, J. E., Robinson, J. L., Lotti, V. L., Goldman, M. E. 1987. Fasting and L-364,718 prevent cholecystokinin-induced elevations of plasma insulin levels. *Eur. J. Pharmacol.* 144:241–43
119. Wisner, Jr., J. R., McLaughlin, R. E., Rich, K. A., Ozawa, S., Renner, I. G. 1988. Effects of L-364,718, a new cholecystokinin receptor antagonist, on camostate-induced growth of the rat pancreas. *Gastroenterology* 94:19–113
120. Schmidt, W. E., Choudhury, A. R., Siegel, E. G., Löser, C., Conlon, J. M., et al. 1989. CCK-antagonist L-364,718: influence on rat pancreatic growth induced by caerulein and bombesin-like peptides. *Regul. Pept.* 24:67–79
121. Lotti, V. J., Pendleton, R. G., Gould, R. J., Hanson, H. M., Chang, R. S. L., et al. 1987. In vivo pharmacology of L-364,718, a new potent nonpeptide peripheral cholecystokinin antagonist. *J. Pharmacol. Exp. Ther.* 241:103–9
122. Liddle, R. A., Gertz, B. J., Kanayama, S., Beccaria, L., Coker, L. D., et al. 1989. Effects of a novel cholecystokinin (CCK) receptor antagonist, MK-329, on gallbladder contraction and gastric emptying in humans. *J. Clin. Invest.* 84:1220–25
123. Decktor, D. L., Pendleton, R. G., Elnitsky, A. T., Jenkins, A. M., McDowell, A. P. 1988. Effect of metoclopramide, bethanechol and the cholecystokinin receptor antagonist. L-364,718, on gastric emptying in the rat. *Eur. J. Pharmacol.* 147:313–16
124. Green, T., Dimaline, R., Peikin, S., Dockray, G. J. 1988. Action of the cholecystokinin antagonist L-364,718 on gastric emptying in the rat. *Am. J. Physiol.* 255:G685–89
125. Pullen, R. G. L., Hodgson, O. J. 1987. Penetration of diazepam and the nonpeptide CCK antagonist, L-364,718, into rat brain. *J. Pharm. Pharmacol.* 39:863–64
126. Soar, J., Hewson, G., Leighton, G. E., Hill, R. G., Hughes, J. 1989. L-364,718 antagonizes the cholecystokinin-induced suppression of locomotor activity. *Pharmacol. Biochem. Behav.* 33:637–40
127. Itoh, S., Takashima, A. 1989. Effect of cholecystokinin octapeptide antagonists on the extinction of an active avoidance task in the rat. *Drug Devel. Res.* 17:83–87

128. Flood, J. F., Morley, J. E. 1989. Cholecystokinin receptors mediate enhanced memory retention produced by feeding and gastrointestinal peptides. *Peptides* 10:809–13
129. Silver, A. J., Flood, J. F., Song, A. M., Morley, J. E. 1989. Evidence for a physiological role for CCK in the regulation of food intake in mice. *Am. J. Physiol.* 256:R646–52
130. Hewson, G., Leighton, G. E., Hill, R. G., Hughes, J. 1988. The cholecystokinin receptor antagonist L-364,718 increases food intake in the rat by attenuation of the action of endogenous cholecystokinin. *Br. J. Pharmacol.* 93: 79–84
131. Leighton, G. E., Griesbacher, T., Hill, R. G., Hughes, J. 1989. Antagonism of central and peripheral anorectic effects of caerulein by L-364,718. *Eur. J. Pharmacol.* 161:255–58
132. Pinnock, R. D., Woodruff, G. N., Boden, P. R. 1990. Cholecystokinin excites dorsal raphe neurones via CCK-A receptor. *Br. J. Pharmacol. (Proc. Suppl.)* 100:349P
133. Vickroy, T. W., Bianchi, B. R., Kerwin Jr., J. F., Kopecka, H., Nadzan, A. M. 1988. Evidence that type A CCK receptors facilitate dopamine efflux in rat brain. *Eur. J. Pharmacol.* 152:371–72
134. Dauge, V., Steimes, P., Derrien, M., Beau, N., Roques, B. P., et al. 1989. CCK8 effects on motivational and emotional states of rats involve CCK-A receptors of the postero-median part of the nucleus accumbens. *Pharmacol. Biochem. Behav.* 34:157–63
135. Gut, S. H., Demoliou-Mason, C. D., Hunter, J. C., Hughes, J., Barnard, E. A. 1989. Solubilization and characterisation of the cholecystokinin$_B$ binding site from pig cerebral cortex. *Eur. J. Pharmacol.* 172:339–46
136. Hill, D. R., Campbell, N. J., Shaw, T. M., Woodruff, G. N. 1987. Autoradiographic localization and biochemical characterization of peripheral type CCK receptors in rat CNS using highly selective nonpeptide CCK antagonists. *J. Neurosci.* 7:2967–76
137. Barrett, R. W., Steffey, M. E., Wolfram, C. A. W. 1989. Type-A cholecystokinin receptors in CHP212 neuroblastoma cells: evidence for association with G protein with activation of phosphoinositide hydrolysis. *Mol. Pharmacol.* 35: 394–400
138. Hill, D. R., Shaw, T. M., Woodruff, G. N. 1987. Species differences in the localization of 'peripheral' type cholecystokinin receptors in rodent brain. *Neurosci. Lett.* 79:286–89
139. Hill, D. R., Shaw, T. M., Woodruff, G. N. 1988. Binding sites for ^{125}I-cholecystokinin in primate spinal cord are of the CCK-A subclass. *Neurosci. Lett.* 89:133–39
140. Bock, M. G., DiPardo, R. M., Evans, B. E., Rittle, K. E., Whitter, W. L., et al. 1989. Benzodiazepine gastrin and brain cholecystokinin receptor ligands: L-365,260. *J. Med. Chem.* 32:13–16
141. Lotti, V. J., Chang, R. S. L. 1989. A new and selective non-peptide gastrin antagonist and brain cholecystokinin receptor (CCK-B) ligand: L-365,260. *Eur. J. Pharmacol.* 162:273–80
142. Chang, R. S. L., Chen, T. B., Bock, M. G., Freidinger, R. M., Chen, R., et al. 1989. Characterization of the binding of [^{3}H]L-365,260: A new potent and selective brain cholecystokinin (CCK-B) and gastrin receptor antagonist radioligand. *Mol. Pharmacol.* 35:803–8
143. Huang, S. C., Zhang, L., Chiang, H-C. V., Wank, S. A., Maton, P. N. 1989. Benzodiazepine analogues L-365,260 and L-364,718 as gastrin and pancreatic CCK receptor antagonists. *Am. J. Physiol.* 257:G169–74
144. Dourish, C. T., Rycroft, W., Iversen, S. D. 1989. Postponement of satiety by blockade of brain cholecystokinin (CCK-B) receptors. *Science* 245:1509–11
145. Kubota, K., Sugaya, K., Koizumi, Y., Toda, M. 1989. Cholecystokinin antagonism by anthramycin, a benzodiazepine antibiotic, in the central nervous system in mice. *Brain Res.* 485:62–66
146. Lotti, V. J., Cerino, D. J., Kling, P. J., Chang, R. S. L. 1986. A new simple mouse model for the in vivo evaluation of cholecystokinin (CCK) antagonists: comparative potencies and durations of action of nonpeptide antagonists. *Life Sci.* 39:1631–38
147. Von Schrenck, T., Moran, T. H., Heinz-Erian, P., Gardner, J. D., Jensen, R. T. 1988. Cholecystokinin receptors on gallbladder muscle and pancreatic acinar cells: a comparative study. *Am. J. Physiol.* 255:G512–21
148. Niederau, M., Niederau, C., Strohmeyer, G., Grendell, J. H. 1989. Comparative effects of CCK receptor antagonists on rat pancreatic secretion in vivo. *Am. J. Physiol.* 256:G150–57
149. Karlsson, S., Ahren, B. 1989. Effect of three different cholecystokinin receptor antagonists on basal and stimulated in-

sulin and glucagon secretion in mice. *Acta Physiol. Scand.* 135:271–78

150. Kerwin Jr., J. F., Nadzan, A. M., Kopecka, H., Lin, C. W., Witte, T. M. D., et al. 1989. Hybrid cholecystokinin (CCK) antagonists: New implications in the design and modification of CCK antagonists. *J. Med. Chem.* 32:739–42
151. Watkins, L. R., Kinscheck, I. B., Mayer, D. J. 1984. Potentiation of opiate analgesia and apparent reversal of morphine tolerance by proglumide. *Science* 224:395–96
152. Watkins, L. R., Kinscheck, I. B., Kaufman, E. F. S., Miller, J., et al. 1985. Cholecystokinin antagonists selectively potentiate analgesia induced by endogenous opiates. *Brain Res.* 327: 181–90
153. Kubota, K., Sugaya, K., Matsuda, I., Matsuoka, Y., Terawaki, Y. 1985. Reversal of antinociceptive effect of cholecystokinin by benzodiazepines and a benzodiazepine antagonist, Ro 15-1788. *Jpn J. Pharmacol.* 37:101–5
154. Rattray, M., Jordan, C. C., De Belleroche, J. 1988. The novel CCK antagonist L-364,718 abolishes caerulein—but potentiates morphine-induced antinociception. *Eur. J. Pharmacol.* 152: 163–66
155. Watkins, L. R., Kinscheck, I. B., Mayer, D. J. 1985. Potentiation of morphine analgesia by the cholecystokinin antagonist proglumide. *Brain Res.* 327: 169–80
156. O'Neill, M. F., Dourish, C. T., Iversen, S. D. 1989. Morphine-induced analgesia in the rat paw pressure test is blocked by CCK and enhanced by the CCK antagonist MK-329. *Neuropharmacology* 28:243–47
157. Tang, J., Chou, J., Iadarola, M., Yang, H.-T., Costa, E. 1984. Proglumide prevents and curtails acute tolerance to morphine in rats. *Neuropharmacology* 23: 715–18
158. Dourish, C. T., Hawley, D., Iversen, S. D. 1988. Enhancement of morphine analgesia and prevention of morphine tolerance in the rat by the cholecystokinin antagonist L-364,718. *Eur. J. Pharmacol.* 147:469–72
159. Weisenfeld-Hallin, Z., Xu, X-J, Hughes, J., Horwell, D. C., Hokfelt, T. 1991. PD 134308, a new selective CCK-B antagonist enhances the analgesic effect of morphine and synergistically interacts with intrathecal galanin to depress spinal nociceptive reflexes. *Proc. Natl. Acad. Sci. USA* 87:7105–9
160. Rovati, L. A. 1987. Views on possible therapeutical use of gastrin and cholecystokinin antagonists. See Ref. 76, 225–34
161. Gertz, B. J. 1988. Potential clinical applications of a CCK antagonist. In *Cholecystokinin Antagonists*, ed. R. Y. Wang, R. Schoenfeld, pp. 327–42. New York: Liss
162. Makovec, F., Bani, M., Cereda, R., Chiste, R., Revel, L., et al. 1986. Protective effect of CR 1409 (cholecystokinin antagonist) on experimental pancreatitis in rats and mice. *Peptides* 7:1159–64
163. Otsuki, M., Tani, S., Okabayshi, Y., Nakamura, T., Fukii, M., et al. 1989. Effect of a new cholecystokinin receptor antagonist CR 1392 on caerulein-induced acute pancreatitis in rats. *Pancreas* 4:237–43
164. Sjövall, S., Ahren, B., Stenram, U., 1988. Effects of the specific cholecystokinin antagonist L-364,718 in experimental acute pancreatitis in the rat. *Eur. Surg. Res.* 20:325–29
165. Silverman, M., Ilardi, C., Bank, S., Kranz, V., Lendvai, S. 1989. Effects of the cholecystokinin receptor antagonist L-364,718 on experimental pancreatitis in mice. *Gastroenterology* 96:186–92
166. Modlin, I. M., Bilchik, A. J., Zucker, K. A., Adrian, T. E., Sussman, J., et al. 1989. Cholecystokinin augmentation of 'surgical' pancreatitis. *Arch. Surg.* 124:574–578
167. Douglas, B. R., Wouteren, R. A., Jansen, J. B. M. J., de Jong, A. J. L., Rovati, L. C., et al. 1989. Modulation by CR-1409 (Lorglumide), a cholecystokinin receptor antagonist of trypsin inhibitor-enhanced growth of azaserine-induced putative preneoplastic lesions in rat pancreas. *Cancer Res.* 49:2438–41
168. Beglinger, C., Dill, S., Meyer, B., Werth, B., Adler, G. 1989. Treatment of biliary colic with loxiglumide. *Lancet* July 15:167
169. Reidelberger, R. D., Kalogeris, T. J., Solomon, T. E. 1989. Plasma CCK levels after food intake and infusion of CCK analogues that inhibit feeding in dogs. *Am. J. Physiol.* 256:R1148–54
170. Kellstein, D. E., Mayer D. T. 1990. Chronic administration of cholecystokinin antagonists reverses the enhancement of spinal morphine analgesia induced by acute pretreatment. *Brain Res.* 516:263–70
171. Suzuki, T., Moroji, T. 1989. Cholecystokinin binding sites in the rat forebrain: effects of acute and chronic methamphetamine administration. *J. Neural Transm.* 77:181–95
172. Jiang, L. H., Kasser, R. J., Wang, R.

Y. 1988. Cholecystokinin antagonist lorglumide reverses chronic haloperidol-induced effects on dopamine neurons. *Brain Res.* 473:165–68

173. Pellow, S., Chopin, P., File, S. E., Briley, M. 1985. Validation of open: closed arm entries in an elevated plus maze as a measure of anxiety in the rat. *J. Neurosci. Meth.* 14:149–67
174. File, S. E. 1980. The use of social interaction as a method for detecting anxiolytic activity of chlordia-zepoxide-like drugs. *J. Neurosci. Meth.* 2:219–28
175. Costall, B., Jones, B. J., Kelly, M. E., Naylor, R. J., Tomkins, D. M. 1989. Exploration of mice in a black and white test box: validation as a model of anxiety. *Pharmacol. Biochem. & Behav.* 32:777–85
176. Woodruff, G. N., Hill, D. R., Singh, L., Boden, P., Pinnock, R. D., et al. 1990. Functional CCK receptor in brain. *Neuropeptides*. In press
177. Singh, L., Lewis, A. S., Field, M. J., Hughes, J., Woodruff, G. N. 1990. Evidence for an involvement of the central CCK-B receptor in anxiety. *Proc. Natl. Acad. Sci. USA*. In press
178. Ravard, S., Dourish, C. T. 1990. Cholecystokinin and anxiety. *Trends Pharmacol. Sci.* 11:271–73
179. Hays, S. E., Meyer, D. K., Paul, S. M. 1981. Localization of cholecystokinin receptors on neuronal elements in rat caudate nucleus. *Brain Res.* 219:208–13
180. Praissman, M., Walden, M. E., Pellecchia, C. 1983. Identification and characterization of a specific receptor for cholecystokinin on isolated fundic glands from guinea-pig gastric mucosa using a biologically active ^{125}I-CCK-8 probe. *J. Recept. Res.* 3(5):647–65
181. Zarbin, M. A., Innis, R. B., Wamsley, J. K., Snyder, S. H., Kuhar, M. J. 1983. Autoradiographic localization of cholecystokinin receptors in rodent brain. *J. Neurosci.* 3:877–906
182. Finkelstein, J. A., Steggles, A. W., Martinez, P. A., Praissman, M. 1984. Cholecystokinin receptor binding levels in the genetically obese rat brain. *Peptides* 5:11–14
183. Praissman, M., Walden, M. 1984. The binding characteristics of ^{125}I-gastrin and ^{125}I-CCK to guinea pig fundic gastric glands differ: is there more than one binding site for peptides of the CCK-gastrin family? *Biochem. Biophys. Res. Commun.* 123:641–47
184. Hommer, D. W., Stoner, G., Crawley, J. N., Paul, S. M., Skirboll, R. L. 1986. Cholecystokinin-dopamine coexistence: Electrophysiological actions corresponding to cholecystokinin receptor subtype. *J. Neurosci.* 6:3039–43
185. Akesson, T. R., Micevych, P. E. 1986. Binding of ^{125}I-cholecystokinin-octapeptide in the paraventricular but not the supraoptic nucleus is increased by ovariectomy. *Brain Res.* 385:165–68
186. Fourmy, D., Zahidi, A., Fabre, R., Guidet, M., Pradayrol, L., et al. 1987. Receptors for cholecystokinin and gastrin peptides display specific binding properties and are structurally different in guinea-pig and dog pancreas. *Eur. J. Biochem.* 165(3):683–92
187. Robinson, P. H., Moran, T. H., Goldrich, M., McHugh, P. R. 1987. Development of cholecystokinin binding sites in rat upper gastrointestinal tract. *Am. J. Physiol.* G529–34
188. Dietl, M. M., Probst, A., Palacios, J. M. 1987. On the distribution of cholecystokinin receptor binding sites in the human brain: An autoradiographic study. *Synapse* 1:169–83
189. Grider, J. R., Makhlouf, G. M. 1987. Regional and cellular heterogeneity of cholecystokinin receptors mediating muscle contraction in the gut. *Gastroenterology* 92:175–80
190. Beresford, I. J. M., Hall, M. D., Clark, C. R., Hill, R. G., Hughes, J., et al. 1987. Striatal lesions and transplants demonstrate that cholecystokinin receptors are localized on intrinsic striatal neurones: a quantitative autoradiographic study. *Neuropeptides* 10:109–36
191. Gaudreau, P., Quirion, R., St-Pierre, S., Chiueh, C. C., Pert, A. 1987. Localization of cholecystokinin receptors in relation to the nigrostriatal and mesolimbic dopaminergic pathways. *Neuropeptides* 9:283–93
192. Moran, T. H., Smith, G. P., Hostetler, A. M., McHugh, P. R. 1987. Transport of cholecystokinin (CCK) binding sites in subdiaphragmatic vagal branches. *Brain Res.* 415:149–52
193. Pelaprat, D., Peschanski, M., Broer, Y., Besson, J. M., Roques, B. P. 1987. Postsynaptic receptors for cholecystokinin in the thalamic reticular nucleus: a possible modulatory system for sensory transmission. *Neurosci. Lett.* 80:16–20
194. Schjoldager, B., Shaw, M. J., Powers, S. P., Schmalz, P. F., Szurszewski, J., et al. 1988. Bovine gallbladder muscularis: source of a myogenic receptor for cholecystokinin. *Am. J. Physiol.* 254: G294–99
195. Beresford, I. J. M., Davenport, A. P., Sirinathsinghji, D. J. S., Hall, M. D.,

Hill, R. G., et al. 1988. Experimental hemiparkinsonism in the rat following chronic unilateral infusion of MPP into the nigrostriatal dopamine pathway—II. Differential localization of dopamine and cholecystokinin receptors. *Neuroscience* 27:129–43

196. Kritzer, M. F., Innis, R. B., Goldman-Rakic, P. S. 1988. Regional distribution of cholecystokinin receptors in macaque medial temporal lobe determined by in vitro receptor autoradiography. *J. Comp. Neurol.* 276:219–30
197. Durieux, C., Pham, H., Charpentier, B., Roques, B. P. 1988. Discrimination between CCK receptors of guinea-pig and rat brain by cyclic CCK_8 analogues. *Biochem. Biophys. Res. Commun.* 154: 1301–7
198. Bone, E. A., Rosenzweig, S. A. 1988. Characterization of cholecystokinin receptors in toad retina. *Peptides* 9:373–81
199. Dahl, D. 1988. Central administration of cholecystokinin potentiates evoked potential amplitude in the hippocampal dentate gyrus. *Neuropeptides* 11:147–51
200. Takashima, A., Itoh, S. 1989. Effect of V-9-M, a peptide fragment derived from procholecystokinin, on memory processes in the rat. *Can. J. Physiol.* 67:228–31
201. Barone, F. C., Bondinell, W. E., Labosh, T. J., White, R. F., Ormsbee, H. S. 1989. Cholecystokinin stimulates neuronal receptors to produce contraction of the canine colon. *Life Sci.* 44:533–42
202. Day, N. C., Hall, M. D., Hughes, J. 1989. Modulation of hypothalamic cholecystokinin receptor density with changes in magnocellular activity: a quantitative autoradiographic study. *Neuroscience* 29:371–83
203. Pearson, R. K., Hadac, E. M., Miller, L. J. 1989. Structural analysis of a distinct subtype of CCK receptor on human gastric smooth muscle tumors. *Am. J. Physiol.* 256:G1005–10
204. Hyde, T. M., Peroutka, S. J. 1989. Distribution of cholecystokinin receptors in the dorsal vagal complex and other selected nuclei in the human medulla. *Brain Res.* 495:198–202
205. Schjoldager, B., Molero, X., Miller, L. J. 1989. Functional and biochemical characterization of the human gallbladder muscularis cholecystokinin receptor. *Gastroenterology* 96:1119–25
206. Noguchi, M., Adachi, H., Sato, S., Honda, T., Ohnishi, S., et al. 1987. Cholecystokinin-stimulated pepsinogen secretion and cholecystokinin receptors on gastric chief cells in guinea pigs. *Endocrinol. Jpn.* 34:727–36
207. Barrett, R. W., Steffey, M. E., Wolfram, C. A. W. 1989. Type-A cholecystokinin binding sites in cow brain: characterization using (-)-[^{3}H]L364718 membrane binding. *Mol. Pharmacol.* 36:285–90