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REGULATION OF CHOLECYSTOKININ SECRETION AND PANCREATIC GENE EXPRESSION IN THE CONSCIOUS RAT

by

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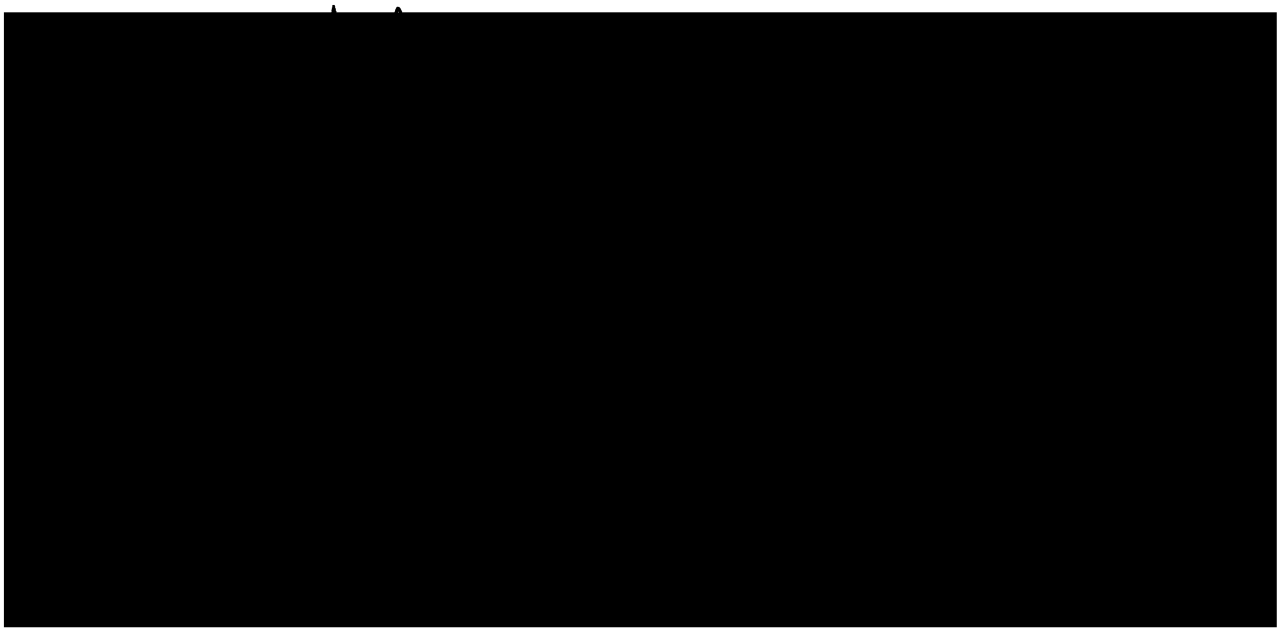

Dedicated to my father

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Regulation of Cholecystokinin Secretion and Pancreatic Gene Expression in the Conscious Rat

by Laura Dunbar Lewis

ABSTRACT

Regulation of cholecystokinin (CCK) secretion was studied in conscious, unrestrained rats by simultaneous duodenal perfusion with nutrients, intravenous infusion of hormones or neural agents and arterial blood sampling for CCK bioassay. Duodenal infusion of casein, but not casein hydrolysate, resulted in elevation of plasma CCK from fasting levels of 0.5 ± 0.1 to 3.8 ± 0.4 pM. Infusion of oleate also increased plasma CCK to 3.7 ± 0.6 pM, while intact fat had a small, but nonsignificant, effect (1.4 ± 0.4 pM). The CCK response to protein was inhibited by somatostatin but not by peptide YY; intravenous infusion of 1 or 10 μg/kg-hr somatostatin-14 decreased casein-stimulated CCK levels to 1.5 ± 0.2 pM and 0.9 ± 0.3 pM, respectively. Stimulation of vagal discharge with 2-D-deoxyglucose or cholinergic blockade with atropine had no effect on basal or protein-stimulated plasma CCK levels. Administration of the cholinergic agonist, bethanechol, while having no effect on fasting CCK levels, inhibited protein-stimulated plasma CCK to 1.3 ± 0.3 pM. Gastrin-releasing peptide increased fasting plasma CCK levels to 1.6 ± 0.1 pM. Thus CCK release in the rat is stimulated by dietary protein or fatty acid, and by gastrin-releasing peptide, and inhibited by somatostatin and bethanechol.

The physiologic role of cholecystokinin in the regulation of pancreatic gene expression was studied in conscious rats maintained at a uniform nutritional status by duodenal perfusion with an elemental diet which does not stimulate CCK release. Plasma CCK was then elevated endogenously by intraduodenal perfusion with soybean trypsin inhibitor (SBTI) or exogenously by intravenous infusion of CCK-8. Changes in pancreatic mRNA levels were quantified using cloned cDNA probes for ornithine

decarboxylase (ODC), as an index of growth stimulation, and for several digestive enzymes. SBTI administration for 48 hours stimulated a 2-fold increase in ODC mRNA and 4-5 fold increases in trypsinogen and chymotrypsinogen mRNA, while having no effect on amylase mRNA and resulting in ~50% less ribonuclease mRNA. CCK-8 infusion for 24 hours reproduced the effects of SBTI administration. The effects on pancreatic gene expression of both CCK infusion and SBTI administration were blocked by the CCK receptor antagonist L-364,718. These data indicate that physiologic levels of CCK regulate pancreatic ODC and digestive enzyme gene expression at the pretranslational level. CCK can therefore mediate, at least in part, the quantitative and qualitative adaptation of the pancreas to changes in the diet by regulating pancreatic gene expression.

CONTENTS

INTRODUCTION

General

Cholecystokinin (CCK) is a gastrointestinal peptide hormone first identified on the basis of its ability to stimulate gallbladder contraction (Ivy, 1928) and pancreatic enzyme secretion (Harper, 1943). Isolation and purification of the peptide revealed that both these functions were stimulated by the same hormone (Mutt, 1968). Early investigations showed that ingestion of a meal, or perfusion of the duodenum with nutrients, stimulated gallbladder contraction and pancreatic enzyme secretion, and were therefore thought to stimulate the release of CCK. This type of study suggested that CCK release is stimulated in man, dog and pig, by fat, fatty acids and amino acids (Go, 1970, Meyer, 1974, Lilja, 1984), and in rats, by intact protein (Green, 1983). Interpretation of these studies, however, was complicated by possible pleiotropic actions of the duodenal infusions, including release of more than one hormone, stimulation of neural pathways, and effects of the absorbed nutrients on the target organs. Development of direct assays for circulating hormone concentration was necessary to learn more about the physiology of CCK.

Initial attempts to develop radioimmunoassays were complicated by the close homology between CCK and gastrin, the presence of several biologically active forms of CCK and difficulty preparing labeled peptide (Rehfeld, 1984), while *in vitro* bioassays lacked sensitivity (Brand, 1981). Results of studies using these assays were therefore also difficult to interpret. Only recently have sensitive, specific assays for CCK become available which have facilitated the direct study of the physiology of CCK (Jansen, 1983, Liddle, 1984, Cantor, 1986a, Kanayama, 1987), including identification of the source and structure of the circulating forms, understanding the regulation of secretion, characterization of receptors and the mechanism of action, and identification of target tissues and actions.

Physiology of Cholecystokinin

Source

The intestine has been thought to be the source of CCK following the original descriptions of a substance present in extracts of proximal small intestine mucosa which stimulated gallbladder contraction or pancreatic enzyme secretion (Ivy, 1929, Harper, 1943). Isolation and purification of CCK from intestinal mucosa proved that this hormone is present in the intestine (Mutt, 1968). The CCK-containing cells have been localized by immunocytochemistry in the mucosal layer of mammalian duodenum and jejunum (Polak, 1975, Buffa, 1976, Dubois, 1976, Larsson, 1978). These elongated enteroendocrine cells are scattered in the epithelium lining the intestinal villi and crypts (Buffa, 1976, Usellini, 1985). Electron microscopy and immunostaining of serial sections showed that the CCK cells are polarized and have apical processes with microvilli contacting the intestinal lumen (Polak, 1975, Usellini, 1985). The CCK is stored in round, moderately electron dense, 210 nm (dog) to 330 nm (human) secretory granules located at the basal pole of the cell; this granule morphology corresponds the gut endocrine cell designation of I-cell (Polak, 1975, Buchan, 1978, Usellini, 1985). The morphology of the CCK cell has been described as "open type" and may permit these endocrine cells to function as chemoreceptors, sensing the luminal contents and responding by secreting CCK basolaterally into the circulation (Fujita, 1981, Solcia, 1987).

CCK is also present in vagal afferent nerve fibers and in enteric neurons (particularly in the myenteric plexus), but these are not likely to be the source of the circulating hormone (Polak, 1981, Dockray, 1987). In addition, CCK is abundant in the brain; CCK can be extracted in large amounts from cerebral cortex, caudate, hippocampus, thalamus, amygdala, striatum, and hypothalamus (Lamers, 1980, Beinfeld, 1981, Dockray, 1981, Eng, 1982). CCK in brain has been localized by

immunohistochemistry to nerve cell bodies and fibers and is not present in glia or other cell types (Innis, 1979). Brain CCK likely functions as a neurotransmitter: it is synthesized in neurons, can be released from synaptosomes, and binds to CCK receptors in brain (Williams, 1982, Williams, 1985). The physiologic significance of CCK in the brain is not known.

Biosynthesis

The CCK gene is present in a single copy (in man and mice) and is located on the short arm of human chromosome 3 (Friedman, 1985, Takahashi, 1986). The human and rat gene spans 7 kb and contains three exons; exon 1 is in the nontranslated region, exon 2 contains the initiation codon and signal sequence, and exon 3 includes the biologically active C-terminal domain (Takahashi, 1986, Deschenes, 1985). The gene is transcribed into a CCK mRNA which codes for a 114 (rat) or 115 (human, pig) amino acid preprohormone (Deschenes, 1984, Gubler, 1984, Takahashi, 1985). The predicted peptide contains a 20 amino acid signal sequence and a 12 amino acid carboxy-terminal extension containing a Gly-Arg-Arg amidation site. Little is known about the post-translational processing of CCK but it appears to be tissue-specific. In the intestine, post-translational processing includes cleavage of the prohormone, tyrosine sulfation and C-terminal amidation; these covalent modifications are required for biological activity.

The predicted peptide sequence also contains several dibasic and monobasic cleavage sites which can result in the generation of several forms including CCK-83, CCK-58, CCK-39, CCK-33, CCK-22, CCK-12, and CCK-8 (Deschenes, 1984, Gubler, 1985, Takahashi, 1985). [This notation refers to the number of amino acids in the peptide with the amidated carboxyl terminal considered number one.] Determination of the relative amounts of these various forms synthesized, secreted and circulating in the blood has been difficult due to differences depending on the method of extraction, differential detection of various forms by sequence-specific antibodies, possible

modifications made in the circulation, and species differences. It is important to note that an *in vitro* bioassay for CCK detects all biologically active forms regardless of size, while radioimmunoassay permits detection of the different forms regardless of activity.

The largest form of CCK predicted from the mRNA sequence is CCK-83, an 83 amino acid peptide resulting from cleavage of the signal sequence and carboxy terminal extension. This peptide is biologically active and has been extracted from human intestine (Eberlein, 1989). CCK-58 has been extracted from canine, porcine and human intestinal mucosa, and from canine, porcine and human plasma (Eysselein, 1982, 1987, 1988, Eberlein, 1987, Cantor, 1989). CCK-58 is therefore at least one of the secreted forms. CCK-58 itself is biologically active (Eysselein, 1983), but it may also serve as a precursor and be further cleaved either before secretion or in the circulation or both giving rise to several different forms.

CCK-33 was the form originally isolated from pig mucosal extracts (Mutt, 1968), while CCK-22 has been identified by bioassay and radioimmunoassay as a major form in rat intestine and rat plasma (Brand, 1981, Eng, 1984, Liddle, 1984). In one study, CCK in peripheral human plasma, after ingestion of a liquid fat meal, was composed of primarily two immunoreactive forms corresponding to CCK33/39 (indistinguishable in this assay) and CCK-22, with a smaller amount of CCK-58 and no CCK-8 (Jansen, 1987). A different study also using radioimmunoassay and chromatography found mostly CCK-33 and CCK-8 with smaller amounts of CCK-22 and CCK-58 in peripheral plasma after ingestion of a mixed meal (Cantor, 1987b). Bioassay of plasma CCK revealed a majority of CCK-33 along with some CCK-22 and CCK-8 after ingestion of a mixed meal in human volunteers, but the same investigators found CCK-22 was the major form in rat plasma after stimulation with soybean trypsin inhibitor (Liddle, 1984, 1985). These studies demonstrate the variability with species and assay in the determination of the relative amounts of the various circulating forms.

One recent study in pigs utilizing numerous sequence-specific antibodies found CCK-58, CCK-33, CCK-22, CCK-12 and CCK-8 present in intestinal mucosa, in venous effluent of isolated perfused duodenum and in both portal and peripheral plasma indicating that all of these forms are secreted and circulate (Cantor, 1989). Comparison of the relative ratios of these peptides in the intestine and portal and peripheral blood suggested that in pigs the larger forms, CCK-58 and CCK-33, are cleaved in the circulation, and CCK-8 (both secreted and produced in the circulation from larger forms) is cleared to some extent by the liver, resulting in a predominance (42%) of CCK-22 in the peripheral plasma. Large proCCK fragments (lacking the biologically active COOH-terminal CCK-8) have also been detected in intestinal extracts, in venous effluent of isolated perfused duodenum, and in plasma suggesting that these fragments are co-secreted with the active peptides (Eng, 1982, Eysselein, 1988, Cantor, 1989). Thus current evidence suggests that all of the CCK peptides predicted from the mRNA sequence can be synthesized in the CCK-cell and are secreted into the circulation where they can be further cleaved.

Regulation of Secretion

Nutrient Stimulated Secretion

Ingestion of a meal or perfusion of the duodenum with nutrients and direct measurement of plasma CCK levels has shown that fat, fatty acid, protein, and amino acids are potent stimulants of CCK release in man (Himeno, 1983, Jansen, 1983, Liddle, 1985, Schaffalitzky de Muckadell, 1986, Watanabe, 1986). In dogs, plasma CCK levels increase in response to meat feeding or intraduodenal infusion of fat, fatty acids, or amino acids (Fried, 1983, Konturek, 1986). Stimulation of CCK release by fat, however, requires adequate digestion by pancreatic enzymes, thus it is the fatty acid which stimulates CCK release (Watanabe, 1988).

Regulation of CCK release in the rat, in contrast, is less well-studied. It has long been known that administration of trypsin inhibitors stimulates pancreatic secretion in the rat (Lyman, 1957), and it was subsequently discovered that diversion of pancreatico-biliary juice from the duodenum also stimulates pancreatic secretion in the rat (Green, 1972). Intestinal infusion of trypsin or chymotrypsin in pancreatic juice-diverted rats reversed the stimulation of pancreatic secretion suggesting that trypsin inhibitors and pancreatic juice diversion stimulate pancreatic secretion through a common mechanism – effective removal of intestinal trypsin activity (Green, 1972). These authors concluded that in the rat pancreatic enzyme secretion is under negative feedback regulation.

Investigation of the mechanism for this negative feedback regulation of pancreatic enzyme secretion suggested involvement of CCK. Administration of trypsin inhibitor or diversion of pancreatic juice from the duodenum not only stimulates pancreatic secretion but also elevates plasma CCK (Liddle, 1984, Louie, 1986, Shiratori, 1986a, Folsh, 1987, Rausch, 1987a). Perfusion of the duodenum with trypsin blocks the stimulation of both pancreatic secretion and plasma CCK levels in response to diversion of pancreatic juice (Louie, 1986, Shiratori, 1986a, Folsch, 1987). Furthermore administration of the potent

and specific CCK receptor antagonist L-364,718 blocks the increase in pancreatic secretion in response to diversion of pancreatic juice (Louie, 1988). These results demonstrate that negative feedback regulation of pancreatic secretion in the rat is mediated by CCK.

CCK release in the rat must therefore also be subject to negative feedback regulation. Thus the presence of trypsin (and/or other serine proteases) in the duodenum normally suppresses CCK release and keeps pancreatic secretion at a low, basal level. A decrease in trypsin activity as a result of pancreatic juice diversion or addition of trypsin inhibitor removes this inhibition and stimulates CCK release which stimulates pancreatic secretion thereby restoring duodenal trypsin activity. This feedback mechanism also appears to exist in pig, while its existence in man and dog is controversial (Ihse, 1979, Owyang, 1986, Liener, 1988). The relative importance of this mechanism in these species is not clear.

The mediator of negative feedback regulation of CCK release is not known, but there is some evidence in the rat for the involvement of a luminal CCK-releasing peptide. Rapid (3 ml/min) perfusion of the small intestine of a rat prevents both the increase in plasma CCK and the increase in pancreatic secretion stimulated by diversion of pancreatic juice (Miyasaka, 1983, Lu, 1989). Rapid infusion of this perfusate into the duodenum of a second rat with diverted pancreatic juice reverses the inhibition of plasma CCK level and pancreatic secretion (Guan, 1988, Lu, 1989). This CCK-releasing activity of the perfusate is unaffected by boiling, but is completely destroyed by trypsin digestion, suggesting the CCK-releasing activity is due to the presence in the intestinal lumen of a CCK-releasing peptide. Stimulation of CCK release and pancreatic secretion in response to bile-pancreatic juice diversion may therefore be mediated by this trypsin-sensitive, CCK-releasing peptide secreted by the proximal small intestine.

Another group of experiments suggested the presence of a different peptide synthesized in the pancreas and secreted in pancreatic juice which also stimulates CCK

release (Fushiki, 1984, Fukoaka, 1986, Iwai, 1986). This peptide has been purified and sequenced and is also trypsin-sensitive (Iwai, 1987). It has been termed "monitor peptide" since it could function as a monitor of the level of trypsin activity in the duodenum and stimulate CCK release when trypsin activity is reduced. "Monitor peptide", however, cannot account for the stimulation of CCK release by pancreatic juice diversion, since it is removed with the pancreatic juice.

In an intact animal, either or both of these peptides would be present in the intestinal lumen and could regulate CCK release by sensing or monitoring the trypsin activity in the duodenum. Thus basal trypsin activity digests the peptide(s) and maintains a low, basal rate of CCK release. Reduction of trypsin activity by administration of trypsin inhibitor or pancreatic juice diversion prevents or reduces degradation of the peptide(s). The peptide(s) can then stimulate CCK release, leading to rise in plasma CCK, increased pancreatic secretion, and increased trypsin activity in the duodenum. Ingestion of substrate would similarly reduce trypsin activity and stimulate CCK release, while duodenal infusion of trypsin degrades the peptide(s) and therefore prevents stimulation of CCK release. This mechanism tends to maintain a balanced trypsin:substrate ratio thus ensuring adequate digestion of dietary protein.

Physiologically, the stimulus for CCK release is clearly not pancreatic juice diversion or ingestion of trypsin inhibitors, but most likely the ingestion of nutrients which interact with this regulatory system. In a previous study intragastric administration of protein, but not amino acids stimulated CCK release in the rat (Liddle, 1986b). The ability of different proteins to stimulate CCK release paralleled the ability of the proteins to compete for trypsin activity *in vitro* consistent with the negative feedback regulation of CCK release just described. The role of fat is not as clear; intragastric administration results in small (Douglas, 1988) or no (Liddle, 1986b) elevation in plasma CCK, while intraduodenal fat has been reported to result in a small

stimulation (Green, 1987). Studies using intragastric administration of nutrients, however, may give misleading results. Since CCK itself slows gastric emptying (Liddle, 1986a, Dektor, 1988, Green, 1988), stimulation of CCK release will alter the subsequent rate of delivery of gastric contents to the duodenum. Intragastric administration may also trigger gastric reflexes which could alter CCK release. Since the CCK-containing cells are located in the duodenum, to assess the role of various nutrients in the regulation of CCK release it seems most appropriate to deliver the nutrients directly to the duodenum. The studies described in chapter three investigate the regulation of CCK release in the rat using intraduodenal administration of various nutrients.

Neural Regulation

Ingestion of a meal or the presence of nutrients in the duodenum is clearly the primary stimulus for CCK release, but little is known about the possible neural or hormonal modulation of CCK release. For example, the vagus nerve coordinates many postprandial functions including gastric and pancreatic secretion and could also modulate CCK release. Vagal stimulation of pancreatic secretion could be mediated in part by stimulation of CCK release. One investigation of the role of the vagus nerve in the regulation of CCK secretion showed that truncal vagotomy had no effect on basal or tryptophan-stimulated CCK release in dogs (Singer, 1989). On the other hand, patients with either truncal vagotomy or highly selective vagotomy had an increased CCK response to ingestion of a liquid fat meal (Hopman, 1984b). These studies are difficult to interpret, however, since vagotomy likely interrupts several regulatory and compensatory mechanisms which could indirectly effect CCK release (e.g. gastric emptying). Few studies have directly measured the plasma CCK response to vagal stimulation, although one recent study in the pig demonstrated CCK release in response to electrical stimulation of the vagus nerve (Cantor, 1986b). The plasma CCK response to vagal stimulation in other species is not known.

The role of cholinergic innervation in the regulation of CCK secretion is also unknown. In man, one study reported that atropine treatment abolished the increase in plasma CCK observed in response to drinking a fatty acid meal (Maton, 1984). A different study, however, showed that the increase in plasma CCK observed after intraduodenal instillation of corn oil was delayed but not inhibited by atropine treatment (Hopman, 1984a). Similarly, atropine treatment had no effect on tryptophan-stimulated CCK release in dogs (Singer, 1989). Thus the role of cholinergic pathways in the regulation of CCK release remains unclear.

CCK release could also be modulated by the extensive peptidergic enteric innervation. For example, gastrin-releasing peptide (GRP), mammalian bombesin, is abundant throughout the enteric nervous system (Moghimzadeh, 1983) and stimulates the release of several hormones including gastrin (Giraud, 1987, Guo, 1987, Holst, 1987, Sugano, 1987). It also stimulates pancreatic secretion (Hosotani, 1987a), acting directly via GRP (bombesin) receptors on the acinar cell (Jensen, 1978, Otsuki, 1987). A study in dogs suggested GRP could also stimulate pancreatic secretion indirectly by stimulating CCK release (Nealon, 1987). Similar studies in rats, however, found little evidence for this hypothesis, since treatment with the CCK receptor antagonist L-364,718 did not alter bombesin-stimulated pancreatic secretion (Anderson, 1988, Herzig, 1988, Wisner, 1988b). Yet direct studies using arterial perfusion of the isolated porcine or rat duodenum have demonstrated increased CCK release in response to GRP (Cantor, 1987a, Nakano, 1988) and infusion of bombesin has been shown to increase plasma CCK levels in man (DeJong, 1987). Thus the effect of GRP on CCK secretion is not clear. Since the role of vagal, cholinergic or peptidergic pathways in regulation of CCK release is poorly understood, the studies described in chapter three were undertaken to investigate the neural regulation of basal and protein-stimulated CCK secretion in the conscious rat.

Hormonal Regulation

CCK release may also be hormonally regulated; two hormones which are potential regulators of CCK release are somatostatin and peptide YY (PYY). Somatostatin inhibits the release of several gastrointestinal hormones including gastrin, insulin, glucagon and pancreatic polypeptide (Annibale, 1987, Colturi, 1984, Vale, 1987, Yamada, 1987). Somatostatin also inhibits numerous gastrointestinal functions including gastric and pancreatic secretion (Gullo, 1987, Gyr, 1987, Kohler, 1986, Yamada, 1987). Somatostatin inhibition of gastric secretion has been shown to be mediated by locally produced and secreted somatostatin acting in a paracrine fashion to inhibit gastrin release and by endocrine and paracrine inhibition of parietal cells (Yamada, 1987). By analogy, inhibition of pancreatic secretion could be mediated by both a direct action on the acinar cell and by inhibition of CCK release. In support of a direct action on acinar cells, somatostatin receptors have been demonstrated and characterized on pancreatic acini (Esteve, 1984, Sakamoto, 1984, Susini, 1986). Although these receptors are functional and when occupied inhibit secretin- or VIP-stimulated increases in cAMP formation, somatostatin has no effect on amylase release from isolated acini (Esteve, 1983). Thus the mechanism for somatostatin inhibition of pancreatic secretion is poorly understood. Somatostatin could inhibit pancreatic secretion by inhibiting CCK release. In support of this hypothesis, somatostatin has been shown to inhibit bombesin-stimulated CCK release in man (DeJong, 1987). There are no other studies directly investigating the effect of somatostatin on plasma CCK levels.

Peptide YY is a gastrointestinal peptide found in the distal small intestine which is released after eating or in response to perfusion of the distal intestine with fatty acid (Taylor, 1985, Adrian, 1985, Pappas, 1985, Greeley, 1987). It has been postulated to function as an "enterogastrone", an intestinal factor which inhibits postprandial gastric secretion, and as a "pancreotone" or the "anti-CCK hormone", an intestinal factor which inhibits pancreatic secretion (Pappas, 1985). PYY inhibits pancreatic secretion indirectly

through a poorly understood mechanism (Lluis, 1987, Inoue, 1988, Konturek, 1988a, Putnam, 1989), which could include inhibition of CCK release. This hypothesis is supported by the demonstration of PYY inhibition of intraduodenal oleate-stimulated CCK release in dog (Lluis, 1988), but little is known concerning the effect of PYY on CCK secretion in man or rat. Thus, an understanding of the hormonal regulation of CCK release also requires additional investigation. The studies described in chapter three investigate the regulation of CCK release in the rat by nutrients, hormones and neural pathways.

Additional information about CCK biosynthesis and secretion could be gained from studies with cultured CCK cells. Although some tumors have been reported to secrete CCK (Deschenes, 1984, Rehfeld, 1987), there are no reports of a CCK-secreting cell line of duodenal origin. There has been one report of an isolated cell preparation obtained by counterflow elutriation of dispersed canine jejunum mucosa (Barber, 1986). Although this preparation contained only 2-3% CCK cells, this was a 20-fold enrichment over the originally dispersed cells. This cell preparation contained immunoreactive CCK8 and CCK33/39 which were released upon stimulation by elevated extracellular potassium. The more physiological stimulus L-tryptophan also stimulated a dose-dependent and stereospecific increase in CCK release, and this stimulation was inhibited by carbachol. No other studies on isolated CCK cells have been reported to date.

Receptor and Mechanism of Action

CCK released from the enteroendocrine cell exerts its actions on peripheral tissues by binding to peripheral CCK receptors. Specific CCK binding has been demonstrated in pancreas, on both acinar (Jensen, 1980, Sankaran, 1980, Rosenzweig, 1983, Sakamoto, 1983b) and islet cells (Sakamoto, 1985, Verspohl, 1986), on gallbladder smooth muscle (Steigerwalt, 1984, Schjoldager, 1988b, Von Schrenck 1988) and on the pyloric sphincter (Robinson, 1987). The CCK receptor on pancreatic acinar cells has been particularly well characterized and is highly selective for biologically active forms of CCK with the binding preference CCK-33 = CCK-8 > CCK-7 >> desulfated CCK-8 >> CCK-6 (Sankaran, 1980, Solomon, 1984, Otsuki, 1986, Vinayek, 1987). Similar agonist selectivity has been demonstrated for the receptor on pancreatic islets (Verspohl, 1986) and gallbladder muscularis (Steigerwalt, 1984, Von Shrenck, 1988). Although the biologically active C-terminal peptides of CCK and gastrin are highly homologous, the apparent affinity of the CCK receptor for gastrin is about 1000 times lower than that for CCK. This selectivity has been exploited to develop a highly sensitive and specific *in vitro* bioassay for CCK using isolated pancreatic acini (Liddle, 1984). Binding of CCK to its receptor can be inhibited by several receptor antagonists including dibutyryl cyclic GMP, benzotript, proglumide and its derivatives, asperlicin, and the recently developed nonpeptide compound L-364,718 (Gardner, 1984, Niedarau, 1986, Chang, 1985, 1986a). The most potent and specific of these is L-364,718, a benzodiazepine derivative with an apparent affinity for peripheral CCK receptors similar to that of CCK itself (Chang, 1986b).

Affinity labeling studies have shown that the peripheral CCK receptor is a glycosylated protein of apparent molecular weight of 80-95 kDa (Sakamoto, 1983a, Rosenzweig, 1983, Pearson, 1987, Schjoldager, 1988a). The CCK receptor appears to be coupled to a guanine nucleotide-binding protein; binding of labeled CCK is inhibited in the presence of GTPγS, and guanine nucleotides increase the dissociation of CCK from

its receptor (Williams, 1987). Binding of CCK to its receptor results in release of intracellular calcium and activation of protein kinase C mediated, at least in part, by breakdown of phosphotidyl inositol by phospholipase C releasing 1,4,5-inositol-tris-phosphate and diacylglycerol (Hootman, 1987). Most actions of CCK are thought to be mediated by these two intracellular messengers directly or indirectly through subsequent activation of various kinases and phosphatases.

Target Tissues

Post-prandial Actions

CCK is an integrative hormone acting on several target tissues with many different actions. Establishing the physiologic hormonal actions of CCK on various target tissues requires the development of sensitive, specific CCK assays which make it possible to measure endogenous changes in circulating CCK levels and to reproduce accurately these physiological concentrations with exogenous administration of CCK. The availability of a potent, specific receptor antagonist L-364,718, enables further elucidation of the role of endogenous CCK. Studies using these tools to determine the physiologic role of CCK have demonstrated that CCK released in response to ingestion of a meal plays a role in the regulation of gallbladder contraction, pancreatic exocrine and endocrine secretion, gastric empyting and satiety.

Ingestion of a high protein, high fat meal elevates plasma CCK in human volunteers from fasting levels of 1-2 pM to approximately 6 pM, while gallbladder volume, measured by ultrasonography, decreases concommitantly (Liddle, 1985, Hopman, 1987). Similarly, intraduodenal administration of emulsified corn oil results in both increased plasma CCK and increased rate of gallbladder emptying in dogs (Shiratori, 1986b). Exogenous infusion of CCK-8 or CCK -33 at rates which reproduce postprandial levels also results in decreased gallbladder volume (Liddle, 1985, Hopman, 1985). Furthermore, administration of the CCK receptor antagonist L-364,718 blocks gallbladder emptying stimulated by endogenous or exogenous CCK (Chang, 1986a, Pendleton, 1987). These results demonstrate that one physiologic action of CCK is regulation of gallbladder contraction, the action for which this hormone was named.

Probably the most studied action of CCK is stimulation of pancreatic exocrine secretion. Although numerous studies both *in vivo* and *in vitro* have clearly demonstrated

CCK stimulation of pancreatic secretion, the physiologic significance of endogenously released CCK in the regulation of pancreatic secretion has not yet been well-established. In particular, it has not been clearly shown that exogenous CCK infusion at doses which reproduce postprandial physiologic levels stimulates pancreatic secretion. Yet there is some evidence for a physiologic role of CCK in stimulating pancreatic secretion. For example, infusion of CCK-33 at a dose which resulted in a circulating level of CCK slightly lower than that found after ingestion of a high fat meal, stimulated pancreatic enzyme secretion in human volunteers (Kerstens, 1985). This study, however, was done with a background infusion of a supraphysiologic dose of secretin, thus obscuring the role of CCK alone.

Studies with CCK receptor antagonists support a physiologic role of CCK in stimulation of pancreatic secretion. The pancreatic secretory response to ingestion of a meal or intraduodenal administration of amino acids, fatty acid, or fat was inhibited by administration of proglumide or its derivative CR-1409 (Stubbs, 1985, Konturek, 1988b). Recent development of the more potent and specific antagonist L-364,718 should facilitate study of the role of endogenously released postprandial circulating CCK. For example, it has recently been observed in rats that the increase in pancreatic secretion in response to pancreatic juice diversion can be blocked by administration of L-364,718, clearly demonstrating the role of endogenous CCK release in this model of stimulated pancreatic secretion (Louie, 1988). Further studies utilizing this CCK receptor antagonist with potent *in vivo* specificity, in addition to studying the effects on pancreatic secretion of physiologic levels of circulating CCK, should soon establish the physiologic action of CCK in stimulation of pancreatic secretion.

CCK is also an incretin: an intestinal hormone which enhances endocrine pancreatic secretion. CCK-8 has been shown to increase glucose-stimulated release of insulin, glucagon and somatostatin from isolated perfused pancreas and isolated islets (Okabayashi, 1983, Hermansen, 1984, Zawalich, 1986, Verspohl, 1987). This effect on

isolated islets can be blocked by administration of L-364,718 (Zawalich, 1988). In addition CCK-8 infusion resulted in elevated pancreatic polypeptide (PP) levels in conscious dogs; this response was increased by additional infusion of amino acids and blocked by treatment with L-364,718 (Schusdziarra, 1986, Hosotani, 1987). This incretin action of CCK may play a role in maintenance of postprandial normoglycemia and coordination of post-prandial exocrine and endocrine secretion.

The physiologic role of CCK in regulation of postprandial insulin and glucagon secretion and therefore postprandial normoglycemia has recently been demonstrated in man and rats. Exogenous infusion of CCK-8 at doses which reproduce those measured after ingestion of a mixed liquid meal potentiated amino-acid induced insulin and glucagon release in human volunteers (Rushakoff, 1987). Similarly in rats, intraduodenal infusion of a protein/glucose mixture stimulated release of insulin and glucagon, and this response was significantly reduced by pretreatment with L-364,718 (Rosetti, 1987). Thus postprandial insulin and glucagon levels are regulated not only by the products of digestion, glucose and amino acids, but also hormonally by CCK.

Regulation of gastric emptying is another physiologic action of CCK. Exogenous infusion of CCK-8 at doses which reproduced those measured after ingestion of a test meal significantly delayed the emptying of technetium-labeled water in human volunteers (Liddle, 1986a). Elevation of plasma CCK either endogenously by feeding trypsin inhibitor or exogenously by infusion of a physiologic dose of CCK-8 inhibited gastric emptying of saline in rats, and this response was blocked by administration of L-364,718 (Green, 1988). L-364,718 treatment also increased the emptying rate of a semisolid test meal containing protein or a peptone meal, but had no effect on the emptying rate of inert pellets, saline, acid or hyperosmolal saline (Dektor, 1988, Green, 1988). These results demonstrate that CCK released in response to ingestion of a meal plays an important physiologic role in the regulation of gastric emptying.

CCK may also play a role in satiety. Although most studies of satiety effects have used nonphysiologic doses and/or routes of administration, there are some suggestive results from recent physiologic studies. Administration of L-364,718 increased intake of a palatable diet in non-deprived rats (Hewson, 1988). Similarly, pigs immunized with a CCK-linked antigen ate more and gained more weight than pigs immunized with a control peptide hormone (Pekas, 1988). Another interesting study showed that women with the eating disorder bulimia nervosa reported feeling less satiated and had a reduced CCK response to a standard meal compared to age-, sex- and weight-matched controls, while in a subgroup of patients treated with tricyclic antidepressants, both the CCK response and the feeling of satiety were improved (Geracioti, 1988). Although the cause and effect of this altered CCK response in bulimia is not clear, it is nevertheless highly suggestive of a role for CCK in induction of satiety.

In summary, the target tissues and actions of CCK released after ingestion of a meal include stimulation of gallbladder contraction, stimulation of pancreatic exocrine and endocrine secretion, inhibition of gastric emptying and possible induction of satiety. CCK is thus an integrative hormone. All of these postprandial actions coordinate digestion, ensuring delivery to the duodenum an appropriate amount of food, digestive enzymes and bile, as well as efficient peripheral uptake of the absorbed nutrients.

Regulation of Pancreatic Growth and Gene Expression

In addition to these immediate post-prandial actions, CCK also regulates adaptive long-term changes including pancreatic growth and the synthesis of some digestive enzymes thereby providing a mechanism for adjustment of pancreatic secretion to match different diet compositions. For example, increasing the protein content of the diet stimulates CCK release in the rat and also stimulates pancreatic growth, resulting in increased pancreatic weight and DNA content (Solomon, 1978, Green, 1986). Similarly, feeding of trypsin inhibitors or chronic diversion of pancreatic juice, which stimulates CCK release via the negative feedback loop, also stimulates pancreatic growth (Goke, 1986, Levan, 1986b). Trypsin-inhibitor stimulated growth is blocked by treatment with the potent CCK receptor antagonist, L-364,718, demonstrating that CCK mediates pancreatic growth stimulated by trypsin inhibitor feeding (Wisner, 1988a). Stimulation of pancreatic growth by CCK is a direct effect since it has also been demonstrated *in vitro* using primary acinar cell cultures (Logsdon, 1986).

CCK-induced pancreatic growth may involve induction of ornithine decarboxylase (ODC), an enzyme which catalyzes the rate-limiting step in the biosynthesis of the polyamines putrescine, spermidine, and spermine (Luk,1987). These compounds have been shown to facilitate nearly all aspects of DNA, RNA, and protein synthesis and are thought to be closely involved in the regulation of growth and proliferation (Pegg, 1982) Increases in pancreatic DNA content stimulated by treatment with the CCK-like peptide, caerulein, are accompanied by increased cellular polyamine content and ODC immunoreactivity (Morisset, 1984, 1986). Furthermore, the irreversible ODC inhibitor α-difluoromethylornithine significantly reduced caerulein-induced pancreatic growth, and this reduction was reversed by the addition of exogenous putrescine (Benrezzak, 1984, Morisset, 1985). Little is known, however, about the physiologic role of CCK or the mechanism whereby CCK stimulates ODC activity. Thus

the studies described in chapter four investigate the physiologic role of CCK in stimulating pancreatic growth via induction of ODC mRNA.

Pancreatic growth in response to changes in diet or alteration of CCK levels is accompanied by changes in the amount of mRNA coding for digestive enzymes, and in the synthesis and content of these proteins. The pancreata of rats fed a high (70-75%) protein diet for 1-2 weeks have an increased protein content and increased mRNA levels for the serine proteases trypsin, chymotrypsin and elastase (Wicker, 1984, Giorgi, 1985, Green, 1986). Similarly the pancreatic content and mRNA level of amylase are increased in animals fed a high carbohydrate diet (Giorgi, 1984, Wicker, 1984).

These adaptive changes in pancreatic enzyme content in response to different diets are mediated, at least in part, by CCK since stimulation of CCK release by feeding of trypsin inhibitors or chronic pancreatic juice diversion also results in increased protein content and mRNA levels of the serine proteases along with decreased amounts of amylase and amylase mRNA (Goke, 1986, Levan, 1986b, Rausch, 1987b, Keim, 1988). In addition, the trypsin inhibitor-induced increase in pancreatic chymotrypsin content is blocked by administration of L-364,718 (Wisner, 1988a). A 24 hour intravenous infusion (250 ng/kg-hr) of the CCK-like peptide caerulein, also results in increased content, synthesis, and amounts of mRNA for the serine pancreatic proteases, and a decrease in amylase content, synthesis and mRNA level (Schick, 1984, Wicker, 1985), while chronic administration (i.p. injection, twice daily for 7 days) of caerulein or a crude preparation of CCK causes similar changes (Renaud, 1986). These results further support a role for CCK in regulating pancreatic digestive enzyme gene expression and thereby mediating pancreatic adaptation to a changing diet.

A direct investigation, however, of the physiologic role of CCK in mediating the adaptation of the pancreas to changes in diet has not been reported. In particular, the effect of physiologic concentrations of circulating CCK on pancreatic digestive enzyme

gene expression has not been investigated. Previous studies have employed crude preparations of CCK which contain contaminants with unknown effects, or caerulein which is not a physiological stimulus. Circulating plasma CCK levels resulting from administered CCK were not measured, and equivalent plasma CCK levels achieved with caerulein administration were likely supraphysiologic. In the aforementioned studies animals were permitted access to food *ad libitum*; yet exogenous administration of CCK can induce satiety and decrease gastric emptying resulting in decreased food intake. Since changes in food intake are likely to be important in the regulation of pancreatic gene expression, it is important to control the nutritional status of the animals. Also, several studies have examined the changes in pancreatic digestive enzyme levels, but it is not clear if these changes are pre- or post-translational. Thus the research described in chapter four also addresses the physiologic role of CCK in regulating pancreatic digestive enzyme gene expression.

Goal of Research

The goal of this research is to learn more about the physiology of CCK, in particular the regulation of CCK secretion and the regulation of pancreatic growth and gene expression by CCK. The rat was chosen as an experimental model because many of the actions of CCK are well described in the rat and it is small enough for easy study, yet just large enough for accurate measurement of circulating CCK levels. The regulation of CCK release in rats was studied using a newly developed experimental model which permits simultaneous duodenal and intravenous infusion, as well as arterial blood sampling, in conscious, unrestrained rats. This model maintains the animals in as physiologically normal conditions as possible in order to minimize any extraneous effects resulting from anesthesia, or stress due to restraint or manipulation.

Regulation of pancreatic gene expression by CCK was also studied in conscious rats, with careful attention to maintenance of uniform nutritional status and physiologic levels of CCK stimulation, and using the techniques of molecular biology to quantitate changes in pancreatic gene expression.

METHODS

Regulation of CCK secretion

Animal Surgery

Male, 250-350 g, Wistar rats (Charles River, Wilmington, DE) were chronically instrumented with arterial, venous, duodenal and intraperitoneal cannulae. Under sodium pentobarbital anesthesia, a cannula made of polyvinyl tubing (0.5 mm ID, Dural Plastics, New South Wales, Australia) fused to Tygon S-54-HL tubing (0.02" ID, Norton Plastics, Akron OH) was inserted into the femoral artery and advanced to the abdominal aorta, just below the renal artery. The venous cannula (0.025" ID, silicone elastomer, Baxter Scientific Products, McGaw Park, IL) was inserted into the femoral vein and advanced into the inferior vena cava. The femoral artery and vein were ligated and severed distal to the insertion point. Both cannulae were tunneled subcutaneously to exit between the scapulae.

An abdominal midline incision was made and the duodenal cannula (0.03" ID, silicone elastomer, Baxter Scientific Products, MacGaw Park IL) was inserted 5 mm distal to the pylorus and secured with a purse-string suture. The peritoneal cannula (0.02" ID, silicone elastomer, Baxter Scientific Products, McGaw Park, IL) was tied to the abdominal wall and both cannulae were tunneled subcutaneously from the abdominal midline to the exit point behind the head. All four cannulae exited at the top of the cage protected by a flexible, stainless steel spring (0.182" ID, Plastic Products, Roanoke, VA). The spring was secured so as to permit the animal free range of motion within the cage (including normal access to food and water) and to permit the investigator access to the cannulae without disturbing the animal. Rats were treated with chloramphenicol (50 mg/kg, i.p.) at the time of surgery and for 3 days post-operatively. Animals were housed with 12 hour light:dark cycle, *ad lib* food and water and were allowed to recover for 4 days before experiments.

Food Studies

To study the effect of various food components on CCK release, rats were fasted overnight, and then infused intraduodenally at 3.5 ml/hr with either casein (Casec, Mead Johnson, Evansville,IN), casein hydrolysate (pH 7.2, ICN Nutrition Chemicals, Cleveland, OH), oleic acid (pH adjusted to 7 with NaOH, ICN Nutrition Chemicals, Cleveland, OH), Lipomul (gift of Upjohn, Kalamazoo, MI), soybean trypsin inhibitor (Type II-S, Sigma, St.Louis, MO), $CaCl_2$ or glucose. During the duodenal infusion, a 3 ml sample of arterial blood was withdrawn through the arterial cannula (1 ml/min) into a heparinized syringe and placed immediately on ice. This rate and amount of blood withdrawal does not elevate heart rate or cause the release of the stress hormones ACTH, norepinephrine, or epinephrine (Darlington, 1986). Within 5 minutes, the blood was separated by centrifugation (1000 x g, 10 min, 4°C), the plasma extracted for bioassay (see below), and the cells resuspended in sterile saline and reinfused into the animal through the venous cannula. Animals were allowed to recover for 3 days between experiments.

Hormone Studies

Animals were fasted overnight and infused intravenously (0.9 ml/hr) with somatostatin or peptide YY (Bachem, Torrance, CA) dissolved in sterile saline containing 0.1% bovin serum albumin (ICN Bichemicals, Lisle, IL). Casein (Casec) was infused intraduodenally (500 mg/hr) starting 5 minutes after the hormone infusion reached the venous circulation. Blood was withdrawn as described above after 10 minutes of casein infusion, plasma separated for bioassay and the blood cells reinfused.

Neural Studies

To stimulate vagal discharge, 2-D-deoxyglucose (Sigma, St. Louis, MO) was administered to fasted animals as a 100 mg/kg bolus i.v. This dose stimulates pancreatic secretion within 20 minutes (Roze, 1977). After 25 minutes arterial blood was collected for bioassay of plasma CCK from animals with either no additional treatment, or duodenal casein infusion during the last 10 minutes. To mimic cholinergic pathways, fasted animals were infused intravenously with bethanechol (100 μg/kg-hr). Blood was collected after 10 minutes of bethanechol stimulation, with or without a concomitant duodenal casein infusion. To block cholinergic pathways, atropine (100 μg/kg-hr) was infused intravenously. Blood was collected after 15 minutes of atropine treatment with or without duodenal casein during the last 10 minutes. To study the effect of the neurotransmitter gastrin-releasing peptide (GRP), animals were fasted overnight and then infused intravenously with gastrin-releasing peptide (1 μg/kg-hr GRP 18-27, Bachem, Torrance, CA) using the same protocol described for atropine.

Bioassay of CCK

Plasma was assayed for CCK bioactivity essentially as previously described (Liddle, 1984). Isolated pancreatic acini were prepared by collagenase digestion of the pancreas of a fasted or fed male Sprague-Dawley rat (Williams, 1978) and incubated in a Tris-buffered Ringer solution containing 40 mM Tris(hydroxymethyl)aminomethane, 103 mM NaCl, 1 mM NaH_2PO_4, 4.7 mM KCl, 1.28 mM $CaCl_2$, 0.56 mM $MgCl_2$, 11.1 mM glucose, 0.1 mg/ml soybean trypsin inhibitor (type I-S), minimal Eagle's medium amino acid supplement, glutamine, and 5 mg/ml bovine serum albumin (BSA). The buffer was equilibrated with 100% O_2 and adjusted to pH 7.58 at 22°C (equivalent to pH 7.4 at 37°C).

CCK was extracted from plasma by adsorption onto a Sep-Pak C-18 cartridge (Waters Associates, Milford, MA). Cartridges were prewet first with 3 ml methanol, then

with 20 ml distilled water. A 1-6 ml (usually 1.5 ml) plasma sample was applied, and the cartridge washed with 20 ml water, dried and stored at - 20°C. For bioassay, cartridges were defrosted, hydrated with 5 ml H_2O, eluted with 1 ml 0.2% TFA/80% ethanol into flat-bottomed blood dilution vials (Baxter Scientific Products, McGaw Park, IL), and dried under nitrogen at 45°C.

Acini were incubated with standard concentrations of CCK-8 or plasma extracts. A standard curve of the amount of amylase released as a function of CCK8 concentration was used to determine the concentration of CCK in the plasma sample expressed as CCK8 equivalents. Recovery of CCK was estimated by adding known amounts of CCK-8 (dissolved in saline containing 1 mg/ml BSA) to a Sep-Pak cartridge as described for plasma samples, eluting and assaying by comparison to the standard curve. Recovery of 3 pM CCK-8 was 97 ± 11 % (mean ± SE, n=6).

Pancreatic acini also have GRP (bombesin) and muscarinic receptors which when occupied stimulate amylase release from isolated acini (Williams, 1978, Jensen, 1978, Otsuki, 1987). Although these secretagogues are not normally present in plasma, GRP and bethanechol were infused in some experiments. To permit assay of CCK in plasma samples containing these secretagogues, the following modifications were made. Plasma samples containing GRP were assayed for CCK in the presence of 10 μM GRP receptor antagonist, [Leu^{13}-ψ-CH_2NH-Leu^{14}]Bombesin (gift of Dr. David Coy, Coy, 1988), added to both CCK8 standards and plasma samples. This concentration of antagonist was sufficient to block stimulation of acini by up to 300 pM GRP (Fig. 1), while retaining the full range of dose-response to CCK8 (Fig. 2).

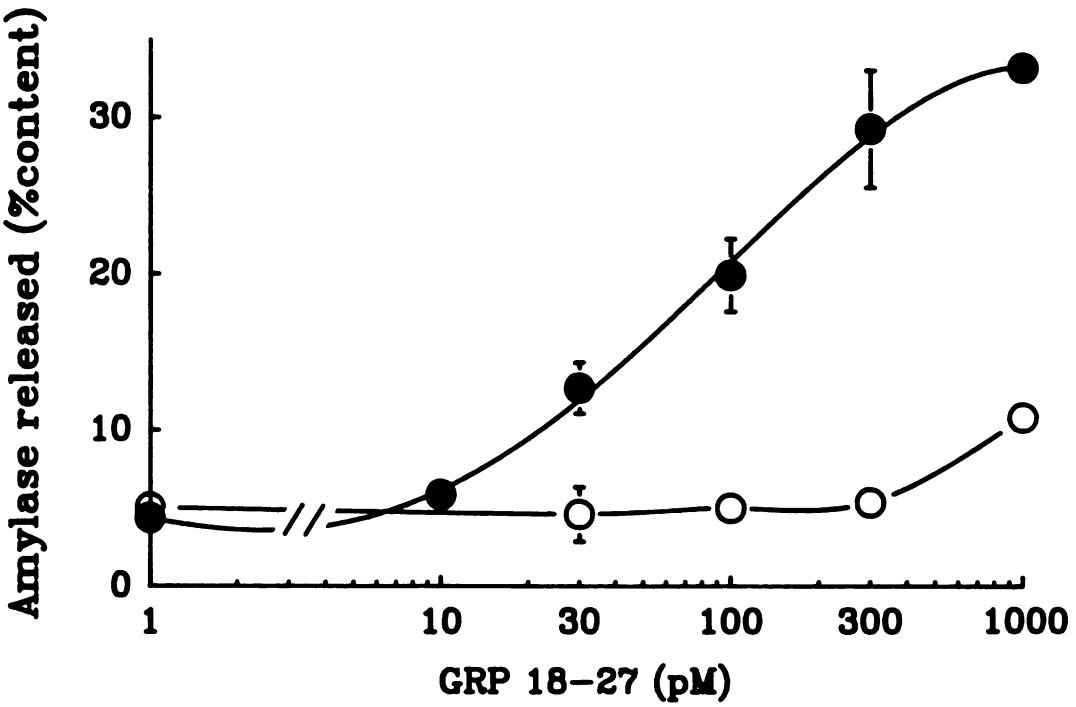

Figure 1 *Gastrin-releasing peptide (GRP) stimulates amylase release from isolated pancreatic acini.* Isolated rat pancreatic acini were incubated with the indicated concentration of GRP in the absence (filled circles) or presence (open circles) of 10 μM GRP receptor antagonist [Leu^{13}-ψ-CH_2NH-Leu^{14}]Bombesin for 30 minutes and the medium assayed for amylase released, expressed as % total content. Each point represents mean ± SD of triplicates. Data are representative of 3 experiments.

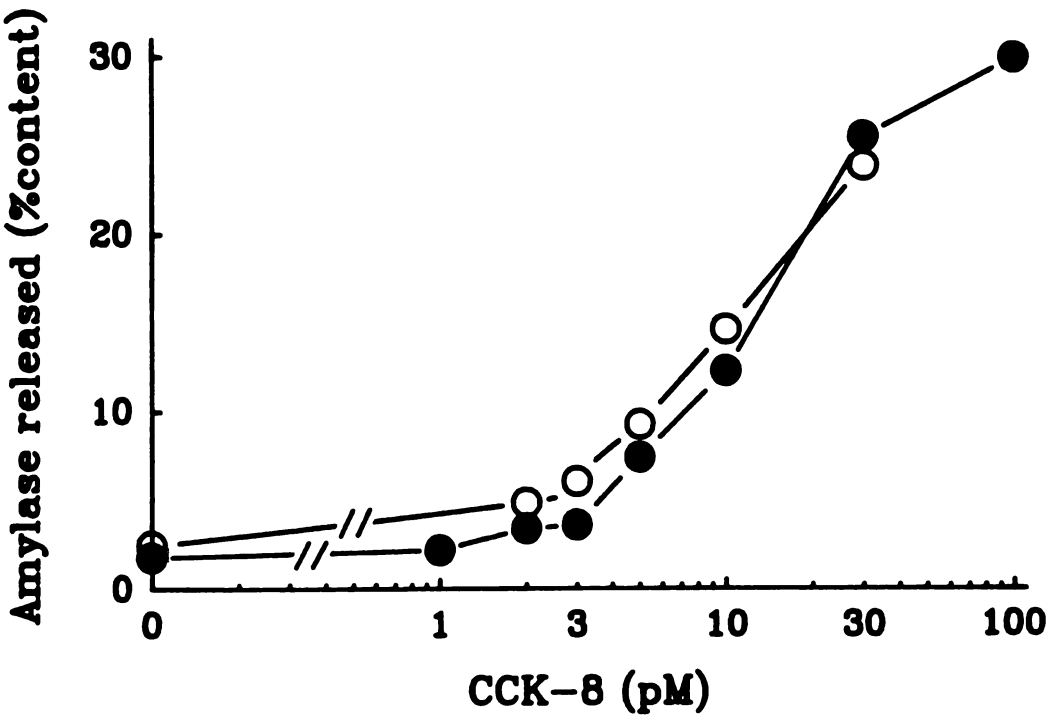

Figure 2 *Gastrin-releasing peptide (GRP) antagonist has no effect on CCK-stimulated amylase release from isolated pancreatic acini.* Isolated rat pancreatic acini were incubated with the indicated concentration of CCK-8 in the absence (filled circles) or presence (open circles) of 10 μM GRP receptor antagonist [Leu^{13}-ψ-CH_2NH-Leu^{14}]Bombesin for 30 minutes and the medium assayed for amylase released, expressed as % total content. Each point represents mean of duplicate determinations, representative of 3 experiments.

In addition, since GRP stimulates amylase release from isolated acini directly, GRP in plasma can be extracted by the same procedure used to extract CCK and plasma GRP levels assayed by comparison with a GRP dose-response standard curve in the presence of the CCK receptor antagonist L-364,718 (Fig. 3). Recovery of GRP was estimated by applying 30, 100 or 300 pM GRP_{18-27} (dissolved in saline containing 1 mg/ml BSA) to a Sep-Pak and assaying by comparison to the GRP_{18-27} standard curve; recovery averaged 103 ± 13% (mean ± SE, n=6). Using this *in vitro* bioassay the plasma level of GRP during 1 μg/kg-hr intravenous infusion was 34 ± 5 pM (mean ± SE, n=5).

Plasma samples containing bethanechol were assayed in the presence of 10 μM atropine. This dose of atropine had no effect on the response to CCK and was sufficient to block the stimulation by up to 1 μM bethanechol.

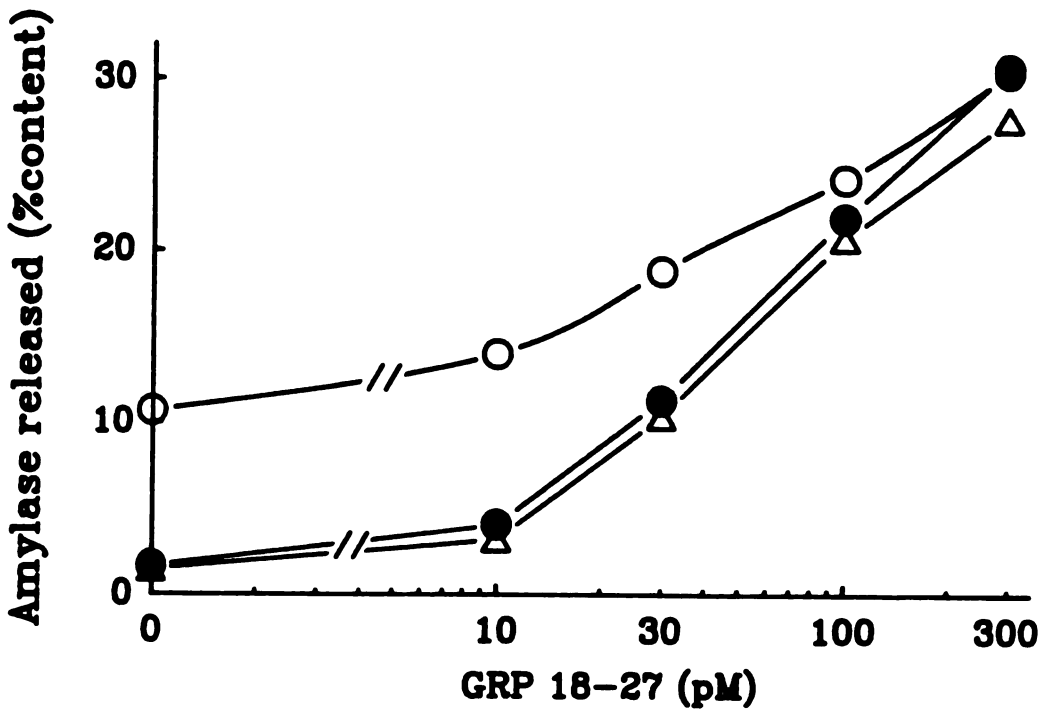

Figure 3 *CCK receptor antagonist L-364,718 blocks CCK-, but not GRP-stimulated amylase release from isolated pancreatic acini.* Isolated rat pancreatic acini were incubated with the indicated concentration of GRP in the absence (filled circles) or presence (open circles) of 5 pM CCK-8 for 30 minutes and the medium assayed for amylase released, expressed as % total content. Addition of 100 nM CCK receptor antagonist, L-364,718, blocked the effect of CCK-8 (open triangles). Each point represents mean of duplicates.

Half-life of CCK

Throughout this study stimulation of CCK secretion was assayed by measuring plasma CCK levels. Plasma levels, however, can change as a result of changes in the rate of secretion or changes in the rate of degradation or both. Preliminary studies were conducted to estimate the half-life of CCK in conscious rats. Fasted rats were infused intravenously with 300 ng/kg-hr CCK-8 for 30 minutes, and arterial blood was sampled just prior to stopping the infusion and during 0-1, 1-2 and 2-3 minutes after stopping the infusion. Samples were placed immediately on ice, plasma separated by centrifugation at 4°C and processed for bioassay of plasma CCK levels. Analysis of the fall in measured plasma CCK level resulted in a calculated half-life of CCK-8 in conscious rats of 0.6 ± 0.1 min (mean ± SE, n=4). Similar experiments with infusion of 390 ng/kg-hr or 450 ng/kg-hr CCK-8, which resulted in supraphysiologic plasma CCK levels, gave an estimated half-life of CCK-8 of 0.6 and 0.9 minutes, respectively (mean of 2 animals each). Thus the half-life of CCK-8 in conscious rats is extremely short and observed changes in plasma levels likely reflect primarily changes in the rate of secretion.

Statistical Analysis

All values are expressed as mean ± SE. All results were analyzed for differences by one-way analysis of variance (ANOVA) followed by Newman-Keuls (food and hormone studies) or Scheffe (neural studies) multiple comparison test using the CRISP statistical analysis program on an IBM PC (Miller, 1981).

Regulation of pancreatic gene expression

Animal preparation

Male Wistar rats (300-400 g, Charles River Laboratories, Wilmington, DE) were prepared with indwelling intraduodenal and intraperitoneal cannulas, as described above. Most animals, including all those receiving intravenous CCK-8, were prepared with additional arterial and venous cannulae. All cannulae were tunneled subcutaneously to exit from a 1-cm incision on the back near the base of the tail. Rats were maintained in modified Bollman cages for a total of 4 days and received either 5 mg/kg ampicillin and 10 mg/kg chloramphenicol or 50 mg/kg chloramphenicol daily for 3 days post-operatively. All animals had free access to water. An elemental diet of Vivonex (gift of Norwich Eaton Pharmaceuticals, Norwich, NY) was administered by continuous intraduodenal infusion (3.5 ml/hr) starting 18 hours after surgery. This diet was maintained throughout the entire study period and provided a daily caloric intake of 84 calories consisting of 0.27 g nitrogen, 19.4 g carbohydrate, and 0.12 g fat.

Elevation of plasma CCK levels

Endogenous CCK release was stimulated by adding crude soybean trypsin inhibitor (SBTI, type II, Sigma Chemical, St. Louis, MO) to the regular dose of Vivonex and perfusing the duodenum at a dose of 50 mg/h for 1, 4, 12, 24, or 48 hours prior to sacrifice. Plasma CCK levels were exogenously elevated by 24 hour intravenous infusion of 300 ng/kg-hr CCK-8 (gift of Squibb Institute, Princeton, NJ) dissolved in saline containing 1% bovine serum albumin (ICN Biomedicals, Naperville, IL). This dose resulted in plasma CCK levels similar to those achieved with SBTI administration.

Administration of CCK receptor antagonist

The specific CCK receptor antagonist, L-364,718, (Merck Sharp & Dohme, Rahway, NJ) was dissolved in dimethyl sulfoxide (100 mg/ml), diluted with water to 6 mg/ml, and administered as a bolus intraduodenal infusion (3mg/rat) every 12 hours starting 12 hours before SBTI feeding or CCK infusion. Control animals received the same dose of antagonist every 12 hours for 36 hours prior to sacrifice.

Collection of plasma for bioassay of CCK

For most studies, including all CCK infusions, blood was drawn through the arterial cannula at 2 ml/min into a heparinized syringe and collected into iced heparinized tubes. Otherwise trunk blood was collected into iced heparinized tubes. Blood was immediately centrifuged at 1000 x g for 10 minutes, plasma extracted and concentrated by adsorption onto Sep-Pak C-18 cartridges, and CCK assayed by bioassay as described.

Isolation and quantification of RNA

RNA was isolated by the method of Chirgwin *et al* (Chirgwin, 1979). Animals were killed by decapitation, and pancreata were removed, trimmed of fat and other tissue, and immediately lysed by high-speed homogenization with a Polytron in 10 ml of a buffer containing 4 M guanidine thiocyanate, 50 mM Tris(hydroxymethyl)aminomethane (pH 7.5), 10 mM EDTA, 0.5% sodium laurylsarcosine, and 0.1 M mercaptoethanol. RNA was separated from DNA and protein by centrifugation through cesium chloride (Glisin, 1974). Total RNA was phenol-chloroform extracted, ethanol precipitated, redissolved in distilled water, and quantified spectrophotometrically (A_{260}unit=40 μg RNA/ml).

Total RNA was qualitatively analyzed using Northern transfers. RNA was denatured, subjected to electrophoresis through 1% agarose in the presence of formaldehyde, and transferred to nitrocellulose exactly as described (Maniatis, 1982).

An RNA ladder (Bethesda Research Laboratories, Bethesda, MD) was used for size determination.

For quantitative comparisons, specific mRNA was measured using slot-blot analysis. RNA samples were denatured (1 μg/50 μl) in 50 % diethylpyrocarbonate-treated water, 30 % 20 X SSC (1 X SSC is 0.15 M sodium chloride and 0.015 M sodium citrate), and 20 % deionized formaldehyde by heating at 65°C for 15 min. After cooling on ice, denatured RNA samples were passed through a nitrocellulose filter equilibrated with 20 X SSC using a Schleicher and Schuell (Keene, NJ) slot blot apparatus (0.25, 0.5, or 1 μg RNA per slot). Filters were air dried then baked at 80°C in a vacuum oven for 2 hours. RNA was hybridized with the following plasmids: pKP39A, containing a 0.9 kilobase (kb) cDNA for rat pancreatic amylase (Logsdon, 1987); pCSp6-105, containing a 0.6 kb cDNA for rat pancreatic ribonuclease (MacDonald, 1982); pCSp33, containing a 0.35 kb cDNA for rat chymotrypsinogen B (Bell, 1984); pCXP4-78, containing a 0.7 kb cDNA for trypsinogen Ia, an anionic form of trypsinogen (Craik, 1984); pOD 48, containing a 1.5 kb cDNA for mouse kidney ornithine decarboxylase (ODC) (McConlogue, 1984); and pHF β-actin-3UT, containing a 2 kb cDNA for β-actin (Cleveland, 1980).

cDNAs were labeled with [α-^{32}P]dCTP (New England Nuclear, Boston, MA) using a random primer kit (Amersham, Arlington, VA). The specific activity of the probes was 1-2.5 x 10^9 cpm/μg. Filters were prehybridized at 42°C for 6-8 hours in 5-10 ml solution containing 5 X SSC, 50% formamide, 5 X Denhardt's reagent, 0.1% sodium dodecyl sulfate (SDS), and 100 μg/ml sonicated salmon sperm DNA. Hybridization was carried out at 42°C overnight with gentle mixing in 5 ml of the above solution containing the random primed probe (1-5 x 10^7 cpm/ml).

After hybridization, filters were washed in 4 X SSC with 0.1% SDS at 55°C for 30 min, then in 2 X SSC with 0.1% SDS at 55°C for 30 min, in 1 X SSC with 0.1% SDS at 55°C for 30 min, and finally in 0.1 X SSC with 0.1% SDS at 60°C for 30 min. Filters

were blotted dry and exposed to X-ray film, and bound radioactivity was determined by scanning with a densitometer (Hoeffer Scientific Instruments, San Francisco, CA). Slopes of the slot intensities of each dilution series were calculated for the linear portion of the curves by linear regression and used for comparisons.

Statistical Analysis

Differences in mRNA levels were analyzed by one-way analysis of variance (ANOVA) followed by a Dunnet multiple comparison test using the CRISP statistical analysis program run on an IBM PC. mRNA values are expressed as percent of the average control value. All values are given as mean ± SE.

Results

Nutrient Stimulation of Cholecystokinin Release

To determine which components of food are intestinal stimulants for CCK release, conscious rats were perfused intraduodenally with various nutrients. Duodenal infusion of casein (500 mg/hr) resulted in elevation of plasma CCK from the fasting level of 0.8 ± 0.1 to 4.1 ± 0.8 pM within four minutes. Plasma CCK remained elevated at 4.0 ± 0.6 pM at 10 minutes and then declined to 1.8 ± 0.4 pM after 80 minutes of continuous infusion (Fig. 4). This amount and rate of casein infusion resulted in an approximately half-maximal CCK response. Duodenal infusion of soybean trypsin inhibitor (140 mg/hr), a well-established stimulus for CCK release, increased plasma CCK levels to 8.6 ± 1.6 pM (n=9), compared to casein infusion which increased plasma CCK from 0.5 ± 0.05 (n=39) to only 3.8 ± 0.4 (n=38). Intact protein, and not its component amino acids, is the component of the casein solution (infused as calcium caseinate) which stimulates CCK release since perfusion of the duodenum for 10 or 40 minutes with the equivalent amount of amino acids present in the casein solution (casein hydrolysate) failed to elevate plasma CCK levels above fasting (Fig. 5). Infusion of the equivalent amount of calcium present in the casein solution (as calcium chloride) also had no effect on plasma CCK (Fig. 5).

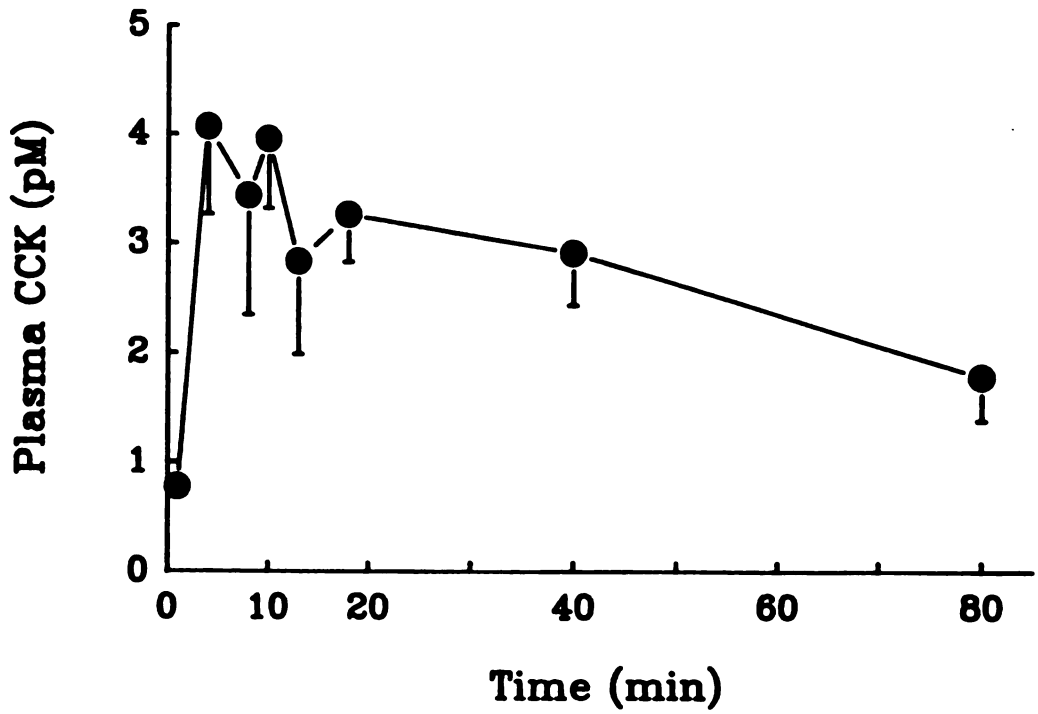

Figure 4 *Intraduodenal casein stimulates CCK release.* Plasma CCK was measured as a function of the duration of duodenal casein infusion (500 mg/hr). Plasma CCK was measured by bioassay and expressed as pM CCK-8 equivalents. Each point represents the mean ± SE of 5-8 experiments.

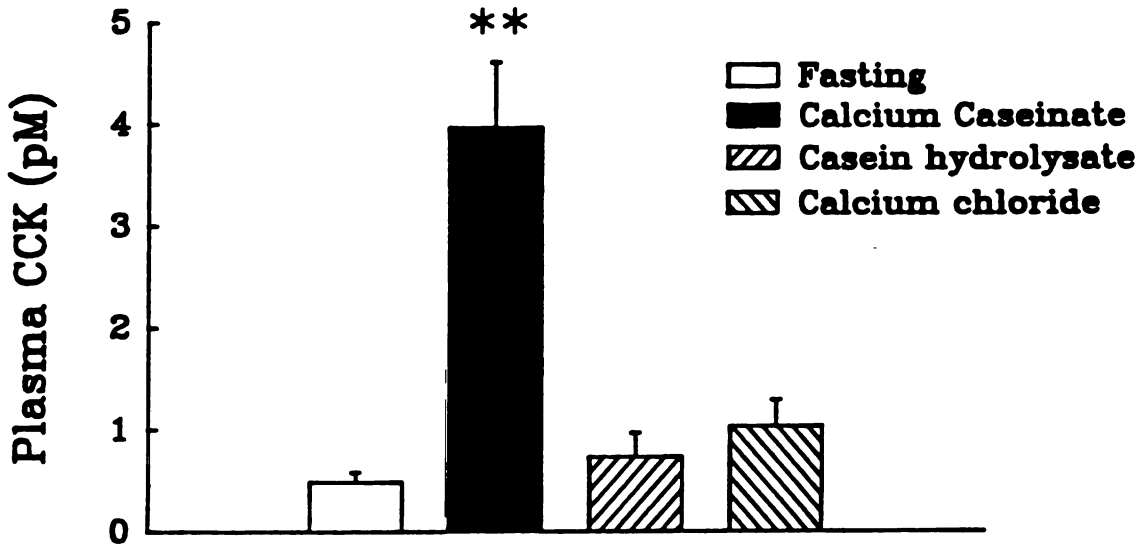

Figure 5 *Intact protein stimulates CCK release.* Plasma CCK was measured in fasted animals infused intraduodenally for 10 minutes with casein (500 mg/hr, n=9), casein hydrolysate (500 mg/hr, n=3) or calcium chloride (62.5 mM, equivalent to the amount of calcium in the casein solution, n=6). Each bar represents mean ± SE. **, $p<0.01$ versus fasting

The fatty acid oleate stimulates pancreatic secretion in the conscious rat (Fukoaka, 1987, Miyasaka, 1988) and is a classical stimulus for CCK release in other species (Go, 1970, Meyer, 1974, Watanabe, 1988); we therefore investigated the effect of oleate on plasma CCK levels. Duodenal infusion of oleate for 10 minutes elevated plasma CCK levels from 0.5 ± 0.1 to 3.6 ± 0.6 pM; similar results were seen with a 40 minute infusion (Fig. 6). Infusion of intact fat (Lipomul, an emulsion of primarily corn oil) had a small (1.4 ± 0.4 pM), but nonsignificant effect on plasma CCK levels after 10 minutes, and a slightly larger (2.1 ± 1.0 pM), but still nonsignificant, effect after 40 minutes of continuous infusion (Fig. 6). Infusion of glucose had no effect (Fig. 6).

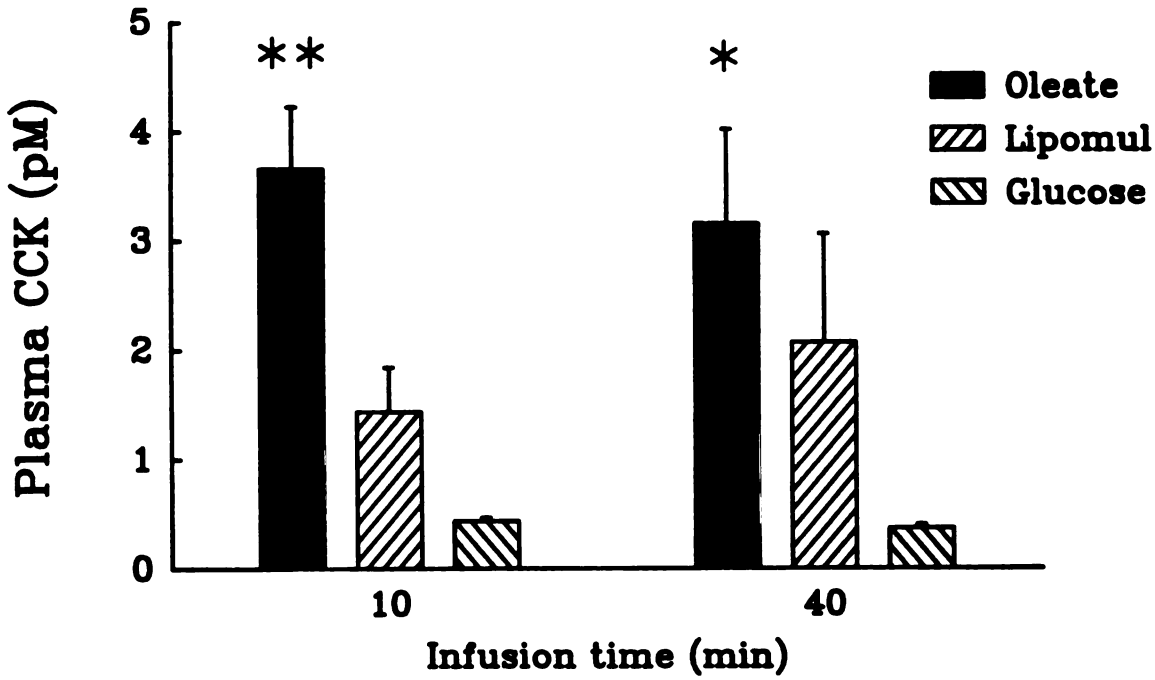

Figure 6 *Fatty acid stimulates CCK release.* Plasma CCK was measured in fasted animals infused intraduodenally for 10 or 40 minutes with oleate (2 mmole/hr, pH 7.2), Lipomul (2.5 mmole/hr), or glucose (2.7 mmole/hr). Each bar represents mean ± SE of 3-5 experiments. *, $p<0.05$, **, $p<0.01$ vs. fasting [Fasting plasma CCK = 0.5 ± 0.1 pM, n=9]

Hormonal Regulation of Protein-Stimulated CCK Release

To study the hormonal regulation of CCK secretion, CCK release was stimulated with an intraduodenal infusion of casein, while hormones were infused intravenously. Since somatostatin inhibits numerous functions in the gastrointestinal tract and the release of several hormones (Colturi, 1984, Yamada, 1987), we investigated the effect of somatostatin on protein-stimulated CCK release. Intravenous infusion of somatostatin resulted in a dose-dependent inhibition of casein-stimulated plasma CCK from 3.7 ± 0.7 pM to 1.5 ± 0.2 pM and 0.9 ± 0.3 pM at the dose of 1 and 10 μg/kg-hr, respectively (Fig. 7).

Peptide YY is a hormone released from the distal intestine which inhibits pancreatic secretion and has been postulated to act in part by inhibiting CCK release (Pappas, 1985, Lluis, 1988). We therefore investigated the effect of PYY on protein-stimulated CCK release. Intravenous infusion of peptide YY at the dose of 0.2 or 2 μg/kg-hr, doses previously shown to inhibit pancreatic secretion in anesthetized rats (Putnam, 1989) and conscious dogs (Pappas, 1985, Lluis, 1988), had no effect on the plasma CCK response to casein stimulation. A ten-fold higher dose, however, increased plasma CCK levels in this group of animals from 2.7 ± 0.6 to 6.5 ± 1.0 pM (Fig. 8). In view of this surprising result, we tested the effect of this dose in the absence of duodenal casein. Intravenous infusion of 20 μg/kg-hr PYY had no effect on fasting plasma CCK concentration (0.5 ± 0.03 pM, n=3).

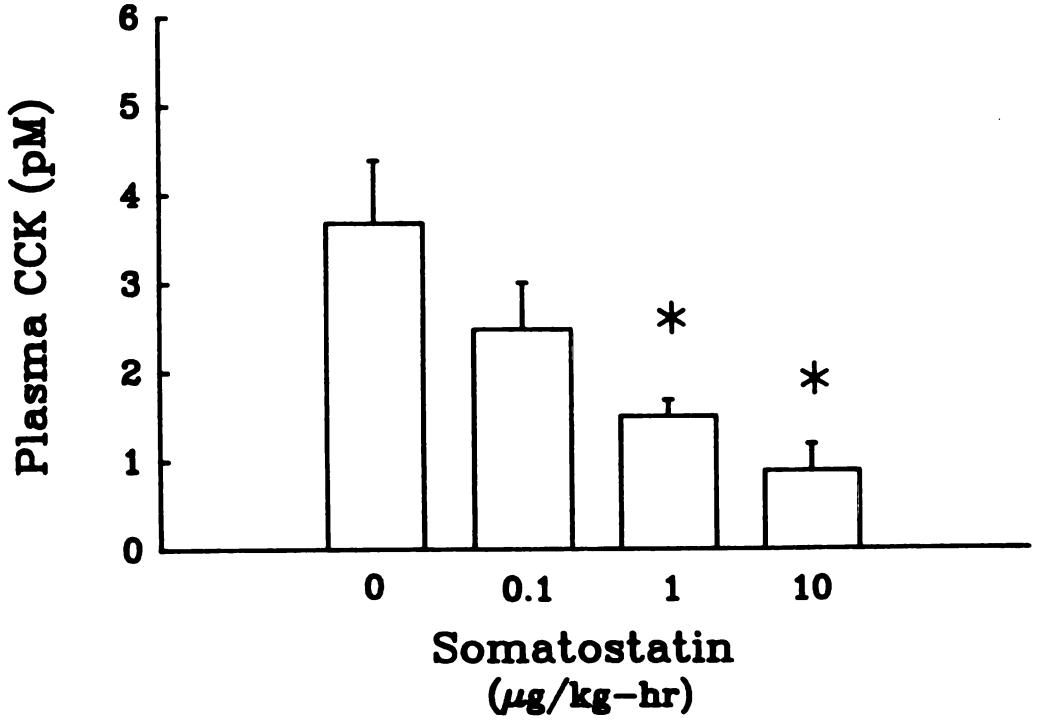

Figure 7 *Somatostatin inhibits casein-stimulated CCK release.* Plasma CCK was measured in rats infused with intraduodenal casein for the final 10 minutes of a 15 minute intravenous infusion with 0.1, 1, or 10 μg/kg-hr somatostatin. Each bar represents mean ± SE of 5-6 experiments. *, $p<0.05$ vs. casein alone

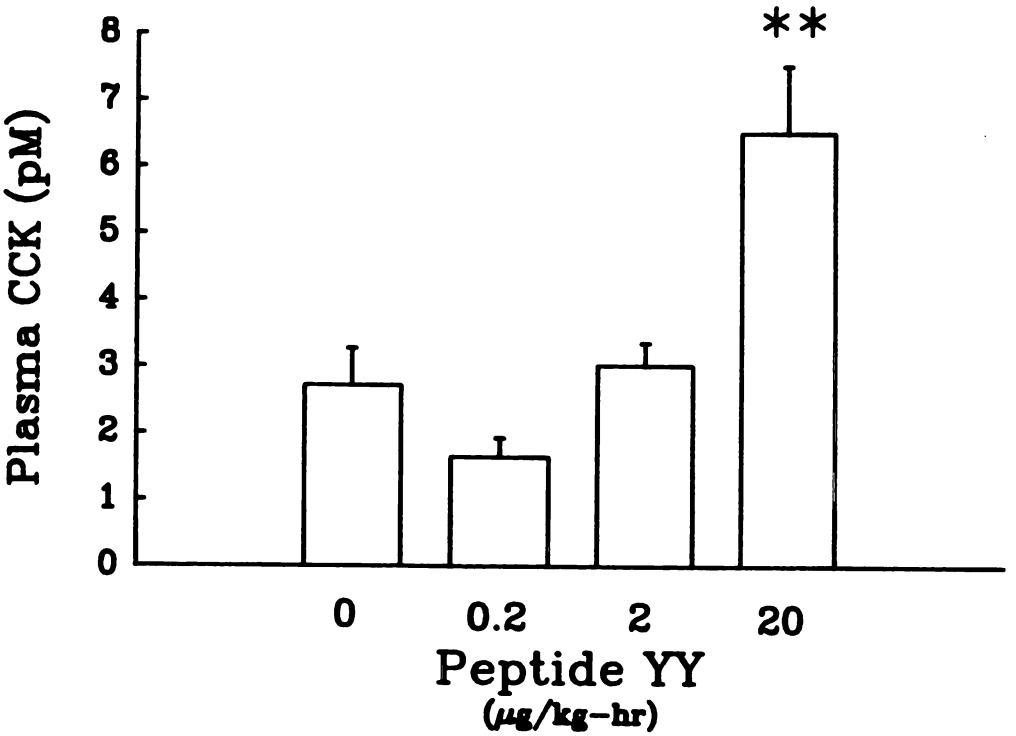

Figure 8 *Effect of peptide YY on casein-stimulated CCK release.* Plasma CCK was measured in rats infused with intraduodenal casein for the final 10 minutes of a 15 minute intravenous infusion with 0.2, 2, or 20 µg/kg-hr peptide YY. Each bar represents the mean ± SE of 5-7 experiments. **, $p<0.01$ vs. casein alone

Neural Regulation of Basal CCK release

To study the role of vagal pathways in the regulation of basal CCK release, fasted animals were treated with 2-D-deoxyglucose (2-DG). This nonmetabolizable glucose analog stimulates vagal discharge by inducing cytoglucopenia in the central nervous system (Brown, 1962, Roze, 1977). Vagal stimulation induced by 2-DG had no effect on fasting plasma CCK release (Fig. 9). To study the role of enteric cholinergic innervation, fasted animals were treated with bethanechol, a cholinergic agonist, or atropine, a muscarinic receptor antagonist. Neither of these agents had an effect on fasting plasma CCK levels (Fig. 9). The role of peptidergic innervation was investigated by treating rats with gastrin-releasing peptide (GRP). This neurotransmitter stimulates the release of several hormones and numerous gastrointestinal functions, including pancreatic secretion (McDonald, 1981, Miyata, 1980). Intravenous infusion of GRP elevated plasma CCK levels from 0.4 ± 0.1 to 1.6 ± 0.1 pM, in the absence of any food in the duodenum (Fig. 9).

Neural Regulation of Protein-Stimulated CCK release

The same agents used to investigate the role of neural pathways in the regulation of basal CCK release were then evaluated for their effect on protein-stimulated CCK release. Neither stimulation of vagal discharge by administration of 2-D-deoxyglucose, nor infusion of GRP or atropine had an effect on casein-stimulated plasma CCK levels. Treatment with bethanechol significantly inhibited casein-stimulated plasma CCK from 3.9 ± 0.6 to 1.3 ± 0.3 pM (Fig. 10).

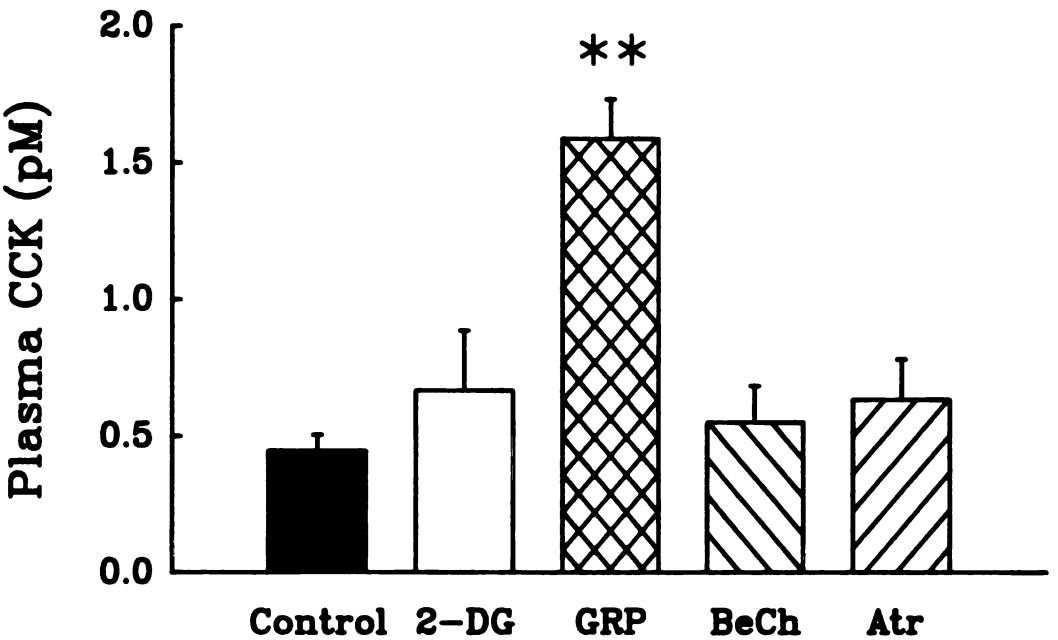

Figure 9 *Neural regulation of basal CCK release.* Plasma CCK was measured in fasted rats (solid bar, n=11) or rats treated with 2-D-deoxyglucose (100 mg/kg, blank bar, n=6), gastrin-releasing peptide (1 μg/kg-hr, hatched bar, n=8), bethanechol (100 μg/kg-hr, right stripes, n=6), or atropine (100 μg/kg-hr, left stripes, n=6). Each bar represents the mean ± SE for each treatment. **, $p<0.01$ vs. fasting

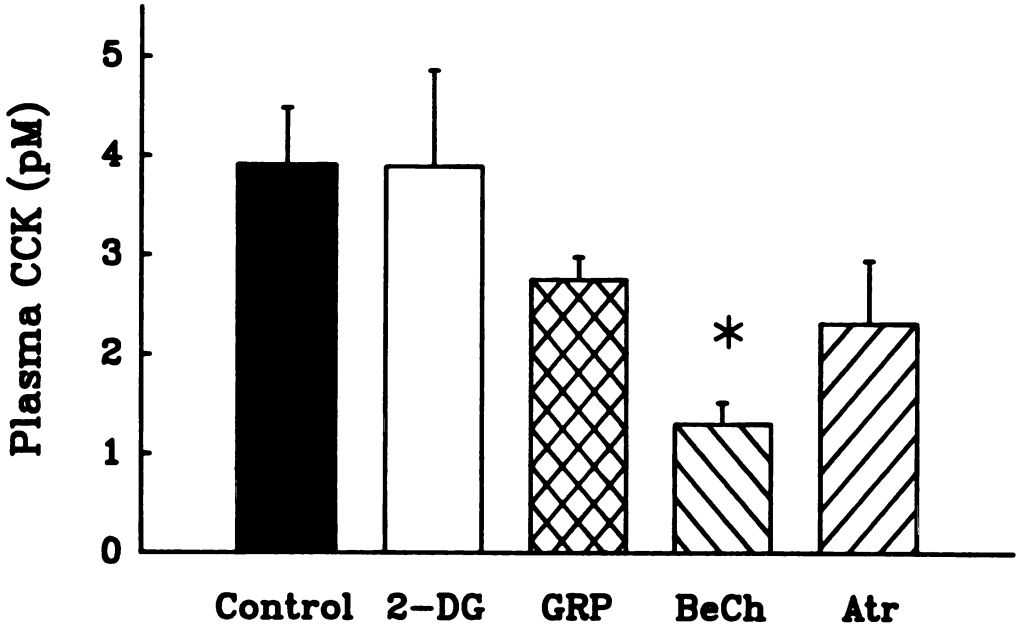

Figure 10 *Neural regulation of casein-stimulated CCK release.* Plasma CCK was measured in rats receiving intraduodenal casein only (solid bar, n=28) or intraduodenal casein and intravenous 2-D-deoxyglucose (100 mg/kg, blank bar, n=9), gastrin-releasing peptide (1 μg/kg-hr, hatched bar, n=8), bethanechol (100 μg/kg-hr, right stripes, n=13), or atropine (100 μg/kg-hr, left stripes, n=9). Each bar represents the mean ± SE for each treatment. *, $p<0.05$ vs. casein alone

Discussion

Previous studies of CCK secretion in rats have used indirect methods of intestinal stimulation, anesthetized, acutely prepared, or restrained animals, and sometimes indirect or imprecise assays for CCK release. We have developed a model to study the regulation of CCK release in conscious, unrestrained rats which permits simultaneous duodenal and intravenous infusion, as well as arterial blood sampling. Direct duodenal perfusion eliminates any complications of intragastric administration of food including different rates of emptying or triggering of gastric reflexes, while arterial blood sampling produces non-hemolyzed, uncontaminated plasma samples and also permits repeated experiments in a given animal. This model maintains the animals in as physiologically normal conditions as possible in order to minimize any extraneous effects resulting from anesthesia, or stress due to restraint or manipulation.

Using direct duodenal perfusion with various nutrients, this study demonstrates that intact protein, but not the component amino acids, stimulates CCK release in the rat. This finding confirms the results of previous studies which demonstrated CCK release in response to intragastric administration of protein (Liddle, 1986b, Douglas, 1988) and extends the finding to direct duodenal stimulation. It has become increasingly clear that protein stimulates pancreatic secretion in the rat via a negative feedback loop in which decreased duodenal trypsin activity due to pancreatic juice diversion, inactivation with trypsin inhibitors, or the presence of excess substrate (in this case casein) stimulates pancreatic secretion (Green, 1983). This regulatory loop is mediated, at least in part, by CCK release (Liddle, 1984, Louie, 1986, Shiratori, 1986a, Folsch, 1987). Although the precise mechanism for protein stimulation of CCK release is not known, it may be mediated by a trypsin-sensitive peptide present in the intestinal lumen which stimulates CCK release (Iwai, 1988, Lu, 1989).

This study also demonstrates, for the first time in the rat, CCK release in response to the fatty acid oleate. Previous studies, in contrast, have found only a small or no increase in plasma CCK levels after a bolus intragastric administration of oleate (Liddle, 1986b, Douglas, 1988). Since CCK has been shown to inhibit gastric emptying in the rat (Dektor, 1988, Green, 1988), it is possible that gastric administration of oleate initially releases CCK which then inhibits gastric emptying, significantly reducing further entry of oleate into the duodenum, and thereby resulting in only a small increase in plasma CCK levels. The present study, in contrast, utilized direct duodenal perfusion, bypassing any indirect effects of gastric administration, and clearly demonstrated oleate-stimulated CCK release. This result further suggests that stimulation of pancreatic secretion observed after intraduodenal administration of oleate (Fukoaka, 1987, Miyasaka, 1988) is mediated by release of CCK.

Although the mechanism by which fatty acids stimulate CCK release is not known, the observation that intact fat does not stimulate CCK release suggests that the mechanism does not involve a fat:lipase feedback loop analogous to the protein:trypsin feedback loop discussed above. The small, but nonsignificant increase in plasma CCK levels observed in response to intact fat may reflect partial hydrolysis of the fat and release of stimulatory fatty acid. A similar study of CCK release in the dog concluded that fat must be hydrolyzed in order to stimulate CCK release (Watanabe, 1988). Intestinal perfusion with glucose had no effect on plasma CCK levels, consistent with the results obtained with intragastric administration demonstrating no effect of glucose or starch on plasma CCK in the rat (Liddle, 1986b). Thus this study has shown directly that the major intestinal stimuli for CCK release are intact protein and fatty acids. Since the typical rat chow diet consists of 20% protein and little or no fatty acid, the primary physiologic dietary stimulus for CCK release is protein.

To determine how the response to the primary dietary stimulus for CCK release is further modulated by hormones, rats were infused simultaneously with casein and either somatostatin or PYY, two hormones which inhibit pancreatic secretion (Kohler, 1986, Gullo, 1987, Gyr, 1987, Pappas, 1985, Inoue, 1988, Konturek, 1988, Lluis, 1987, Putnam, 1989). Since somatostatin inhibits both gastric acid secretion and gastrin release (Yamada, 1987), by analogy inhibition of pancreatic secretion could be mediated by a direct action on acinar cells as well as by inhibition of CCK secretion. Although pancreatic acini have functional somatostatin receptors, somatostatin has no effect on basal or CCK-stimulated amylase release from isolated acini (Esteve, 1983, Sakamoto, 1984). Thus the mechanism for somatostatin inhibition of pancreatic secretion is unclear.

The results of this study demonstrate dose-dependent somatostatin inhibition of protein-stimulated CCK release in the rat. That is, ingestion of protein stimulates CCK release, but circulating somatostatin can decrease the CCK response. Somatostatin has also been shown to inhibit bombesin-stimulated CCK release in man (DeJong, 1987). Thus somatostatin may inhibit pancreatic secretion by inhibition of CCK release analagous to the inhibition by somatostatin of both gastrin release and gastric acid secretion. Somatostatin thereby provides an important mechanism for modulation of plasma CCK levels as well as integration of the entire post-prandial hormonal and secretory response.

The mechanism by which somatostatin inhibits CCK release is not known. The dose of somatostatin which inhibited protein-stimulated CCK release in this study and bombesin-stimulated CCK release in the human study (Ibid.) is 10-20 times higher than the dose which reproduced postprandial circulating levels of somatostatin and inhibited insulin and glucagon release in man (Colturi, 1984, Annibale, 1987). It is also higher than the dose required to inhibit pancreatic secretion in man and dogs (Kohler, 1986,

Gullo, 1987, Gyr, 1987). Thus the inhibition of CCK release by somatostatin is not likely to be a physiologic endocrine effect.

Somatostatin may act in a paracrine fashion similar to the interaction between gastric D (somatostatin) and G (gastrin) cells which have been shown by immunocytochemistry to be closely connected by cytoplasmic processes from the D cell terminating on the G cell (Solcia, 1987, Yamada, 1987). Somatostatin has also been shown to inhibit release of gastrin, neurotensin and enteroglucagon from isolated enteroendocrine cell preparations suggesting a possible similar direct action of somatostatin on the CCK cell (Barber, 1987, Giraud, 1987, Sugano, 1987). Additional dose-response, passive immunization, and somatostatin antagonist studies utilizing isolated duodenum or isolated CCK cells combined with careful ultrastructural investigation of the anatomical relationship between CCK cells and somatostatin cells in the duodenal mucosa will further elucidate the mechanism of somatostatin inhibition of CCK secretion.

Peptide YY inhibition of pancreatic secretion could also be mediated in part by inhibition of CCK secretion, since PYY has no known direct effect on acinar cells. In the present study , however, PYY did not inhibit protein-stimulated CCK release. Although the physiologically relevant dose of PYY in the rat is unclear, the middle dose used in this study (2 μg/kg-hr) is similar to that previously used to inhibit pancreatic secretion in anesthetized rats (Putnam, 1989), and to inhibit both pancreatic secretion and CCK release in conscious dogs (Pappas, 1985, Lluis, 1988). Thus the rat may differ from the dog in that the inhibition of pancreatic secretion observed in response to colonic instillation of oleate and attributed to PYY is not mediated by inhibition of CCK release. A ten-fold higher dose of PYY (20 μg/kg-hr) actually potentiated protein-stimulated plasma CCK levels, while having no effect on basal CCK levels. The observed potentiation may reflect marked inhibition of pancreatic secretion, resulting in reduced

duodenal trypsin activity, and increased CCK release via the feedback loop. From these results, we conclude that PYY has no direct role in the modulation of CCK release.

Little is known about the role of the vagus nerve in regulating CCK release. Stimulation of vagal firing by administration of the non-metabolizable 2-D-deoxyglucose has been shown to stimulate pancreatic secretion in the anesthetized rat (Roze, 1977, Putnam, 1989) and could exert its effect in part by stimulating CCK release. Administration of 2-D-deoxyglucose in the present study, however, did not stimulate CCK release in the conscious, fasted rat; nor did it alter protein-stimulated plasma CCK levels. These results are consistent with the observation that truncal vagotomy has no effect on basal or tryptophan-stimulated CCK release in the dog (Singer, 1989). The current results differ, however, from those obtained using direct, electrical stimulation of the vagus in anesthetized pigs (Cantor, 1986b), which clearly showed vagally-stimulated CCK release. It is not clear if this difference is due to the method of vagal stimulation, anesthesia, or species differences. In conscious rats, however, basal release of CCK does not appear to be controlled by the vagus, and vagal stimulation does not augment or diminish protein-stimulated CCK release. Thus, vagal stimulation of pancreatic secretion is probably direct, and does not involve CCK release.

Cholinergic agonists stimulate many functions in the gastrointestinal tract including pancreatic secretion, yet the role of cholinergic pathways in the regulation of CCK release is unclear. Some studies suggest that the protein:trypsin negative feedback loop is atropine sensitive (Louie, 1986), indicating cholinergic control of CCK release at some level. This effect, however, is not always observed (Levan, 1986a, Katoaka, 1988). In the present study, administration of bethanechol, a cholinergic agonist, or atropine, muscarinic antagonist, had no effect on basal CCK release. Protein-stimulated CCK release, however, was inhibited by bethanechol, while unaffected by atropine treatment. This is consistent with the results of previous studies demonstrating no effect of atropine

on casein- or oleate-stimulated pancreatic secretion (Levan, 1986a, Fukoaka, 1987). The observed inhibition of CCK release in response to bethanechol may be a direct action on the CCK cell, as suggested by a study demonstrating carbachol inhibition of tryptophan-stimulated CCK release from a partially, purified preparation of isolated canine CCK cells (Barber, 1986). Alternatively, since bethanechol stimulates pancreatic secretion directly, the presence of increased trypsin activity in the duodenum may attenuate the strength of the protein stimulus and thereby reduce the CCK response. We cannot currently distinguish these possibilites. In any case, cholinergic pathways do not appear to play a major role in the regulation of CCK release in the rat.

Gastrin-releasing peptide (GRP), mammalian bombesin, stimulates the release of several gastrointestinal hormones, as well as several gastrointestinal functions including pancreatic secretion (McDonald, 1981, Hosotani, 1987a). The results of this study demonstrate that GRP stimulates basal CCK release in the rat. This result is consistent with findings in other species including man (DeJong, 1987), dog (Miyata, 1980), and pig (Cantor, 1987a). On the other hand, GRP did not potentiate protein-stimulated CCK release; in fact, it decreased plasma CCK levels in response to duodenal casein. Although the decrease was not statistically significant, it is suggestive, and may reflect direct stimulation of pancreatic secretion by GRP leading to increased trypsin activity in the duodenum and interaction with the feedback loop as suggested for bethanechol stimulation.

GRP clearly stimulates CCK release in the absence of food in the duodenum. Although the mechanism for GRP stimulation of CCK release is not known, the significance of this result is the possibility for feed-forward control of CCK release mediated by the extensive peptidergic innervation of the gastrointestinal tract. GRP-containing neurons have been demonstrated by immunocytochemistry in the small intestine of several species including rat, pig and rabbit (Moghimzadeh, 1983, Keast, 1987). GRP fibers are numerous in the myenteric plexus and smooth muscle layer

of the rat duodenum ; a few fibers are present in the mucosa (Moghimzadeh, 1983). In addition to the presence of GRP neurons, specific binding of GRP has also been demonstrated in the rat duodenum (Moran, 1988). Thus GRP could act as a neurotransmitter to stimulate CCK release from the duodenum. Additional studies of GRP-stimulated CCK release from isolated CCK cells combined with ultrastructural studies describing the anatomical relationship between CCK cells and GRP nerve endings will help elucidate the mechanism for GRP-stimulated CCK release.

In conclusion, intact protein and fatty acids stimulate CCK release in the conscious fasted rat. Gastrin-releasing peptide also stimulates CCK release in the absence of food, while having no effect on protein-stimulated plasma CCK levels, suggesting a possible mechanism for feed-forward neural regulation of CCK release. Protein-stimulated release is inhibited by somatostatin and bethanechol, thus providing mechanisms for post-prandial modulation of the response to dietary stimuli.

REGULATION OF PANCREATIC GENE EXPRESSION

Results

Plasma CCK levels in response to SBTI administration and CCK infusion

To ensure uniform nutrition, rats were infused for 72 hours with an elemental diet (Vivonex) via an indwelling intraduodenal tube. This diet did not elevate plasma CCK (0.7 ± 0.2 pM, n=6) as predicted from the results of the regulation of CCK secretion studies. To stimulate endogenous CCK secretion, soybean trypsin inhibitor (SBTI) was added to the elemental diet and continuously infused into the duodenum at 50 mg/hr for up to 48 hour. Plasma CCK was increased to 6.9 ± 1.0 pM (n=8, $p<0.01$) after 1 hour of infusion with SBTI, and remained similarly elevated for up to 48 hours of infusion (Fig. 11). Similar plasma CCK levels were achieved with exogenous intravenous infusion of 300 ng/kg-hr CCK-8 for 24 hours (6.0 ± 0.9 pM, n=5).

Effect of SBTI administration on pancreatic ODC mRNA level

Polyamines facilitate nearly all aspects of DNA, RNA, and protein synthesis (Pegg, 1982) and are thought to be closely involved in the regulation of growth. Ornithine decarboxylase (ODC) is the enzyme which catalyzes the rate limiting step in the biosynthesis of the polyamines putrescine, spermidine, and spermine (Luk, 1987). Therefore, to investigate the physiologic role of CCK in the stimulation of pancreatic growth via induction of ornithine decarboxylase (ODC), changes in the amount of ODC mRNA in the pancreas were investigated using a cDNA probe for mouse kidney ODC. To monitor the quality of extracted RNA and specific hybridization of the cDNA probe, total pancreatic RNA was electrophoresed on 1% agarose gels, transferred to nitrocellulose, and hybridized with the labeled cDNA probe. Northern analysis of total RNA from control animals and animals infused with SBTI demonstrated an SBTI-stimulated increase in ODC mRNA levels (Fig. 12).

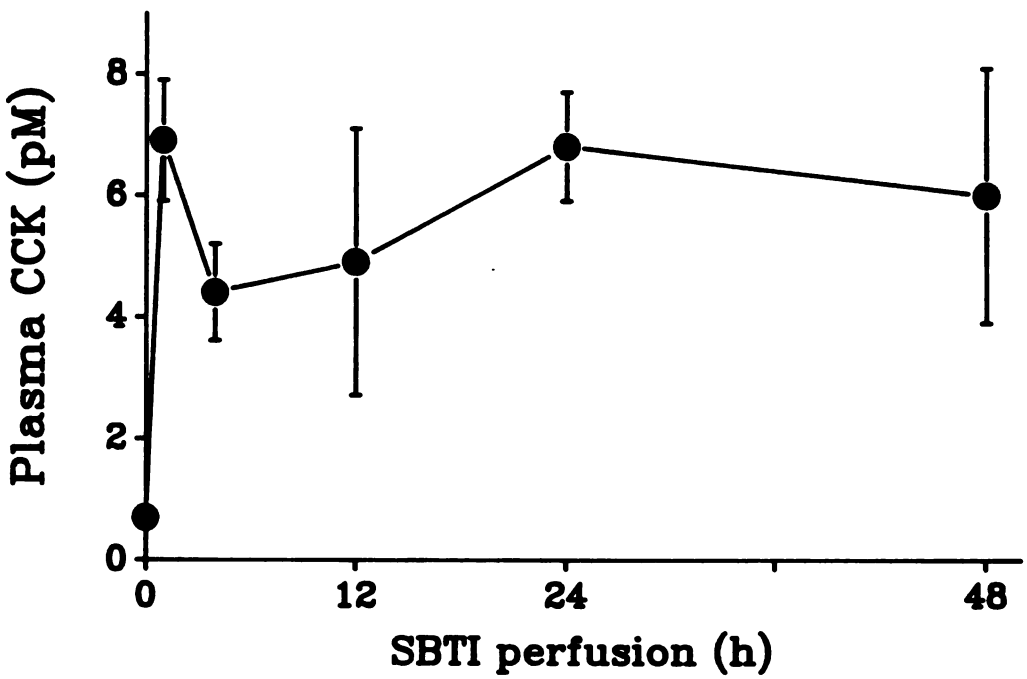

Figure 11 *Soybean trypsin inhibitor (SBTI) administration increases plasma CCK.* Rats were perfused intraduodenally for 72 hours with an elemental diet (Vivonex) to which soybean trypsin inhibitor was added for 1, 4, 12, 24, or 48 hours. Plasma CCK levels were determined by bioassay. Data shown represent mean ± SE (n=3-8 animals for each point).

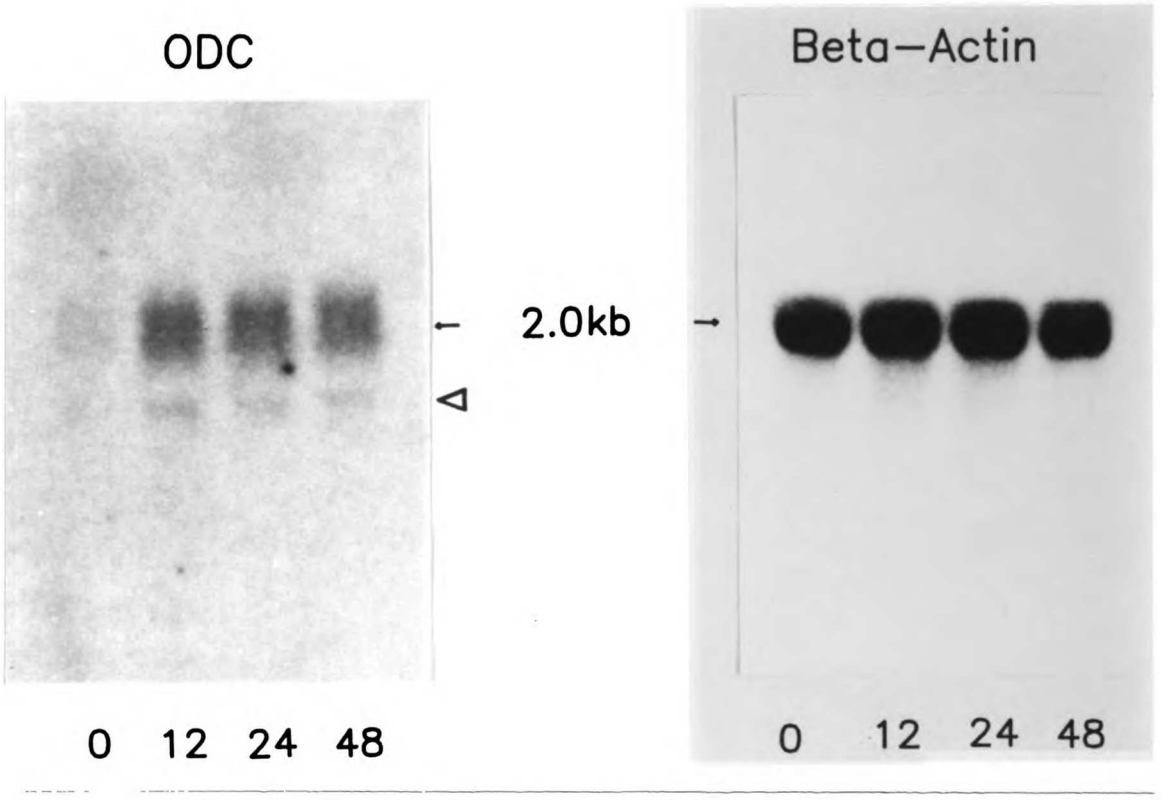
ODC
Beta–Actin
2.0kb
0 12 24 48
0 12 24 48

Figure 12 *Northern blot analysis of pancreatic ornithine decarboxylase (ODC) mRNA.* Rats were infused intraduodenally with soybean trypsin inhibitor (SBTI) for 0, 12, 24, and 48 hours. Total cellular pancreatic RNA was extracted, denatured, separated on a 1% agarose-formaldehyde gel, transferred onto nitrocellulose filters and hybridized with cDNA for ODC or β-actin. Molecular sizes were determined by a parallel mRNA ladder stained with ethidium bromide.

Actin mRNA, used as an internal control did not change during the course of the SBTI infusion. The ODC cDNA probe hybridized to a major class of mRNA of approximately 2.0 kb. Hybridization to a smaller species was also observed (arrowhead , Fig. 12); this smaller mRNA species has been observed for ODC mRNA in other tissues and is a result of different polyadenylation (Katz, 1988, McConlogue, 1984). Changes in mRNA levels after 1, 4, 12, 24, or 48 hour of SBTI treatment were quantified by slot-blot hybridization. mRNA for ODC increased to 191 ± 28% of control after 4 hours of SBTI infusion and remained elevated at about 200% for up to 48 hours (Fig. 13).

Effect of SBTI administration on pancreatic digestive enzyme mRNA levels

Changes in pancreatic digestive enzyme mRNA levels in response to administration of trypsin inhibitor were monitored using cDNA probes for rat amylase, ribonuclease, chymotrypsinogen B, and trypsinogen Ia. Qualitative changes in digestive enzyme mRNA levels after 48 hours of trypsin inhibitor treatment were observed by Northern analysis: mRNA levels of trypsin and chymotrypsin increased, while amylase and ribonuclease appeared to decrease slightly (Fig. 14). Changes in mRNA levels after 1, 4, 12, 24 or 48 hours of SBTI administration were quantified by slot-blot hybridization (Fig. 15). The mRNA levels of the serine proteases trypsinogen Ia and chymotrypsinogen B increased throughout the SBTI infusion, reaching 502 ± 90% and 431 ± 40% of control, respectively, after 48 h ($n=4$, $p<0.01$). Amylase mRNA levels, in contrast, were not significantly affected at any time (88 ± 9% of control at 24 h, $n=8$), while ribonuclease mRNA was significantly decreased to 53 ± 8% of control only after 24 h ($n=8$, $p<0.05$).

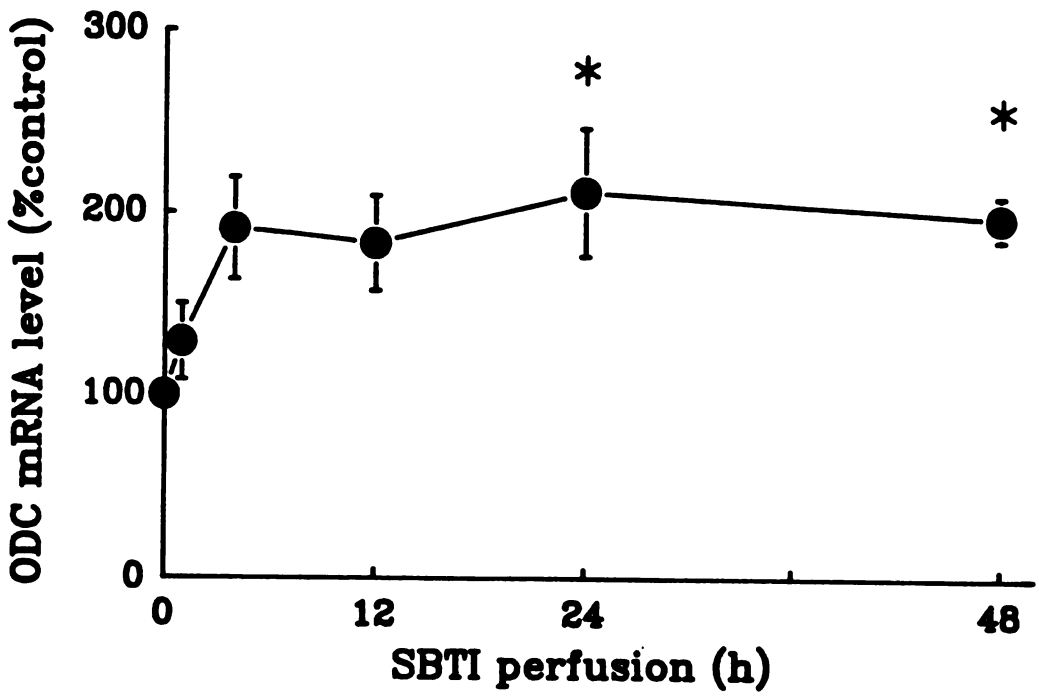

Figure 13 *Soybean trypsin inhibitor (SBTI) administration increases pancreatic ornithine decarboxylase (ODC) mRNA level.* Rats received continuous intraduodenal perfusion of trypsin inhibitor for indicated time periods. Pancreata were then removed and total cellular RNA extracted, denatured, slotted onto nitrocellulose filters, and hybridized with labeled cDNA for ODC. Slot blots were quantified by densitometry. Values obtained were then expressed as percent of untreated controls. Data shown represent mean ± SE for 3-4 animals at each time point. *,$p<0.05$ versus control.

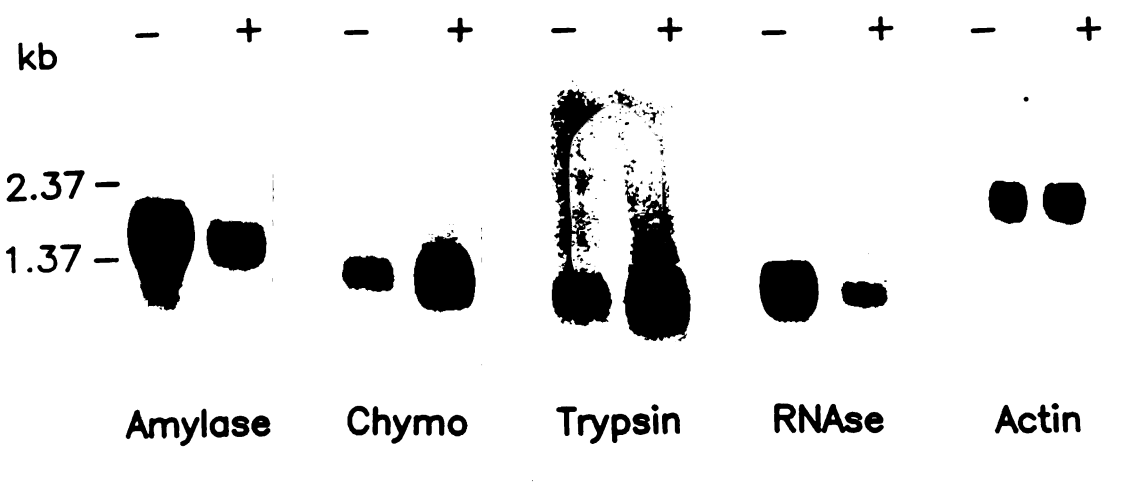
kb
– + – + – + – + – +
2.37 –
1.37 –
Amylase
Chymo
Trypsin
RNAse
Actin

Figure 14 *Northern blot analysis of pancreatic digestive enzyme mRNA.* Total cellular pancreatic RNA was extracted, denatured, separated on a 1% agarose-formaldehyde gel, transferred onto nitrocellulose filters and hybridized with cDNAs for digestive enzymes and β-actin. Shown are mRNA levels of amylase, chymotrypsinogen B, trypsinogen Ia, ribonuclease and β-actin in control rats (–) or rats perfused with trypsin inhibitor for 48 hours (+). Numbers on left indicate molecular size in kilobases as determined by a parallel mRNA ladder.

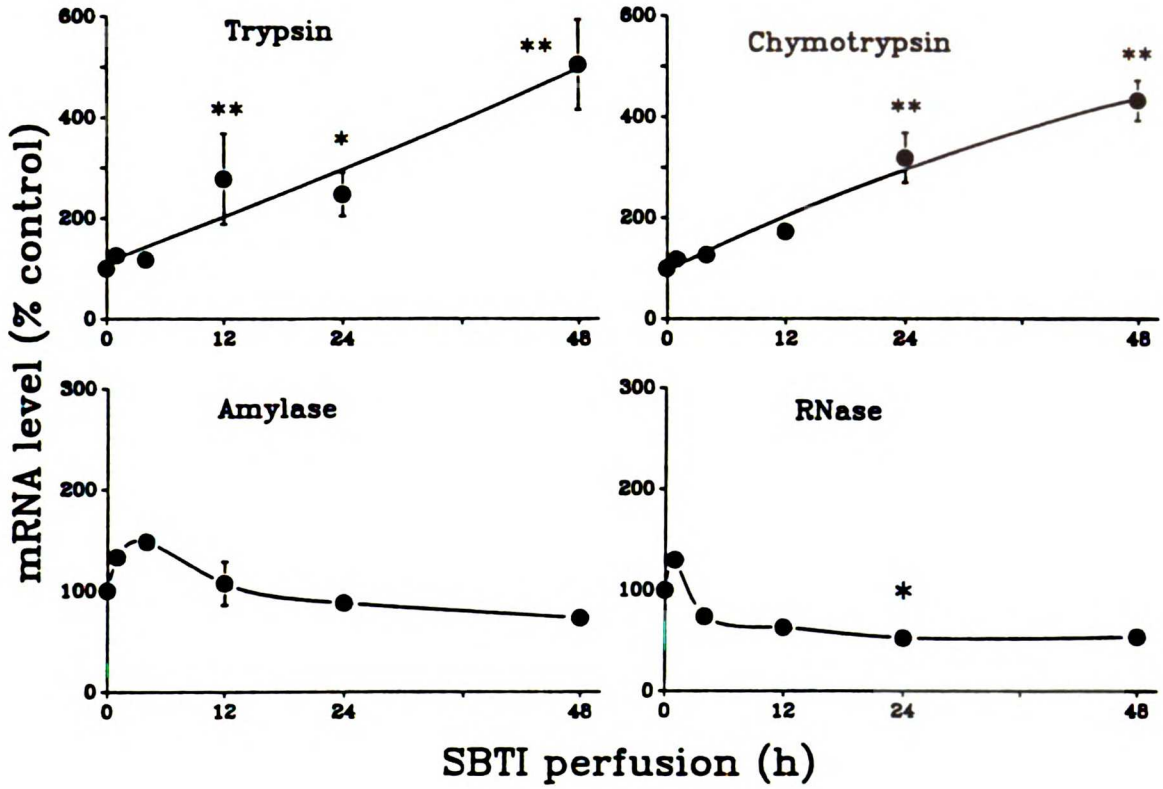

Figure 15 *Effects of soybean trypsin inhibitor (SBTI) administration on digestive enzyme mRNA levels.* Animals received continuous intraduodenal perfusion of trypsin inhibitor for indicated time periods. Pancreata were then removed and total cellular RNA extracted, denatured, slotted onto nitrocellulose filters, and hybridized with labeled cDNAs for trypsinogen Ia, chymotrypsinogen B, amylase, and ribonuclease. Slot blots were quantified by densitometry and the values obtained expressed as percent of untreated controls. Data shown represent mean ± SE for 3-8 animals at each time point. *,$p<0.05$, **,$p<0.01$ versus control.

Effect of CCK infusion on ODC and pancreatic digestive enzyme mRNA levels

Intravenous infusion of CCK for 24 hours reproduced the effects of 24 hour SBTI treatment on both ODC and digestive enzyme mRNA levels (Fig. 16). ODC mRNA level was increased to 256 ± 37%. Similarly, trypsinogen and chymotrypsinogen mRNA levels were increased to 340 ± 9% and 393 ± 25%, respectively. Amylase mRNA level was unchanged in comparison to control animals (81 ± 9%), while ribonuclease was decreased to 46 ± 8% ($p<0.05$).

Specificity of effect of SBTI administration and CCK infusion

To investigate whether the effects of SBTI administration or CCK infusion on pancreatic gene expression were due exclusively to elevated plasma CCK, the highly specific CCK receptor antagonist, L-364,718, was used to block any CCK-mediated actions. L-364,718 (3mg/rat) was administered 12 hours before the administration of SBTI or CCK infusion and every 12 hours during the experiment. This dose resulted in a plasma level of antagonist sufficient to totally abolish *in vitro* plasma CCK bioactivity. Administration of L-364,718 with the elemental diet alone had no effect on mRNA levels.

All the effects of exogenous CCK infusion on pancreatic gene expression were blocked by administration of the CCK receptor antagonist, L-364,718 (Fig. 16). Administration of the antagonist along with CCK-8 infusion returned mRNA levels of ODC, trypsinogen, chymotrypsinogen, amylase, and ribonuclease to 102 ± 11%, 95 ± 17%, 135 ± 41%, 132 ± 24%, and 65 ± 14% of control levels , respectively.

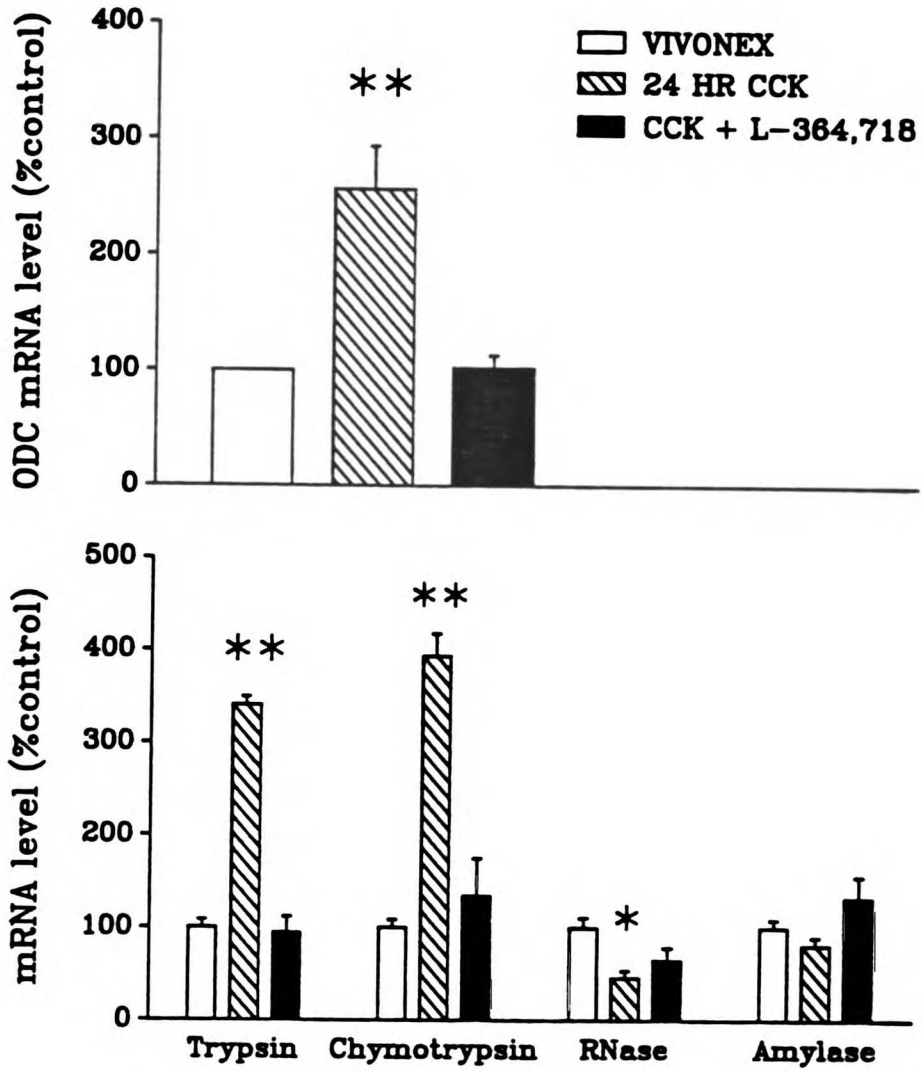

Figure 16 *Effect of CCK infusion and CCK receptor antagonist on pancreatic mRNA levels.* Rats were perfused intraduodenally with an elemental diet (Vivonex, open bars, n=8). CCK-8 was infused intravenously (300 ng/kg-hr) for 24 hours in the absence (CCK, hatched bars, n=6) or presence (filled bars, n=4) of the CCK receptor antagonist, L-364,718. mRNA levels were quantified by by slot-blot analysis and are expressed as % control (mean ± SE). *,$p<0.05$, **, $p<0.01$ versus Vivonex alone.

The same dose of antagonist was then used to block any effects of SBTI administration which are mediated by CCK. Treatment with L-364,718 reversed the effects of SBTI administration on pancreatic ODC and digestive enzyme mRNA levels (Fig. 17). In animals treated with both the CCK receptor antagonist and SBTI for 24 hours, mRNA levels of ODC, trypsinogen, chymotrypsinogen, amylase, and ribonuclease were 129 ± 9%, 124 ± 11%, 96 ± 16%, 129 ± 16%, 122 ± 13% of control levels, respectively.

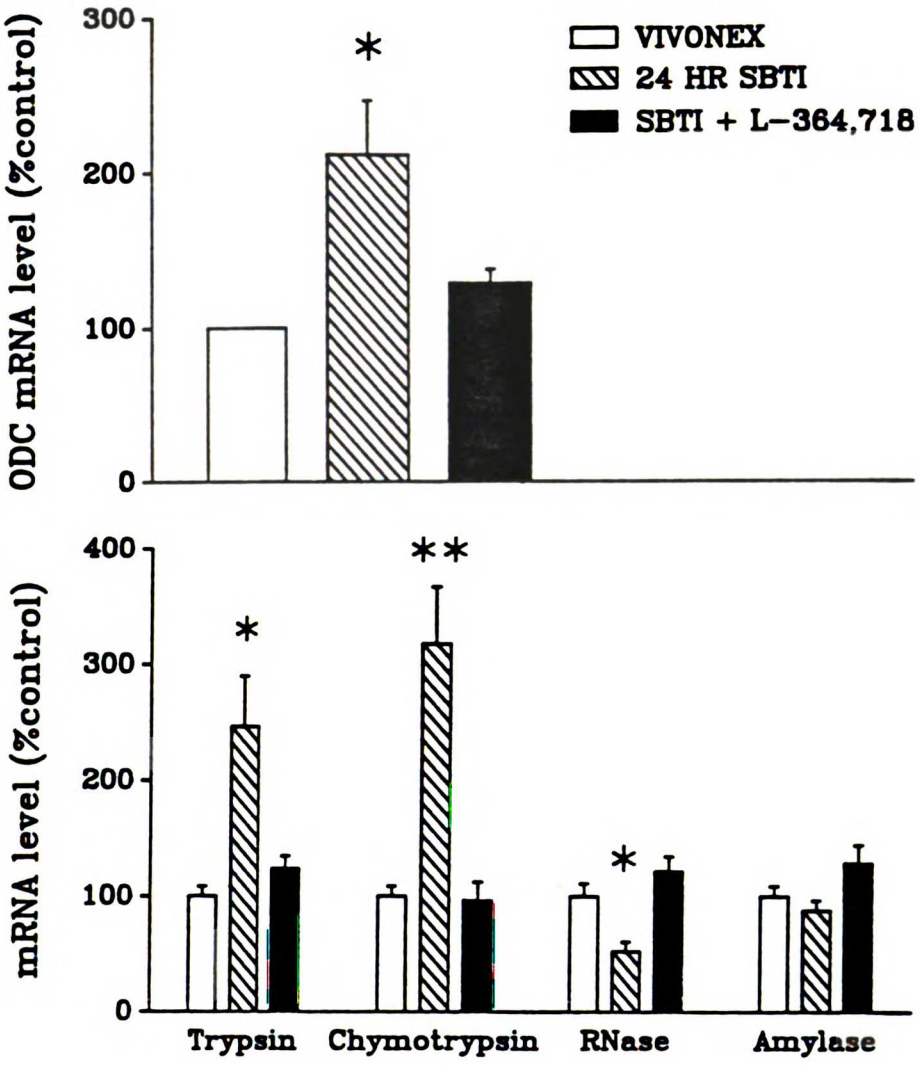

Figure 17 *Administration of CCK receptor antagonist, L-364,718, blocks the effects of SBTI administration on pancreatic mRNA levels.* Rats were perfused intraduodenally with an elemental diet (Vivonex, open bars) or with SBTI added to the diet for 24 hours in the absence (SBTI, hatched bars) or presence (filled bars) of the CCK receptor antagonist, L-364,718. mRNA levels were quantified by by slot-blot analysis and are expressed as % control (mean ± SE, n=8). *,$p<0.05$, **, $p<0.01$ versus Vivonex alone.

Discussion

Several previous studies have suggested that CCK both stimulates pancreatic growth and regulates pancreatic digestive enzyme gene expression (Solomon, 1978, Dembinski, 1980, Wicker, 1985, Green, 1986, Renaud, 1986). These studies, however, did not take into account the effects of CCK on food intake, and did not monitor CCK levels to ensure physiologic relevance of the findings. Thus the role of CCK in the physiological adaptation of the pancreas to increased protein in the diet has not been well-established. In this study, plasma CCK levels were monitored using a highly sensitive and specific CCK bioassay and were maintained within the normal physiologic range. In addition, the nutritional status of the animals was maintained by continuous intraduodenal infusion with an elemental diet which did not increase plasma CCK. Thus the physiologic effects of CCK on pancreatic gene expression were investigated in the absence of dietary variables.

Plasma CCK concentration was elevated by two different methods:
1) endogenously, by duodenal infusion of trypsin inhibitor, which stimulates CCK release via the well-described feedback loop (Louie, 1986, Folsch, 1987, Shiratori, 1987), and 2) exogenously, by intravenous infusion of CCK-8. Both of these methods resulted in similar plasma CCK levels in the physiologic range throughout the study period.

Previous studies have suggested effects of CCK on ODC activity, but it has not been clear whether this regulation is pre- or post-translational. In the current study elevation of plasma CCK by either method increased pancreatic ODC mRNA about 2-fold. This corresponds well with the two-fold increase in ODC immunoreactivity reported in rat pancreas after treatment with caerulein, a potent CCK analogue (Morisset, 1986). Administration of the CCK receptor antagonist, L-364,718, blocked the effect of SBTI treatment or CCK infusion on ODC mRNA level. Thus the increase in ODC mRNA observed in response to SBTI treatment can be completely accounted for by SBTI-stimulated CCK release. In conclusion, trypsin inhibitor feeding or CCK

administration stimulates both pancreatic growth and increased pancreatic expression of ornithine decarboxylase. The exact relationship between these events remains to be determined.

CCK is also thought to mediate the adaptation of the pancreas to high protein diets. A previous study showed that rats fed a high protein diet for 7 days had increased plasma CCK (transiently for 24 hours) along with increased pancreatic weight, DNA, protein, and chymotrypsinogen content (Green, 1986). Similar studies of the effects of increasing dietary protein demonstrated a two-fold increase in mRNA for the serine proteases after 9 days of high protein diet (Wicker, 1984) and a 3-4 fold increase after 2 weeks (Giorgi, 1985). Infusion of the CCK-like peptide caerulein for 24 hours also stimulated a 1.4-fold increase in serine protease mRNA levels, while repeated injection of caerulein (6 μg/kg i.p. twice daily for 7 days) increased pancreatic mRNA levels of trypsinogen and chymotrypsinogen 1.6 and 1.2-fold, respectively (Renaud, 1986). These results suggest that CCK mediates the adaptation of the pancreas to changes in dietary protein by regulation of pancreatic digestive enzyme gene expression.

The results of the present study support the hypothesis that physiological levels of CCK regulate pancreatic digestive enzyme gene expression and could thereby mediate the dietary adaptation of the pancreas. Pancreatic mRNA levels of the serine proteases trypsinogen I and chymotrypsinogen B were increased in response to either endogenous or exogenous elevation of plasma CCK within the physiologic range. The changes in the serine protease mRNA levels observed in this study were larger than those previously observed. Trypsinogen and chymotrypsinogen mRNA levels increased 2-3 fold after 24 hours of elevated plasma CCK, while Wicker *et al* reported a 1.4 fold increase after 24 hours of caerulein treatment. Previous studies also reported 1-2 fold increases after 7-9 days of high protein diet or caerulein injection. Although the present study did not extend past 48 hours, 4-5 fold increases were observed after 2 days of elevated plasma CCK. The reason for the larger increases observed in this study is not clear, but may be due to the method or duration of elevated plasma CCK, since the present study utilized

sustained physiologic elevated levels of endogenously released CCK. Also, the animals in the previous studies were fed *ad libitum* during the caerulein treatments and may have had reduced food intake as a result of the treatment, leading to blunted effects on digestive enzyme mRNA levels.

Previous studies of dietary adaptation of the pancreas have found that adaptation to diets high in protein results in decreased pancreatic amylase mRNA levels (Giorgi, 1984, Wicker, 1984). This decrease does not seem to be mediated by CCK, since elevation of plasma CCK had no effect on amylase mRNA levels in this study. Similarly, an earlier study using chronic adminstration of caerulein or a crude CCK preparation demonstrated only a small effect (Renaud, 1986). Because high protein diets are generally achieved by an isocaloric shift from carbohydrate to protein, changes in amylase gene expression could be mediated by a mechanism sensitive to the decreased dietary carbohydrate content, such as insulin or glucocorticoids (Logsdon, 1987, Osborn, 1987).

Nucleases are a class of digestive enzymes that have not been previously studied with regard to dietary adaptation. In the current study, ribonuclease mRNA levels declined to about 60% of the control level after 12 hours of SBTI administration and further decreased to about 50% after 24 or 48h; this decrease, however, was significant only at 24 hours. CCK therefore has a small inhibitory effect on ribonuclease gene expression. The adaptive significance of this decrease is not clear.

It has been previously reported that administration of the CCK receptor antagonist proglumide failed to fully suppress trypsin inhibitor or pancreatico-biliary diversion-induced pancreatic growth in rats (Miazza, 1985, Goke, 1986). Thus the suggestion has been made that other humoral factors in addition to CCK may be involved in the enteral regulation of the pancreas. In the current study, however, all the effects of SBTI administration were reproduced by infusion of CCK. Furthermore, the effects of either CCK infusion or SBTI administration were blocked by the potent receptor antagonist L-364,718. These results suggest that CCK alone is sufficient for the observed effects on

pancreatic digestive enzyme gene expression. Similary Wisner *et al* recently reported that the more potent antagonist L-364,718 was able to block most of the effects of the trypsin inhibitor camostate on pancreatic growth and chymotrypsin content (Wisner, 1988). The failure of previous studies to block trypsin inhibitor effects was likely due to the lower potency of the earlier antagonists.

In conclusion, CCK at physiologic concentrations has been shown to regulate the cellular level of mRNA for ornithine decarboxylase and pancreatic digestive enzymes. Furthermore, administration of the CCK receptor antagonist reversed the effects of CCK infusion or SBTI treatment on pancreatic gene expression. These data support the conclusion that CCK is the major factor involved in SBTI regulation of pancreatic mRNA levels. In addition, CCK can mediate at a pretranslational level the adaptation of the pancreas to high protein diet.

CONCLUSIONS

One goal of this research was to gain a better understanding of the regulation of cholecystokinin secretion in the rat. The results of these studies demonstrate that intact protein and fatty acids are the nutrients which stimulate CCK release in the rat. The relative physiologic importance of these nutrients depends on the composition of the diet. Since rat chow generally contains 20-25% protein and little fat or fatty acid, the primary physiologic stimulus for CCK release in the laboratory rat is ingestion of protein.

CCK secretion was also found to be regulated by neural pathways. Gastrin-releasing peptide (GRP) stimulated release of CCK in the conscious fasted rat. This peptide is abundant in enteric neurons and likely functions as a neurotransmitter. The action of GRP to stimulate CCK release in the absence of food suggests that it may the transmitter in the neural pathway which mediates positive feedforward regulation of digestion, stimulating delivery of digestive enzymes and bile to the duodenum to meet the incoming food. The food itself, either protein or fatty acid released by digestion of fat, then continues to stimulate CCK secretion as long as necessary. Once the system is "primed" by GRP, a negative feedback loop regulates CCK release and pancreatic secretion, maintaining the appropriate protein:protease ratio for digestion. Infusion of bethanechol inhibited protein-stimulated CCK release. This may reflect direct stimulation of pancreatic secretion by bethanechol resulting in decreased CCK secretion through the negative feedback loop. Alternatively, inhibition of protein-stimulated CCK secretion by bethanechol could be due to an inhibitory cholinergic innervation of the CCK cells; administration of atropine, however, had no effect on basal or protein-stimulated CCK release.

Protein-stimulated CCK release was also shown to be inhibited by somatostatin. Although neither the mechanism nor function of this hormonal regulation is known, somatostatin is known to inhibit the secretion of many hormones and numerous

gastrointestinal functions. Somatostatin inhibits CCK, gastrin, insulin and glucagon release and also inhibits gastric and pancreatic secretion. Circulating somatostatin could therefore coordinate and modulate postprandial secretory functions, keeping several systems working in concert. Alternatively, this broad array of actions could reflect evolutionary conservation of a peptide with general inhibitory action.

In summary, CCK secretion is regulated by food, hormones and neural pathways. Although the mechanisms by which these agents regulate CCK secretion are not well-understood, one model of the regulation of CCK secretion including all the factors investigated in this study is shown in Figure 18. The center of this diagram depicts the fairly well-described negative feedback regulation of CCK secretion mediated by both of the proposed CCK-releasing peptides. Intraduodenal infusion of protein stimulates CCK release by reducing the trypsin activity in the duodenum and preventing degradation of the CCK-releasing peptides. The mechanism for fatty acid stimulation of CCK release is not known, but is depicted as a direct effect on the CCK cell. Cholinergic pathways appear to regulate several points in this scheme including pancreatic secretion and release of the luminal CCK-releasing peptide. Inhibition of protein-stimulated CCK secretion by bethanechol could be direct, as depicted by the dashed line, or indirect through stimulation of pancreatic trypsin secretion thereby partially reducing the effectiveness of the protein stimulus. Gastrin-releasing peptide stimulated CCK release in the absence of food, suggesting that GRP neurons mediate positive feedforward regulation of CCK secretion. Somatostatin inhibited CCK secretion, possibly via paracrine regulation. Peptide YY was also investigated but had no effect at doses approximating physiologic levels of PYY. A larger dose of PYY increased protein-stimulated CCK release presumably through inhibition of pancreatic secretion resulting in reduced duodenal trypsin activity. Thus CCK secretion is stimulated by protein or fatty acid in the duodenum, and by GRP, and inhibited by bethanechol or somatostatin.

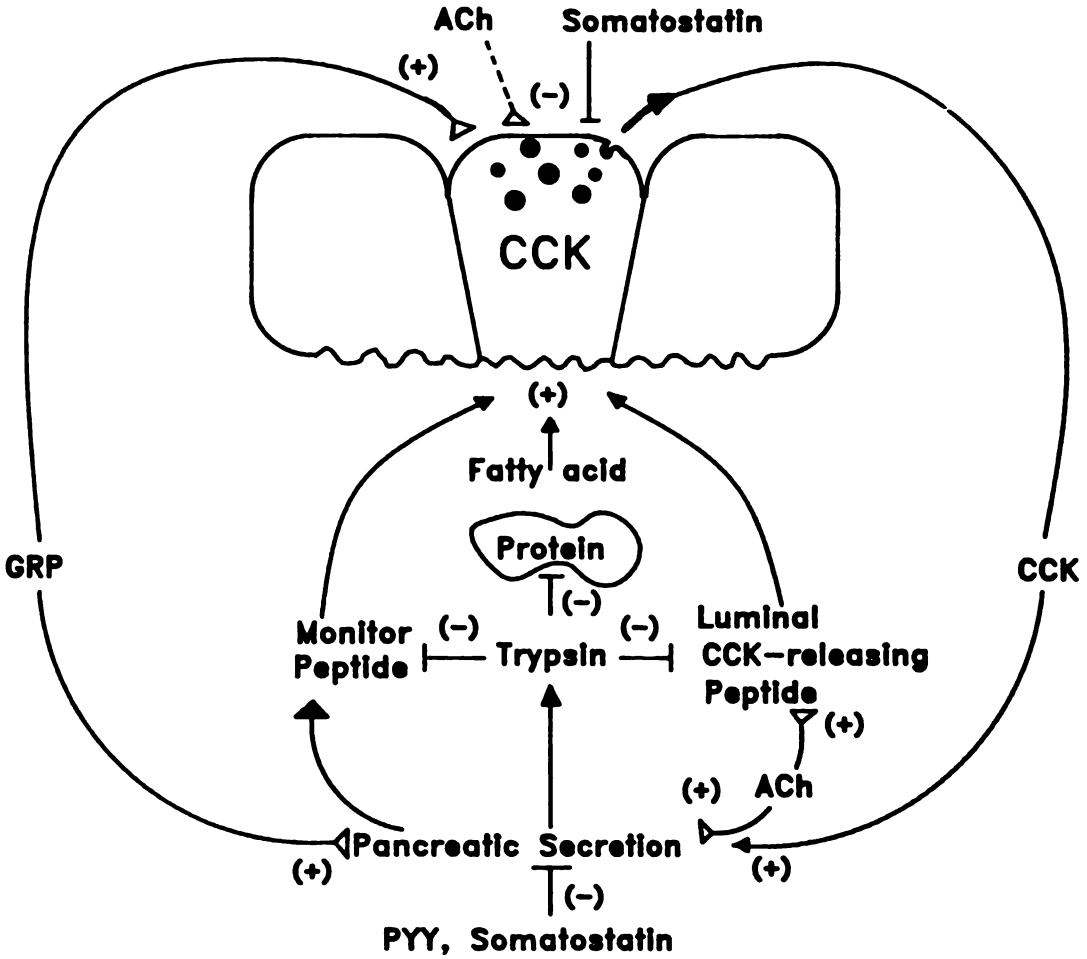

Figure 18 *A model of the regulation of cholecystokinin (CCK) secretion.* CCK secretion is regulated by a negative feedback loop in which the presence of trypsin in the duodenum inhibits CCK release by inactivation of CCK-releasing peptides. Intraduodenal protein stimulates CCK release by reducing the available trypsin activity and thereby preventing degradation of the CCK-releasing peptides. CCK secretion is also regulated by fatty acid, gastrin-releasing peptide (GRP), cholinergic innervation (ACh), and somatostatin. Peptide YY (PYY) inhibits pancreatic secretion and may alter CCK secretion indirectly.

Another goal of this research was to elucidate the physiologic role of CCK in regulating pancreatic gene expression. Since CCK release is sensitive to food content, CCK could mediate the changes in gene expression necessary for the adaptation of the pancreas to different diets. For example, a diet with increased protein content requires additional protease secretion to ensure adequate digestion of the protein. Stimulation of CCK secretion by dietary protein stimulates pancreatic secretion, meeting the immediate need for proteases, but CCK may also stimulate increased production of proteases to replenish or increase the amount of protease available for secretion. The results of this research demonstrated that physiologic levels of CCK stimulate an increase in pancreatic mRNA for ODC, a key enzyme in the regulation of growth, and an increase in mRNA for the serine proteases, trypsin and chymotrypsin. These results suggest that CCK stimulates both pancreatic growth and a specific increase in the amount of protease by regulating pancreatic gene expression at a pretranslational level. CCK can therefore mediate the adaptation of the pancreas to increased dietary protein by increasing its capacity to secrete the proteases necessary to digest the higher protein diet.

CCK is an integrative hormone. CCK secretion is stimulated by the presence of food in the duodenum, and CCK transmits this information to several target tissues thereby coordinating an integrated response to the ingestion of food. In particular, the present research has demonstrated that in the rat, protein stimulates CCK release and that elevation of plasma CCK stimulates increased pancreatic expression of mRNA for ODC, trypsin and chymotrypsin leading to increased capacity to digest ingested protein. This integrative function of CCK demonstrates one of the fundamental concepts of physiology – maintenance of homeostasis. By mediating and coordinating both the immediate and long term responses of several tissues to the ingestion of food, CCK regulates the ability of an animal to digest ingested nutrients and therefore maintains the input side of the energy balance which is fundamental to the maintenance of every other physiologic function.

REFERENCES

Adrian, T. E., G. - L. Ferri, A. J. Bacarese-Hamilton, H. S. Fuessl, J. M. Polak, and S. R. Bloom. Human distribution and release of a putative new gut hormone, peptide YY. Gastroenterology 89:1070-1077, 1985.

Anderson, L., and G. J. Dockray. The cholecystokinin antagonist L-364,718 inhibits the action of cholecystokinin but not bombesin on rat pancreatic secretion in vivo. Eur J Pharmacol 146:307-311, 1988.

Annibale, B., G. Delle Fave, F. Barbetti, L. DeMagistris, M. Puoti, E. Giordano, F. Leonetti, and G. Tamburro. Dose-response effect of somatostatin-14 on human basal pancreatic hormones. Pancreas 2:551-556, 1987.

Barber, D. L., J. H. Walsh, and A. H. Soll. Release and characterization of cholecystokinin from isolated canine jejunal cells. Gastroenterology 91: 627-36, 1986.

Barber, D. L., M. Gregor, and A. H. Soll. Somatostatin and muscarinic inhibition of canine enteric endocrine cells: cellular mechanisms. Am J Physiol 253:G684-G689, 1987.

Beinfeld, M. C., D. K. Meyer, R. L. Eskay, R. T. Jensen, and M. J. Brownstein. The distribution of cholecystokinin immunoreactivity in the central nervous system of the rat as determined by radioimmunoassay. Brain Research 212:51-57, 1981.

Bell, G. I., C. Quinto, M. Quiroga, P. Valenzuela, C. S. Craik, and W. J. Rutter. Isolation and sequence of a rat chymotrypsin B gene. J Biol Chem 259:14265-14270, 1984.

Benrezzak, O., and J. Morisset. Effects of alpha-difluoromethylornithine on pancreatic growth induced by caerulein. Regul Peptides 9:143-153, 1984.

Brand, S. J., and R. G. H. Morgan. The release of rat intestinal cholecystokinin after oral trypsin inhibitor measured by bio-assay. J Physiol 319:325-343, 1981.

Brown, J. Effects of 2-deoxyglucose on carbohydrate metabolism: Review of the literature and studies in the rat. Metabolism 11:1098-1112, 1962.

Buchan, A. M. J., J. M. Polak, E. Solcia, C. Capella, D. Hudson, and A. G. E. Pearse. Electron immunohistochemical evidence for the human intestinal I cell as the source of CCK. Gut 19:403-407, 1978.

Buffa, R., E. Solcia, and V. L. W. Go. Immunohistochemical identification of the cholecystokinin cell in the intestinal mucosa. Gastroenterology 70: 528-532, 1976.

Cantor, P. Evaluation of a radioimmunoassay for cholecystokinin in human plasma. Scand J Clin Lab Invest 46:213-221, 1986a.

Cantor, P., J. J. Holst, S. Knuhtsen, and J. F. Rehfeld. The effect of vagal stimulation on the release of cholecystokinin in anaesthetized pigs. Scand J Gastroenterol 21:1069-1072, 1986b.

Cantor, P., J. J. Holst, S. Knuhtsen, and J. F. Rehfeld. Effect of neuroactive agents on cholecystokinin release from the isolated, perfused porcine duodenum. Acta Physiol Scand 130:627-632, 1987a.

Cantor, P., and J. F. Rehfeld. The molecular nature of cholecystokinin in human plasma. Clinica Chimica Acta 168:153-158, 1987b.

Cantor, P., and J. F. Rehfeld. Cholecystokinin is pig plasma: release of components devoid of a bioactive COOH-terminus. Am J Physiol 256:G53-G61, 1989.

Chang, R. S. L., V. J. Lotti, R. L. Monaghan, J. Birnbaum, E. O. Stapley, M. A. Goetz, G. Albers-Schonberg, A. A. Patchett, J. M. Liesch, O. D. Hensens, and J. P. Springer. A potent nonpeptide cholecysokinin antagonist selective for peripheral tissues isolated from Aspergillus alliaceus. Science 230:177-179, 1985.

Chang, R. S. L., and V. J. Lotti. Biochemical and pharmacological characterization of an extremely potent and selective nonpeptide cholecystokinin antagonist. Proc Natl Acad Sci USA 83:4923-4926, 1986a.

Chang, R. S. L., V. J. Lotti, and K. A. Kunkel. Characterization of the binding of [3H]-(+)-L-364,718: A new potent,nonpeptide cholecystokinin antagonist radioligand selective for peripheral receptors. Molecular Pharmacology 30:212-217, 1986b.

Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. Isolation of a biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294-5299, 1979.

Cleveland, D. W., M. A. Lopata, R. J. MacDonald, N. J. Cowan, W. J. Rutter, and M. W. Kirschner. Number and evolutionary conservation of a- and b- tubulin and cytoplasmic b- and g- actin genes using specific cloned cDNA probes. Cell 20:95-105, 1980.

Colturi, T. J., R. H. Unger, and M. Feldman. Role of circulating somatostatin in regulation of gastric acid secretion, gastrin release, and islet cell function - Studies in healthy subjects and duodenal ulcer patients. J Clin Invest 74:417-423, 1984.

Coy, D. H., P. Heinz-Erian, N. - Y. Jiang, Y. Sasaki, J. Taylor, J. - P. Moreau, W. T. Wolfrey, J. D. Gardner, and R. T. Jensen. Probing peptide backbone function in bombesin - A reduced peptide bond analogue with potent and specific receptor antagonist activity. J Biol Chem 263:5056- 5060, 1988.

Craik, C. S., Q. L. Choo, G. H. Swift, C. Quinto, R. J. MacDonald, and W. J. Rutter. Structure of two related rat pancreatic trypsin genes. J Biol Chem 259:14255-14264, 1984.

Darlington, D. N., J. Shinsuko, and M. F. Dallman. The response of ACTH, epinephrine, norepinephrine, and the cardiovascular system to hemorrhage. Am J Physiol 251:H612-H618, 1986.

DeJong, A. J. L., M. Klamer, J. B. M. J. Jansen, and C. B. H. W. Lamers. Effect of atropine and somatostatin on bombesin-stimulated plasma gastrin, cholecystokinin and pancreatic polypeptide in man. Regulatory Peptides 17: 285-293, 1987.

Dektor, D. L., R. G. Pendleton, A. T. Elnitsky, A. M. Jenkins, and A. P. McDowell. Effect of metoclopramide, bethanechol, and the cholecystokinin receptor antagonist, L-364,718, on gastric emptying in the rat. European J Pharmacol 147:313-316, 1988.

Dembinski, A. B., and L. R. Johnson. Stimulation of pancreatic growth by secretin, caerulein, and pentagastrin. Endocrinology 106:323-328, 1980.

Deschenes, R. J., L. J. Lorenz, R. S. Haun, B. A. Roos, K. J. Collier, and J. E. Dixon. Cloning and sequence analysis of a cDNA encoding rat preprocholecystokinin. Proc Natl Acad Sci USA 81:726-730, 1984.

Deschenes, R. J., R. S. Haun, C. L. Funkes, and J. E. Dixon. A gene encoding rat cholecystokinin - Isolation, nucleotide sequence, and promoter activity. J Biol Chem 260:1280-1286, 1985.

Dockray, G. J. Cholecystokinin in Brain and Gut - Origins, Evolution and Identitity. In Gut Peptides - Secretion, Function and Clinical Aspects, edited by A. Miyoshi, Tokyo: Kodansha Ltd., pp. 237-244, 1979.

Dockray, G. J. Cholecystokinin. In Gut Hormones, edited by S. R. Bloom, and J. M. Polak, New York: Churchill Livingston, pp. 229-239, 1981.

Dockray, G. J. Physiology of Enteric Neuropeptides. In Physiology of the Gastrointestinal Tract, edited by L. R. Johnson, New York: Raven Press, 1, pp. 41-66, 1987.

Douglas, B. R., R. A. Woutersen, J. B. M. J. Jansen, A. J. L. DeJong, and C. B. H. W. Lamers. The influence of different nutrients on plasma cholecystokinin levels in the rat. Experientia 44:21-23, 1988.

Dubois, P. M., C. Paulin, and J. A. Chayvialle. Identification of gastrin-secreting cells and cholecystokinin secreting cells in the gastrointestinal tract of the human fetus and adult man. Cell Tiss Res 175: 351-356, 1976.

Eberlein, G. A., V. E. Eysselein, W. H. Hesse, H. Goebell, M. Schaefer, and J. R. Reeve, Jr. Detection of cholecystokinin-58 in human blood by inhibition of degradation. Am J Physiol 253:G477-G482, 1987.

Eberlein, G. A., V. E. Eysselein, T. D. Lee, J. E. Shively, M. Davis, M. Schaeffer, W. Niebel, J. Zeeh, A. Moessner, H. E. Meyer, D. Grandt, H. Goebell, and J. R. Reeve, Jr. Processing of human preprocholecystokinin by signal peptidase: Formation of cholecystokinin-83 (Abstract). Gastroenterology 96:A134, 1989.

Eng, J., Y. Shiina, E. Straus, and R. S. Yalow. Post-translational processing of cholecystokinin in pig brain and gut. Proc Natl Acad Sci USA 79:6060-6064, 1982.

Eng, J., B. H. Du, Y. C. E. Pan, M. Chang, J. D. Hulmes, and R. S. Yalow. Purification and sequencing of rat intestinal 22 amino acid C-terminal CCK fragment. Peptides 5:1203-1206, 1984.

Esteve, J. P., N. Vaysse, C. Susini, J. M. Kunsch, D. Fourmy, L. Praydol, E. Wunsch, L. Moroder, and A. Ribet. Bimodal regulation of pancreatic exocrine function in vitro by somatostatin-28. Am J Physiol 245:G208-G216, 1983.

Esteve, J. P., C. Susini, N. Vaysse, H. Antoniotti, E. Wunsch, G. Berthon, and A. Ribet. Binding of somatostatin to pancreatic acinar cells. Am J Physiol 247:G62-G69, 1984.

Eysselein, V. E., J. R. Reeve, JR, J. E. Shively, D. Hawke, and J. H. Walsh. Partial structure of a large canine cholecystokinin (CCK-58): Amino acid sequence. Peptides 3:687-691, 1982.

Eysselein, V. E., C. W. Deveney, H. Sankaran, J. R. Reeve, Jr, and J. H. Walsh. Biological activity of canine intestinal cholecystokinin-58. Am J Physiol 245:G313-G320, 1983.

Eysselein, V. E., G. A. Eberlein, W. H. Hesse, M. V. Singer, H. Goebell, and J. R. Reeve, Jr. Cholecystokinin-58 is the major circulating form of cholecystokinin in canine blood. J Biol Chem 262:214-217, 1987.

Eysselein, V. E., G. Eberlein, F. J. Ho, H. Goebell, and J. R. Reeve, Jr. An amino-terminal fragment of cholecystokinin-58 is present in the gut: evidence for a similar processing site of procholecystokinin in canine gut and brain. Regulatory Peptides 22:205-215, 1988.

Folsch, U. R., P. Cantor, H. M. Wilms, A. Schafmayer, H. D. Becker, and W. Creutzfeldt. Role of cholecystokinin in the negative feedback control of pancreatic enzyme secretion in conscious rats. Gastroenterology 92:449-458, 1987.

Fried, G. M., W. D. Ogden, J. Swierczek, G. H. Greeley, Jr, P. L. Rayford, and J. C. Thompson. Release of cholecystokinin in conscious dogs: correlation with simultaneous measurements of gallbladder pressure and pancreatic protein secretion. Gastroenterology 85:1113-1119, 1983.

Friedman, J., B. S. Schneider, and D. Powell. Differential expression of the mouse cholecystokinin gene during brain and gut development. Biochemistry 82:5593-5597, 1985.

Fukoaka, S. I., H. Kawajiri, T. Fushiki, K. Takahashi, and K. Iwai. Localization of pancreatic enzyme secretion-stimulating activity and trypsin inhibitory activity in zymogen granule of the rat pancreas. Biochim Biophys Acta 884:18-24, 1986.

Fukoaka, S. - I., T. Fushiki, M. Kondoh, and K. Iwai. Influence of neural blockages and proglumide on rat pancreatic enzyme secretion in response to intraluminal fatty acid. Proc Soc Exp Biol Med 186:27-35, 1987.

Fujita, T., and S. Kobayashi. The endocrine cell. In Gut Hormones, edited by S. R. Bloom, and J. M. Polak, Edinburgh: Churchill Livingstone, 1981, pp. 90-95.

Fushiki, T., S. I. Fukoaka, and K. Iwai. Stimulatory effect of an endogenous peptide in rat pancreatic juice on pancreatic enzyme secretion in the presence of atropine: Evidence for different mode of action of stimulation from exogenous trypsin inhibitors. Biochem Biophys Res Comm 118:532-537, 1984.

Gardner, J. D., and R. T. Jensen. Cholecystokinin receptor antagonists. Am J Physiol 246:G471-G476, 1984.

Gardner, J. D., and R. T. Jensen. Receptors mediating the actions of secretagogues on pancreatic acinar cells. In The Exocrine Pancreas, edited by V. L. W. Go, New York: Raven Press, 1, pp. 109-122, 1986.

Geracioti, T. D., Jr, and R. A. Liddle. Impaired cholecystokinin secretion in bulimia nervosa. N Engl J Med 319:683-688, 1988.

Giorgi, D., B. R. LaPointe, and J. C. Dagorn. Regulation of amylase messenger RNA concentration in rat pancreas by food content. EMBO J 3:1521- 1524, 1984.

Giorgi, D., W. Renaud, J. P. Bernard, and J. C. Dagorn. Regulation of proteolytic enzyme activities and mRNA concentrations in rat pancreas by food content. Biochem Biophys Res Comm 127:937-942, 1985.

Giraud, A. S., A. H. Soll, F. Cuttitta, and J. H. Walsh. Bombesin stimulation of gastrin release from canine gastrin cells in primary culture. Am J Physiol 252:G413-G420, 1987.

Glisin, V., R. Crkvinjakov, and C. Byus. Ribonucleic acid isolated by cesium chloride centrifugation. Biochemistry 13:2633-2637, 1974.

Go, V. L. W., A. F. Hofman, and H. J. Summerskill. Pancreozymin bioassay in man based on pancreatic enzyme secretion: potency of specific amino acids and other digestive products. J Clin Invest 49:1558-1564, 1970.

Goke, B., H. Printz, I. Koop, U. Rausch, G. Richter, R. Arnold, and G. Adler. Endogenous CCK release and pancreatic growth in rats after feeding a proteinase inhibitor (Camostate). Pancreas 1:509-515, 1986.

Greeley, G. H., Jr, L. C. Hill, A. Spannagel, and J. C. Thompson. Distribution of peptide YY in the gastrointestinal tract of the rat, dog, and monkey. Regulatory Peptides 19:365-372, 1987.

Green, G. M., and R. L. Lyman. Feedback regulation of pancreatic enzyme secretion as a mechanism for trypsin inhibitor-induced hypersecretion in rats. Proc Soc Exp Biol Med 140:6-12, 1972.

Green, G. M., and K. Miyasaka. Rat pancreatic response to intestinal infusion of intact and hydrolyzed protein. Am J Physiol 245:G394-G398, 1983.

Green, G. M., V. H. Levan, and R. A. Liddle. Plasma cholecystokinin and pancreatic growth during adaptation to dietary protein. Am J Physiol 251: G70-74, 1986.

Green, G. M., J. Friestman, S. Taguchi, W. Chey, and R. Liddle. Role of CCK and secretin in pancreatic response to fat in the rat (Abstract). Dig Dis Sci 32:1167, 1987.

Green, T., R. Dimaline, S. Peikin, and G. J. Dockray. Action of the cholecystokinin antagonist L364,718 on gastric emptying in the rat. Am J Physiol 255:G685-G689, 1988.

Grider, J. R., and G. M. Makhlouf. Regional and cellular heterogeneity of cholecystokinin receptors mediating muscle contraction in the gut. Gastroenterology 92:175-180, 1987.

Guan, D., H. Ohta, T. Tawil, R. Liddle, and G. Green. Regulation of rat pancreatic secretion by a putative intraluminal CCK-releasing factor: effect of atropine (Abstract). Gastroenterology 94:A236, 1988.

Gubler, U., A. O. Chua, B. J. Hoffman, K. J. Collier, and J. Eng. Cloned cDNA to cholecystokinin mRNA predicts an identical preprocholecystokinin in pig brain and gut. Proc Natl Acad Sci USA 81:4307-4310, 1984.

Gullo, L., P. Priori, C. Scarpignato, F. Baldoni, G. Mattioli, and L. Barbara. Effect of somatostatin 14 on pure human pancreatic secretion. Dig Dis Sci 32:1065-1070, 1987.

Guo, Y. - S., L. Mok, C. W. Cooper, G. H. Greeley, Jr, J. C. Thompson, and P. Singh. Effect of gastrin-releasing peptide analogues on gastrin and somatostatin release from isolated rat stomach. Am J Physiol 253:G206-G210, 1987.

Gyr, K., C. Beglinger, E. Kohler, U. Trautzl, U. Keller, and S. R. Bloom. Circulating somatostatin - Physiological regulator of pancreatic function?. J Clin Invest 79:1595-1600, 1987.

Harper, A. A., and H. S. Raper. Pancreozymin, a stimulant of the secretion of pancreatic enzymes in the extracts of the small intestine. J Physiol 102:115-125, 1943.

Hermansen, K. Effects of cholecystokinin (CCK)-4, nonsulfated CCK-8, and sulfated CCK-8 on pancreatic somatostatin, insulin, and glucagon secretion in the dog: Studies in vitro. Endocrinology 114:1770-1775, 1984.

Herzig, K. H., D. S. Louie, and C. Owyang. In vivo action of bombesin on exocrine pancreatic secretion in the rat: Independent of cholecystokinin and cholinergic mediation. Pancreas 3:292-296, 1988.

Hewson, G., G. E. Leighton, R. G. Hill, and J. Hughes. The cholecystokinin receptor antagonist L364,718 increases food intake in the rat by attentuation of the action of endogenous cholecystokinin. Br J Pharmacol 93:79-84, 1988.

Himeno, S., S. Tarui, S. Kanayama, T. Kuroshima, Y. Shinomura, C. Hayashi, K. Tateishi, K. Imagawa, E. Hashimura, and T. Hamaoka. Plasma cholecystokinin responses after ingestion of liquid meal and intraduodenal infusion of fat, amino acids, or hydrochloric acid in man: Analysis with a region specific radioimmunoasasy. Am J Gastroenterol 78:703-707, 1983.

Holst, J. J., S. Knuhtsen, C. Orskov, T. Skak-Nielsen, S. S. Poulsen, and O. V. Nielsen. GRP-producing nerves control antral somatostatin and gastrin secretion in pigs. Am J Physiol 253:G767-G774, 1987.

Hootman, S. R., and J. A. Williams. Stimulus-secretion coupling in the pancreatic acinus. In Physiology of the Gastrointestinal Tract, edited by L. R. Johnson, New York: Raven Press, 2, pp. 1129-1146, 1987.

Hopman, W. P. M., J. B. M. J. Jansen, and C. B. H. W. Lamers. Effect of atropine on the plasma cholecystokinin response to intraduodenal fat in man. Digestion 29:19-25, 1984a.

Hopman, W. P. M., J. B. M. J. Jansen, and C. B. H. W. Lamers. Plasma cholecystokinin response to a liquid fat meal in vagotomized patients. Ann Surg 200:693-697, 1984b.

Hopman, W. P. M., P. J. S. M. Kerstens, J. B. M. J. Jansen, G. Rosenbusch, and C. B. H. W. Lamers. Effect of graded physiologic doses of cholecystokinin on gallbladder contraction measured by ultrasonography. Gastroenterology 89:1242-1247, 1985.

Hopman, W. P. M., J. B. M. J. Jansen, G. Rosenbusch, and C. B. H. W. Lamers. Cephalic stimulation of gallbladder contraction in humans: role of cholecystokinin and the cholinergic system. Digestion 38:197-203, 1987.

Hosotani, R., K. Inoue, M. Kogire, T. Suzuki, M. Otsuki, P. L. Rayford, H. Yajima, and T. Tobe. Synthetic neuromedin-C stimulates exocrine pancreatic secretion in dogs and rats. Pancreas 2:414-421, 1987a.

Hosotani, R., P. Chowdhury, D. McKay, and P. L. Rayford. L364718, a new CCK antagonist, inhibits biological actions of CCK in conscious dogs. Peptides 8:1061-1064, 1987b.

Ihse, I., and P. Lilja. Effects of intestinal amylase and trypsin on pancreatic secretion in the pig. Scand J Gastro 14:1009-1013, 1979.

Innis, R. B., F. M. A. Correa, G. R. Uhl, B. Schneider, S. H. Snyder. Cholecystokinin octapeptide-like immunoreactivity: histochemical localization in rat brain. Proc Natl Acad Sci USA 76:521-525, 1979.

Inoue, K., R. Hosotani, K. Tatemoto, H. Yajima, and T. Tobe. Effect of natural peptide YY on blood flow and exocrine secretion of pancreas in dogs. Dig Dis Sci 33:828-832, 1988.

Ivy, A. C., and E. Goldberg. A hormone mechanism for gall-bladder contraction and evacuation. Am J Physiol 86:599-613, 1928.

Ivy, A. C., G. Kloster, H. C. Lueth, and G. E. Drewyer. On the preparation of "cholecystokinin". Am J Physiol 91:336-344, 1929.

Iwai, K., S. I. Fukoaka, T. Fushiki, T. Kodaira, and N. Ikei. Elevation of plasma CCK concentration after intestinal administration of a pancreatic enzyme secretion-stimulating peptide purifed from rat bile-pancreatic juice: Analysis with N-terminal region specific radioimmunoassay. Biochem Biophys Res Comm 136:701-706, 1986.

Iwai, K., S. I. Fukoaka, T. Fushiki, M. Tsujikawa, M. Hirose, S. Tsunasawa, and F. Sakiyama. Purification and sequencing of a trypsin-sensitive cholecystokinin-releasing peptide from rat pancreatic juice. J Biol Chem 262:8956-8959, 1987.

Iwai, K., T. Fushiki, and S. I. Fukuoka. Pancreatic enzyme secretion mediated by a novel peptide: Monitor peptide hypothesis. Pancreas 3:720-728, 1988.

Jansen, J. B. M. J., and C. B. H. W. Lamers. Radioimmunoassay of cholecystokinin in human tissue and plasma. Clinica Chimica Acta 131:305-316, 1983.

Jansen, J. B. M. J., and C. B. H. W. Lamers. Molecular forms of cholecystokinin in plasma from normal and gastrectomized human subjects following a fat meal. Peptides 8:801-805, 1987.

Jensen, R. T., T. Moody, C. Pert, J. E. Rivier, and J. D. Gardner. Interaction of bombesin and litorin with specific membrane receptors on pancreatic acinar cells. Proc Natl Acad Sci USA 75:6139-6143, 1978.

Jensen, R. T., G. F. Lemp, and J. D. Gardner. Interaction of cholecystokinin with specific membrane receptors on pancreatic acinar cells. Proc Natl Acad Sci 77:2079-2083, 1980.

Johnson, C. D., J. A. Chayvialle, M. A. Devaux, and H. Sarles. Neural pathways for the release of gastrin, cholecystokinin, and pancreatic polypeptide after a meal in dogs - Role of gastric and splanchnic nerves. Dig Dis Sci 31:1361-1369, 1986.

Kanayama, S., S. Himeno, Y. Higashimoto, Y. Yamasaki, T. Kitani, and S. Tarui. Plasma cholecystokinin-octapeptide like immunoreactivity in patients with hepatic cirrhosis. Life Sciences 41:1915-1920, 1987.

Kataoka, K. Effect of atropine on feedback regulation of pancreatic enzyme secretion in rats. Gastroenterologia Japonica 23:292-298, 1988.

Katz, A., and C. Kahana. Isolation and characterization of the mouse ornithine decarboxylase gene. J Biol Chem 263:7604-7609, 1988.

Keast, J. R., J. B. Furness, and M. Costa. Distribution of peptide- containing neurons and endocrine cells in the rabbit gastrointestinal tract, with particular reference to the mucosa. Cell Tissue Res 248:565-577, 1987.

Keim, V., B. Goke, and G. Adler. Changes in pattern of enzyme secretion by rat pancreas during repeated trypsin inhibitor treatment. Am J Physiol 255: G236-G241, 1988.

Kohler, E., C. Beglinger, G. Ribes, U. Grotzinger, M. - M. Loubatieres- Mariani, and K. Gyr. Effect of circulating somatostatin on exocrine pancreatic secretion in conscious dogs. Pancreas 1:455-459, 1986.

Konturek, S., J. Tasler, J. Bilski, A. J. DeJong, J. B. M. J. Jansen, and C. B. H. W. Lamers. Physiological role and localization of cholecystokinin release in dogs. Am J Physiol 250:G391-397, 1986.

Konturek, S. J., J. Bilski, W. Pawlik, J. Tasler, and W. Domshke. Adrenergic pathway in the inhibition of pancreatic secretion by peptide YY in dogs. Gastroenterology 94:266-273, 1988a.

Konturek, S. J., J. Tasler, M. Cieszkowski, K. Szewczyk, and M. Hladij. Effect of cholecystokinin receptor antagonist on pancreatic responses to exogenous gastrin and cholecystokinin and to meal stimuli. Gastroenterology 94:1014-1023, 1988b.

Lamers, C. B., J. E. Morley, P. Poitras, B. Sharp, H. E. Carlson, J. M. Hershman, and J. H. Walsh. Immunological and biological studies on cholecystokinin in rat brain. Am J Physiol 239:E232-E235, 1980.

Larsson, L. I., and J. F. Rehfeld. Distribution of gastrin and CCK cells in the rat gastrointestinal tract. Histochemistry 58:23-31, 1978.

Lee, P. C., B. M. Newmann, M. Praissman, D. R. Cooney, and E. Lebenthal. Cholecystokinin: a factor responsible for the enteral feedback control of pancreatic hypertrophy. Pancreas 1:335-340, 1986.

Levan, V. H., and G. M. Green. Effect of atropine on rat pancreatic secretory response to trypsin inhibitors and protein. Am J Physiol 251:G64- G69, 1986a.

Levan, V. H., and G. M. Green. Effect of diversion of bile-pancreatic juice to the ileum on pancreatic secretion and adaptation in the rat. Proc Soc Exp Biol Med 181:139-143, 1986b.

Liddle, R. A., I. D. Goldfine, and J. A. Williams. Bioassay of plasma cholecystokinin in rats: effects of food, trypsin inhibitor, and alcohol. Gastroenterology 87:542-549, 1984.

Liddle, R. A., I. D. Goldfine, M. S. Rosen, R. A. Taplitz, and J. A. Williams. Cholecystokinin bioactivity in human plasma. J. Clin. Invest. 75: 1144-1152, 1985.

Liddle, R. A., E. T. Morita, C. K. Conrad, and J. A. Williams. Regulation of gastric emptying in humans by cholecystokinin. J Clin Invest 77:992-996, 1986a.

Liddle, R. A., G. M. Green, C. K. Conrad, and J. A. Williams. Proteins but not amino acids, carbohydrates, or fats stimulate cholecystokinin secretion in the rat. Am. J. Physiol. 14:G243-G248, 1986b.

Liddle, R. A., J. D. Carter, and A. R. McDonald. Dietary regulation of rat intestinal cholecystokinin gene expression. J Clin Invest 81:2015-2019, 1988.

Liener, I. E., R. L. Goodale, A. Deshmukh, T. L. Satterberg, G. Ward, C. M. DiPietro, P. E. Bankey, and J. W. Borner. Effect of trypsin inhibitor from soybeans (Bowman-Birk) on the secretory activity of the human pancreas. Gastroenterology 94:419-427, 1988.

Lilja, P., I. Wiener, K. Inoue, G. M. Fried, G. H. Greeley, and J. C. Thompson. Release of cholecystokinin in response to food and intraduodenal fat in pigs, dogs and man. Surgery 159:557-561, 1984.

Lluis, F., G. Gomez, M. Fujimara, G. H. Greeley, Jr, and J. C. Thompson. Peptide YY inhibits nutrient-, hormonal-, and vagally-stimulated pancreatic exocrine secretion. Pancreas 2:454-462, 1987.

Lluis, F., G. Gomez, M. Fujimara, G. H. Greeley, Jr, and J. C. Thompson. Peptide YY inhibits pancreatic secretion by inhibiting cholecystokinin release in the dog. Gastroenterology 94:137-144, 1988.

Logsdon, C. D. Stimulation of pancreatic acinar cell growth by CCK, epidermal growth factor, and insulin in vitro. Am J Physiol 251:G487-G494, 1986.

Logsdon, C. D., S. F. Akana, C. R. Meyer, M. F. Dallman, and J. A. Williams. Pancreatic acinar cell amylase gene expression: selective effects of adrenalectomy and corticosterone replacement. Endocrinology 121: 1242-1250, 1987.

Louie, D. S., D. May, P. Miller, and C. Owyang. Cholecystokinin mediates feedback regulation of pancreatic enzyme secretion in rats. Am J Physiol 13:G252-G259, 1986.

Louie, D. S., J. P. Liang, and C. Owyang. Characterization of a new CCK antagonist, L364,718: in vitro and in vivo studies. Am J Physiol 255:G261- G266, 1988.

Lu, L., D. S. Louie, M. Wider, D. May, and C. Owyang. Extraction and characterization of a CCK-releasing peptide mediating feedback regulation of apncreatic secretion. Am J Physiol 256:G430-G435, 1989.

Luk, G. D., and P. Yang. Polyamines in intestinal and pancreatic adaptation. Gut 28:95-101, 1987.

Lyman, R. L., and S. Lepkovsky. The effect of raw soybean meal and trypsin inhibitor diets on pancreatic enzyme secretion in the rat. J Nutr 62:265-284, 1957.

MacDonald, R. J., S. J. Stary, and G. H. Swift. Rat pancreatic ribonuclease mRNA. J Biol Chem 257:14582-14585, 1982.

Maton, P. N., A. C. Selden, and V. S. Chadwick. Atropine inhibits meal-stimulated release of cholecystokinin. Scand J Gastroenterol 19:831-834, 1984.

McConlogue, L., M. Gupta, L. Wu, and P. Coffino. Molecular cloning and expression of the mouse ornithine decarboxylase gene. Proc Natl Acad Sci USA 81:540-544, 1984.

McDonald, T. J., M. A. Ghatei, S. R. Bloom, N. S. Track, J. Radziuk, J. Dupre, and V. Mutt. A qualitative comparison of canine plasma gastroenteropancreatic hormone responses to bombesin and the porcine gastrin-releasing peptide (GRP). Regulatory Peptides 2:293-304, 1981.

Meyer, J. H., and R. S. Jones. Canine pancreatic responses to intestinally perfused fat and products of fat digestion. Am. J. Physiol. 5:1178-1187, 1974.

Miazza, B. M., Y. Turberg, P. Guillaume, W. Hahne, J. R. Chayvialle, and E. Loizeau. Mechanism of pancreatic growth induced by pancreatico-biliary diversion in the rat. Scand J Gastroenterol 20:75-83, 1985.

Miller, R. G., Jr. Simultaneous Statistical Inference. New York: Springer-Verlag, 1981.

Miyasaka, K., and G. M. Green. Effect of rapid washout of proximal small intestine on pancreatic secretion in the conscious rat (Abstract). Gastroenterology 84:1251, 1983.

Miyasaka, K., and K. Kitani. The effect of oleate on pancreatic and bile secretion in the conscious rat. Proc Soc Exp Biol Med 189:94-99, 1988.

Miyata, M., P. L. Rayford, and J. C. Thompson. Hormonal (gastrin, secretin, cholecystokinin) and secretory effects of bombesin and duodenal acidification in dogs. Surgery 87:209-215, 1980.

Moghimzadeh, E., R. Ekman, R. Hakanson, N. Yanahira, and F. Sundler. Neuronal gastrin-releasing peptide in the mammalian gut and pancreas. Neuroscience 10:553-563, 1983.

Moran, T. H., T. W. Moody, A. M. Hostetler, P. H. Robinson, M. Goldrich, and P. R. McHugh. Distribution of bombesin binding sites in the rat gastrointestinal tract. Peptides 9:643-649, 1988.

Morisset, J., S. Chamberalnd, L. Gilbert, A. Lord, and L. Larose. Study of pancreatic DNA synthesis in vivo and in vitro following caerulein treatment in vivo. Biomed Res 3:151-158, 1982.

Morisset, J., and O. Benrezzak. Polyamines and pancreatic growth induced by caerulein. Life Sci 35:2471-2480, 1984.

Morisset, J., and O. Benrezzak. Reversal of alpha-difluoromethylornithine inhibition of caerulein-induced pancreatic growth by putrescine. Regul Peptides 11:201-208, 1985.

Morisset, J., P. Sarfati, and G. Grondin. Immunocytochemical demonstration of ODC in the rat exocrine pancreas using the protein-A gold technique. Can J Physiol Pharmacol 64:444-448, 1986.

Mutt, V., and J. E. Jorpes. Structure of porcine cholecystokinin- pancreozymin 1. Cleavage with thrombin and with trypsin. European J Biochem 6:156-162, 1968.

Nakano, I., K. Miyazaki, A. Funakoshi, K. Tateishi, T. Hamaoka, and H. Yajima. Gastrin-releasing peptide stimulates cholecysokinin secretion in perfused rat duodenum. Regulatory Peptides 23:153-159, 1988.

Nealon, W. H., R. D. Beauchamp, C. M. Townsend, Jr, and J. C. Thompson. Role of cholecystokinin in canine pancreatic exocrine response to bombesin stimulation. American J Surgery 153:96-101, 1987.

Niederau, C., M. Niederau, J. A. Williams, and J. H. Grendell. New proglumide-analogue CCK receptor antagonists: very potent and selective for peripheral tissues. Am J Physiol 251:G856-G860, 1986.

Okabayashi, Y., M. Otsuki, A. Ohki, C. Sakamoto, and S. Baba. Effects of C- terminal fragments of cholecystokinin on exocrine and endocrine secretion from isolated perfused rat pancreas. Endocrinology 113:2210-2215, 1983.

Osborn, L., S. A. Keller, and M. H. Meisler. Tissue-specific and insulin- dependent expression of a pancreatic amylase gene in transgenic mice. Mol Cell Biol 7:326-334, 1987.

Otsuki, M., Y. Okabayahi, A. Ohki, T. Oka, M. Fujii, T. Nakamura, N. Suguira, N. Yanaihara, and S. Baba. Action of cholecystokinin analogues on exocrine and endocrine rat pancreas. Am J Physiol 250:G405-G411, 1986.

Otsuki, M., M. Fujii, T. Nakamura, S. Tani, T. Oka, S. Baba, and H. Yajima. Actions of neuromedin-B and neuromedin-C on amylase release from isolated rat pancreatic acini. Pancreas 2:252-257, 1987.

Owyang, C., D. S. Louie, and D. Tatum. Feedback regulation of pancreatic enzyme secretion suppression of cholecystokinin release by trypsin. J Clin Invest 77:2042-2047, 1986.

Pappas, T. N., H. T. Debas, Y. Goto, and I. L. Taylor. Peptide YY inhibits meal-stimulated pancreatic and gastric secretion. Am J Physiol 248:G118- G123, 1985.

Pearson, R. K., and L. J. Miller. Affinity labeling of a novel cholecystokinin-binding protein in rat pancreatic plasmalemma using new short probes for the receptor. J Biol Chem 262:869-876, 1987.

Pegg, A. E., and P. P. McCann. Polyamine metabolism and function. Am J Physiol 243:C212-C221, 1982.

Pekas, J. C., W. E. Trout, and B. D. Schanbacher. Cholecystokinin octapeptide (CCK-8) immunization; Stimualtion of feed intake and growth of swine (Abstract). FASEB J 2:A1198, 1988.

Pendleton, R. G., R. J. Bendesky, L. Schaffer, T. E. Nolan, R. J. Gould, and B. V. Clineschmidt. Roles of endogenous cholecystokinin in biliary, pancreatic and gastric function: Studies with L-364,718, a specific cholecystokinin receptor antagonist. J Pharmacol Exp Ther 241:110-116, 1987.

Polak, J. M., S. R. Bloom, P. L. Rayford, A. G. E. Pearse, A. M. J. Buchan, and J. C. Thompson. Identification of cholecystokinin-secreting cells. Lancet :1016-1018, 1975.

Polak, J. M., and S. R. Bloom. Organisation of the gut peptidergic innervation. In Gut Hormones, edited by S. R. Bloom, and J. M. Polak, Edinburgh: Churchill Livingstone, pp. 487-494, 1981.

Putnam, W. S., R. A. Liddle, and J. A. Williams. Inhibitory regulation of the rat exocrine pancreas by peptide YY and pancreatic polypeptide. Am J Physiol 256:G698-G703, 1989.

Rausch, U., H. Weidenbach, G. Adler, and H. F. Kern. Stimulation of pancreatic secretory process in the rat by low-molecular weight proteinase inhibitor II. Regulation of total protein and individual enzyme biosynthesis. Cell Tissue Res 249:63-67, 1987a.

Rausch, U., G. Adler, F. Weidenbach, D. Rudolff, I. Koop, and H. F. Kern. Stimulation of pancreatic secretory process in the rat by low-molecular weight proteinase inhibitor I. Dose-response study on enzyme content and secretion, cholecystokinin release and pancreatic fine structure. Cell Tissue Res 247:187-193, 1987b.

Rehfeld, J. F., J. J. Holst, and S. L. Jensen. The molecular nature of vascularly release cholecystokinin from the isolated perfused porcine duodenum. Regulatory Peptides 3:15-28, 1982.

Rehfeld, J. F. How to measure cholecystokinin in plasma?. Gastroenterology 87:434-438, 1984.

Rehfeld, J. F., J. Lindholm, B. N. Andersen, L. Bardram, P. Cantor, M. Fenger, and D. K. Ludecke. Pituitary tumors containing cholecystokinin. New England J Medicine 316:1244-1247, 1987.

Renaud, W., D. Giorgi, J. Iovanna, and J. C. Dagorn. Regulation of concentrations of mRNA for amylase, trypsinogen I and chymotrypsinogen B in rat pancreas by secretagogues. Biochem J 235:305-308, 1986.

Robinson, P. H., T. H. Moran, M. Goldrich, and P. R. McHugh. Development of cholecystokinin binding sites in rat upper gastrointestinal tract. Am J Physiol 252:G259-G534, 1987.

Rosenzweig, S. A., L. J. Miller, and J. D. Jamieson. Identification and localization of cholecystokinin-binding sites on rat pancreatic plasma membranes and acinar cells: A biochemical and autoradiographic study. J Cell Biol 96:1288-1297, 1983.

Rosetti, L., G. I. Shulman, and W. S. Zawalich. Physiological role of cholecystokinin in meal-induced insulin secretion in conscious rats - Studies with L-364,718, a specific inhibitor of CCK-receptor binding. Diabetes 36:1212-1215, 1987.

Roze, C., J. DeLaTour, J. Chariot, M. Souchard, C. Vaille, and C. Debray. La stimulation pancreatique provoquee par le 2-desoxy-D-glucose chez la rat: Analyse des mecanismes nerveux et humeraux. Gastroenterol Clin Biol 1: 435-446, 1977.

Rushakoff, R. J., I. D. Goldfine, J. D. Carter, and R. A. Liddle. Physiological concentrations of cholecystokinin stimulate amino acid- induced insulin release in humans. J Clin Endocrinol Metab 65:395-401, 1987.

Sakamoto, C., I. D. Goldfine, and J. A. Williams. Characterization of cholecystokinin receptor subunits on pancreatic plasma membranes. J Biol Chem 258:12707-12711, 1983a.

Sakamoto, C., J. A. Williams, K. Y. Wong, and I. D. Goldfine. The CCK receptor on pancreatic plasma membranes: Binding characteristics and covalent cross-linking. FEBS Letters 151:63-66, 1983b.

Sakamoto, C., I. D. Goldfine, and J. A. Williams. The somatostatin receptor on isolated pancreatic acinar cell plasma membranes - Identification of subunit structure and direct regulation by cholecystokinin. J Biol Chem 259:9623-9627, 1984.

Sakamoto, C., I. D. Goldfine, E. Roach, and J. A. Williams. Localization of saturable CCK binding sites in rat pancreatic islets by light and electron microscope autoradiography. Diabetes 34:390-394, 1985.

Sankaran, H., I. D. Goldfine, C. W. Deveney, K. Y. Wong, and J. A. Williams. Binding of cholecystokinin to high affinity receptors on isolated rat pancreatic acini. J Biol Chem 255:1849-1853, 1980.

Schaffalitzky de Muckadell, O. B., O. Olsen, P. Cantor, and E. Magid. Concentration of secretin and CCK in plasma and pancreatico-biliary secretion in response to intraduodenal acid and fat. Pancreas 1:536-543, 1986.

Schick, J., H. Hern, and G. Scheele. Hormonal stimulation in the exocrine pancreas results in coordinate and anticoordinate regulation of protein synthesis. J Cell Biol 99:1569-1574, 1984.

Shiratori, K., Y. F. Chen, W. Y. Chey, K. Y. Lee, and T. - M. Chang. Mechanism of increased exocrine pancreatic secretion in pancreatic juice-diverted rats. Gastroenterology 91:1171-1178, 1986a.

Shiratori, K., S. Watanabe, W. Y. Chey, K. Y. Lee, and T. M. Chang. Endogenous cholecystokinin drives gallbladder emptying in dogs. Am J Physiol 251:G553-G558, 1986b.

Schjoldager, B., S. P. Powers, and L. J. Miller. Affinity labeling the bovine gallbladder cholecystokinin receptor using a battery of probes. Am J Physiol 255:G579-G586, 1988a.

Schjoldager, B., M. J. Shaw, S. J. Powers, P. F. Schmalz, J. Szurszewski, and L. J. Miller. Bovine gallbladder muscularis: source of a myogenic receptor for cholecystokinin. Am J Physiol 254:G294-G299, 1988b.

Schusdziarra, V., N. Lenz, R. Schick, and V. Maier. Modulatory effect of glucose, amino acids, and secretin on CCK-8 induced somatostatin and pancreatic polypeptide release in dog. Diabetes 35:523-529, 1986.

Singer, M. V., W. Niebel, J. B. M. J. Jansen, D. Hoffmeister, S. Gotthold, H. Goebell, and C. B. H. W. Lamers. Pancreatic secretory response to intravenous caerulein and intraduodenal tryptophan: Studies before and after stepwise removal of the extrinsic nerves of the pancreas in dogs. Gastroenterology 96:925-934, 1989.

Solcia, E., C. Capella, R. Buffa, L. Usellini, R. Fiocca, and F. Sessa. Endocrine cells of the digestive system. In Physiology of the Gastrointestinal Tract, edited by L. R. Johnson, New York: Raven Press, 1, 1987, pp. 111-130.

Solomon, T. E., H. Petersen, J. Elashoff, and M. I. Grossman. Interaction of caerulein and secretin on pancreatic size and composition in the rat. Am J Physiol 235:E714-E719, 1978.

Solomon, T. E., T. Yamada, J. D. Elashoff, J. Wood, and C. Beglinger. Bioactivity of cholecystokinin analogues: CCK-8 is not more potent than CCK-33. Am J Physiol 247:G105-G111, 1984.

Steigerwalt, R. W., I. D. Goldfine, and J. A. Williams. Characterization of cholecystokinin receptors on bovine gallbladder membranes. Am J Physiol 247:G709-G714, 1984.

Stubbs, R. S., and B. E. Stabile. Role of cholecystokinin in pancreatic exocrine response to intraluminal amino acids and fat. Am J Physiol 248: G347-352, 1985.

Sugano, K., J. Park, A. H. Soll, and T. Yamada. Stimulation of gastrin release by bombesin and canine gastrin-releasing peptides - Studies with isolated canine G cells in primary cultures. J Clin Invest 79:935-942, 1987.

Susini, C., A. Bailey, J. Szecowka, and J. A. Williams. Characterization of covalently cross-linked pancreatic somatostatin receptors. J Biol Chem 261:16738-16743, 1986.

Takahashi, Y., K. Kato, Y. Hayashizaki, T. Wakabayashi, E. Ohtsuka, S. Matsuki, M. Ikehara, and K. Matsubara. Molecular cloning of the human cholecystokinin gene by use of a synthetic probe containing deoxyinosine. Proc Natl Acad Sci USA 82:1931-1935, 1985.

Takahashi, Y., S. Fukushige, T. Murotsu, and K. Matstubara. Structure of human cholecystokinin gene and its chromosomal location. Gene 50:353-360, 1986.

Taylor, I. L. Distribution and release of peptide YY in dog measured by specific radioimmunoassay. Gastroenterology 88:731-737, 1985.

Usellini, L., C. Capella, A. Malesci, G. Rindi, and E. Solcia. Ultrastructural localization of cholecystokinin in endocrine cells of the dog duodenum by the immunogold technique. Histochemistry 83:331-336, 1985.

Vale, W., C. Rivier, and M. Brown. Regulatory peptides of the hypothalamus. In Annual Review of Physiology, edited by E. Knobil, R. R. Sonnenschein and I. S. Edelman, Palo Alto:Annual Reviews, 39, pp. 473-527, 1977.

Verspohl, E. J., H. P. T. Ammon, J. A. Williams, and I. D. Goldfine. Evidence that cholecystokinin interacts with specific receptors and regulates insulin release in isolated rat islets of Langerhans. Diabetes 35:38-43, 1986.

Verspohl, E. J., and H. P. T. Ammon. Cholecystokinin (CCK8) regulates glucagon, insulin, and somatostatin secretion from isolated rat pancreatic islets: interaction with glucose. Pflugers Archives 410:284-287, 1987.

Vinayek, R., R. T. Jensen, and J. D. Gardner. Role of sulfate ester in influencing biologic activity of cholecystokinin-related peptides. Am J Physiol 252:G178-G181, 1987.

Von Shrenck, T., T. H. Moran, P. Heinz-Erian, J. D. Gardner, and R. T. Jensen. Cholecystokinin receptors on gallbladder muscle and pancreatic acinar cells: a comparative study. Am J Physiol 255:G512-G521, 1988.

Watanabe, S., K. Shiratori, T. Takeuchi, W. K. Chey, C. H. You, and T. M. Chang. Release of cholecystokinin and exocrine pancreatic secretion in response to an elemental diet in human subjects. Dig Dis Sci 31:919-924, 1986.

Watanabe, S., K. Y. Lee, T. M. Chang, L. Berger-Ornstein, and W. Y. Chey. Role of pancreatic enzymes on release of cholecystokinin-pancreozymin in response to fat. Am J Physiol 254:G837-G842, 1988.

Wicker, C., A. Puigserver, and G. Scheele. Dietary regulation of levels of active mRNA coding for amylase and serine protease zymogens in the rat pancreas. Eur J Biochem 139:381-387, 1984.

Wicker, C., A. Puigserver, U. Rausch, G. Scheele, and H. Kern. Multiple level caerulein control of the gene expression of secretory proteins in the pancreas. Eur J Biochem 151:461-466, 1985.

Williams, J. A., M. Korc, and R. L. Dormer. Action of secretagogues on a new preparation of functionally intact, isolated pancreatic acini. Am J Physiol 235:E517-E524, 1978.

Williams, J. A. Cholecystokinin: A hormone and a neurotransmitter. Biomedical Research 3:107-121, 1982.

Williams, J. A., S. R. Vigna, C. Sakamoto, and I. D. Goldfine. Brain cholecystokinin receptors - Binding characteristics, covalent-crosslinking and evolutionary aspects. Ann NY Acad Sci 448:220-230, 1985.

Williams, J. A., and D. J. McChesney. Cholecystokinin induces the interaction of its receptor with a guanine nucleotide binding protein. Regulatory peptides 18:109-117, 1987.

Wisner, J. R., Jr, R. E. McLaughlin, K. A. Rich, S. Ozawa, and I. G. Renner. Effects of L-364,718, a new cholecystokinin receptor antagonist, on camostate-induced growth of the rat pancreas. Gastroenterology 94:109- 113, 1988a.

Wisner, J. R., Jr, S. Ozawa, and I. G. Renner. Evidence against cholecystokinin mediation of basal and bombesin-stimulated pancreatic secretion in the rat. Gastroenterology 95:151-155, 1988b., 1977

Yamada, T. Local regulatory actions of gastrointestinal peptides. In Physiology of the Gastrointestinal Tract, edited by L. R. Johnson, New York: Raven Press, 1, pp. 131-142, 1987.

Zawalich, W. S., S. B. Cote, and V. A. Diaz. Influence of cholecystokinin on insulin output from isolated perifused pancreatic islets. Endocrinology 119:616-621, 1986.

Zawalich, W. S., V. A. Diaz, and K. C. Zawalich. Stimulatory effcts of cholecystokinin on isolated perfused islets inhibited by L-364,718 potent and specific antagonist. Diabetes 37:1432-1437, 1988.


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