

Peptone Stimulates CCK-releasing Peptide Secretion by Activating Intestinal Submucosal Cholinergic Neurons

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Abstract

In this study we tested the hypothesis that peptone in the intestine stimulates the secretion of the CCK-releasing peptide (CCK-RP) which mediates CCK secretion, and examined the enteric neural circuitry responsible for CCK-RP secretion. We used a "donor-recipient" rat intestinal perfusion model to quantify the CCK-RP secreted in response to nutrient stimulation. Infusion of concentrated intestinal perfusate collected from donor rat perfused with 5% peptone caused a $62 \pm 10\%$ increase in protein secretion and an elevation of plasma CCK levels to 6.9 ± 1.8 pM in the recipient rat. The stimulatory effect of the intestinal washings was abolished when the donor rats were pretreated with atropine or hexamethonium but not with guanethidine or vagotomy. Mucosal application of lidocaine but not serosal application of benzalkonium chloride which ablates the myenteric neurons in the donor rats also abolished the stimulatory action of the intestinal washings. Furthermore, treatment of the donor rats with a 5HT₃ antagonist and a substance P antagonist also prevented the secretion of CCK-RP. These observations suggest that peptone in the duodenum stimulates serotonin release which activates the sensory substance P neurons in the submucous plexus. Signals are then transmitted to cholinergic interneurons and to epithelial CCK-RP containing cells via cholinergic secretomotor neurons. This enteric neural circuitry which is responsible for the secretion of CCK-RP may in turn play an important role in the postprandial release of CCK. (*J. Clin. Invest.* 1996; 97:1463–1470.) Key words: substance P • serotonin • enteric nervous system • cholecystokinin • cholecystokinin-releasing peptide

Introduction

Cholecystokinin (CCK)¹ plays an important role in the mediation of postprandial pancreatic secretion and gallbladder contraction. In the rat, protein is the major dietary intestinal stimulus for CCK release (1). Little, however, is known about the mechanisms regulating CCK secretion.

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1. Abbreviations used in this paper: CCK, cholecystokinin; RP, releasing peptide.

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Recently it was reported that the release of CCK into the circulation is mediated by a "CCK-releasing peptide" (CCK-RP) secreted into the intestine of the rat (2, 3). When trypsin is present, this peptide is cleaved and inactivated. It has been proposed that this newly discovered CCK-RP may also act as a mediator of pancreatic enzyme secretion in response to dietary protein (4). Dietary protein in the intestine competes for trypsin (1), which would otherwise inactivate the CCK-RP. The resulting increase of active CCK-RP in the intestine releases CCK and stimulates pancreatic enzyme secretion. On the other hand, if a dietary protein such as bovine serum albumin or lactalbumin is a poor substrate for pancreatic proteases, it will not prevent proteolytic inactivation of the CCK-RP and therefore will not evoke an increase in CCK secretion (1). Hence the ability of intact protein to stimulate CCK release and pancreatic secretion has been attributed to its ability to bind with pancreatic proteases and reverse protease-induced feedback inhibition of CCK release. In a recent study, however (5) it was reported that peptic digests of lactalbumin not native lactalbumin were potent stimuli of pancreatic secretion. It is conceivable that digests of protein in the intestine may stimulate the secretion of CCK-RP. To test this hypothesis we used a "donor-recipient" rat intestinal perfusion model that has been previously used to identify the CCK-RP (2) and examined the ability of peptone, a pancreatic protease digest of protein in the intestine, to stimulate the secretion of CCK-RP. Furthermore, we examined the enteric neural circuitry mediating CCK-RP secretion. The relative importance of extrinsic versus intrinsic neural pathways were studied and the location of the local neural plexus identified. In addition, we delineated the individual components of the enteric neural circuitry which mediates CCK-RP secretion.

Methods

Materials

The following were purchased from Sigma Chemical Co. (St. Louis, MO): Peptone, maltose, L-amino acids, atropine sulfate, hexamethonium and benzalkonium chloride.

Animal preparation

Male Sprague-Dawley rats, weighing between 250 and 300 grams, were fasted overnight and anesthetized with a mixture of xylazine and ketamine (13 and 87 mg/kg body wt, respectively). Supplemental doses were used every 2 h as needed to maintain adequate anesthesia. Through a midline incision, a polyethylene catheter was inserted into the common bile pancreatic duct at the ampulla. A second catheter was placed in the duodenum, slightly above the ampulla for the intestinal perfusion of bile pancreatic juice. The abdominal wound was covered with a saline gauge and the animal body temperature was monitored by a rectal thermal probe and maintained at 37°C with a heating pad. After 60 min of stabilization, combined bile and pancreatic secretions were collected every 15 min. The volume was measured, and an aliquot was taken and diluted with distilled water for protein determination. The remainder of the undiluted bile pancre-

atic juice was returned to the intestine via the duodenal cannula during the next collection period. Bile pancreatic juice protein was measured spectrophotometrically using the assay method of Bradford (6). Our previous study (7) has confirmed that the increase in protein output in the bile pancreatic juice following CCK-8 stimulation mainly reflected protein from the pancreatic source. Biliary juice protein did not increase with CCK stimulation.

Experimental protocols

Effect of intraluminal nutrients on CCK-RP secretion after 5-h diversion of BPI. To investigate the effect of intraluminal peptone on CCK-RP secretion, we utilized the donor-recipient rat model described previously (2). The common bile-pancreatic duct was cannulated at the ampulla for diversion of bile pancreatic juice. Two cannulas were placed into the small bowel: one in the proximal duodenum for the infusion of saline or nutrient solutions and the other in the jejunum 20 cm distal to the ligament of Treitz for collection of intestinal perfusates. We have shown previously that diversion of bile pancreatic juice from the intestine resulted in a marked increase in plasma CCK levels and pancreatic secretion. These increases gradually returned to basal after 5 h of diversion due to a reduction in the luminal CCK-RP (8). This period is optimal to test the ability of peptone or other nutrients to stimulate the secretion of CCK-RP. After a 5-h diversion of bile pancreatic juice, donor rats received an intraduodenal perfusion of phosphate-buffered saline, 5% peptone (mol wt < 1000, pH 6.0, osmolality 300 mOsm/liter), 18% casein, maltose (300 mM pH 6.0, osmolality 300 mOsm/liter) or 5% L-amino acids (pH 6.0, osmolality 300 mOsm/liter) at the rate of 3 ml/min for 3 h. The 5% peptone test solution was prepared by ultrafiltration using an Amicon membrane (YM1) and the filtrate which only contained peptides with mol wt < 1000 was used as a test solution to infuse into the donor rat. The intestinal perfusate collected from the donor rat was filtered with Whatman No. 54 paper to remove any large particles such as intestinal debris. The filtrate was then ultrafiltered with an Amicon membrane (YM1) and the residue on the Amicon membrane which contained peptides with mol wt > 1000 was washed with 250 ml of water until the filtrate gave a negative ninhydrin test (~5 washes) indicating that all the peptones had been removed from the residue. The residue which contained the CCK-RP (mol wt > 1000) was then dissolved in water and lyophilized. The latter was then suspended in phosphate buffered saline and adjusted to pH 7.4 for intraduodenal administration in the recipient rats. The recipient rats received concentrated intestinal perfusate (1 ml) collected from donor rats and infused over 2 min at 5 h after bile pancreatic juice diversion when the plasma CCK has returned to basal level. Pancreatic protein output and plasma CCK levels were monitored every 10 min after the administration of concentrated intestinal perfusate in the recipient rats. The response of pancreatic secretion and plasma CCK levels in the recipient rats following the administration of intestinal perfusates collected from the donor rats served as a surrogate for the quantification of CCK-RP secreted in response to nutrient stimulation.

Effects of atropine, hexamethonium, guanethidine, and acute vagotomy on intraduodenal peptone-stimulated CCK-RP secretion. To determine the role of cholinergic pathways in the mediation of peptone induced CCK-RP secretion, atropine (100 µg/kg per hour) was administered intravenously to the donor rats 30 min before intestinal perfusion of 5% peptone as described earlier. Similar studies were performed with hexamethonium treatment in the donor rats (15 mg/kg bolus plus 7.5 mg/kg per hour continuous infusion) to determine the role of presynaptic cholinergic neurons in the mediation of peptone-stimulated CCK-RP secretion. To evaluate the role of adrenergic pathways, a separate group of donor rats received two daily injections of guanethidine i.p. at a dose of 20 mg/kg before peptone administration. This dosage regimen has previously been shown to deplete cardiac norepinephrine by 90% (9). In separate studies, we evaluated the role of the vagal nerve in mediating peptone-induced CCK-RP secretion. After a 5-h diversion of bile pancreatic juice,

acute bilateral subdiaphragmatic vagotomy was performed in donor rats. Through a midline incision of the abdominal wall, the stomach was carefully manipulated to expose the esophagus. The subdiaphragmatic vagal trunks were exposed halfway between the diaphragm and the gastric cardia. Both anterior and posterior trunks of the vagal nerves were transected. For control experiments, the abdominal vagal nerves were exposed but not cut. Intraluminal peptone perfusion studies were performed following acute vagotomy. Pancreatic amylase output and plasma CCK levels were monitored in the recipient rats following the same protocol as described earlier.

Effects of mucosal anesthetic and serosal application of benzalkonium chloride (BAC) on peptone-stimulated CCK-RP secretion. The enteric nervous system is composed of the myenteric and submucous plexuses. To investigate if the submucous plexus is involved in the mediation of CCK-RP secretion, 1% lidocaine was used as a topical anesthetic (2 ml/kg bolus plus 0.2 ml/min for 5 min at hourly intervals intraduodenally) ten minutes before the intraduodenal perfusion of 5% peptone. This method has previously been shown to inhibit the increase in superior mesenteric artery blood flow evoked by intraduodenal perfusion of 0.1N HCl (10). To investigate if myenteric neurons are important in mediating peptone-stimulated CCK-RP secretion, myenteric neurons were ablated with the surfactant benzalkonium chloride (BAC) applied to the serosa of the intestine every 5 min for 30 min (six applications) in the donor rats 15 d before the experiment (11). Serosal application of BAC has been shown to destroy more than 90% of the ganglia in the myenteric plexus, without affecting the submucous plexus (12). In control rats, 0.9% saline was applied in a similar manner. After 15 d, intestinal perfusion studies with 5% peptone were performed. Pancreatic protein output and plasma CCK levels of the recipient rats in response to infusion of intestinal perfusate collected from the donor rats were monitored following the same protocol as described earlier.

Effects of 5HT antagonist and substance P antagonist on peptone-stimulated CCK-RP secretion. To delineate the local neural reflex in the submucous plexus which mediates CCK-RP secretion, we examined the effect of a 5HT₃ receptor antagonist, ICS 205-930 which has been shown to prevent 5HT-induced vagal responses (13). ICS 205-930 (200 µg/kg iv) was administered to donor rats 30 min before the intraduodenal infusion of 5% peptone. Similar studies were performed with donor rats pretreated with the 5HT receptor antagonists such as 5HTP-DP-acetyl (1 mg/kg IV, 5HT_{1p} antagonist), and ketanserine (200 µg/kg IV, 5HT₂ antagonist). This will test the hypothesis that peptone in the intestine releases serotonin which in turn sets up a local reflex cascade to stimulate CCK-RP secretion from intestinal epithelial cells.

In separate studies we examined the effect of a highly specific, nonpeptide sub P antagonist, CP-96,345 (14). It has been previously shown that the administration of CP-96,345 markedly reduces the vascular inflammatory response to an allograft (15) and reduces toxin-A-induced secretion and mucosal permeability mediated by sub P (16). The sub P antagonist CP-96,345 (2.5 mg/kg i.p.) or its inactive enantiomer CP-96,344 was given to the donor rats 1 h before intraduodenal administration of 5% peptone.

Bioassay of plasma CCK. Plasma CCK was extracted and bioassayed as previously described (17). Plasma CCK was absorbed onto C18 Sep-Pak cartridges (Waters Division of Millipore, Millford, MA), washed, and the CCK eluted with 1 ml acetonitrile/water (1:1) into a polyethylene scintillation vial and dried in a 45°C water bath under a flow of nitrogen. Previous studies involving the addition of CCK-8 and CCK-33 to the C18 Sep-Pak cartridges showed recoveries of 87±6% and 84±5%, respectively (18).

1-ml aliquots of acini suspension were added to the vials containing the plasma extracts or known amounts of CCK-8 and incubated for 30 min at 37°C. Amylase released into the medium and total acinar amylase content were measured using porcion yellow starch as a substrate. The percentage of total amylase released by plasma extracts was compared with the dose-response curve to CCK-8. Plasma CCK was expressed as CCK-8 equivalents.

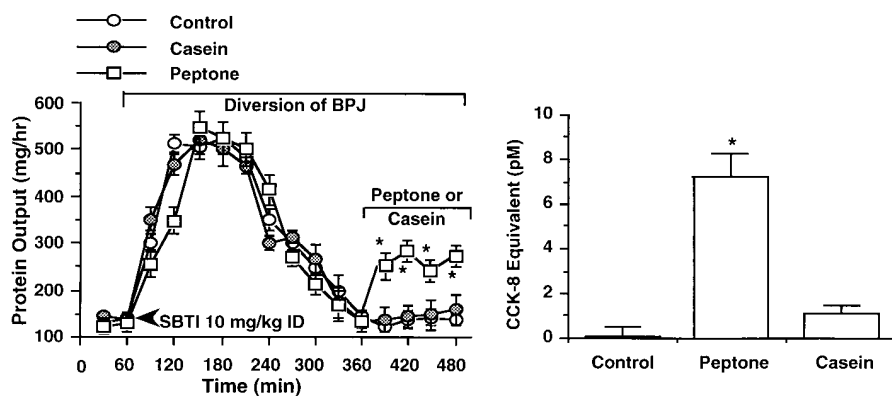


Figure 1. Pancreatic protein output and plasma CCK levels in rats perfused with casein or peptone solutions between the fifth and eighth hour post diversion of bile pancreatic juice. Values are mean $\pm$ SE for six rats in each group. * $P < 0.01$.

Statistical analysis. Results were expressed as mean $\pm$ SE. The multivariate analysis of variance method was used to evaluate the effects of the repeated measurement over time, treatment effects, and the interaction between them. Significance was accepted at the 5% level.

Results

Effects of intraluminal nutrients on CCK-RP secretion. To investigate the effects of intraluminal nutrients on the secretion of CCK-RP, we used the donor-recipient rat model described previously (2). The recipient rats were used as test rats for the measurement of CCK-RP.

5 h after diversion of bile pancreatic juice, the pancreatic protein output has returned to basal levels and was stable, averaging 120 ± 7 mg/h. The basal plasma CCK was 0.5 ± 0.1 pM.

Intraduodenal infusion of 5% peptone caused a significant increase in pancreatic protein output accompanied by an increase in plasma CCK levels to 7.1 ± 1.3 pM (Fig. 1). In contrast intraduodenal administration of 18% casein failed to increase pancreatic secretion and plasma CCK levels (Fig. 1).

In separate studies, infusion of concentrated intestinal perfusate (1 ml) collected from the donor rat during the fifth to the eighth hour after diversion resulted in no significant increase in pancreatic protein output or plasma CCK levels. This indicates that the concentrated intestinal perfusate collected from the donor rat contained no significant amounts of CCK-RP. In contrast, infusion of concentrated intestinal perfusate (1 ml) collected from the donor rat when it was perfused with 5% peptone caused a $62 \pm 10\%$ increase in protein secretion (Fig. 2A) and an elevation of plasma CCK levels to 6.9 ± 1.8 pM (Fig. 2B). Note that the peptone (mol wt < 1000) in the intestinal perfusate collected from the donor rat was removed by ultrafiltration which was confirmed by a negative ninhydrin staining.

In separate studies, we showed that intraduodenal infusion of concentrated perfusate collected from donor rats which were perfused with maltose (300 mM) or 5% L-amino acids did not significantly increase pancreatic protein secretion or plasma CCK levels (Fig. 2, A and B). These observations indicate that peptone but neither maltose nor L-amino acids stimulated CCK-RP secretion.

Effects of vagotomy, atropine, guanethidine and hexamethonium. To investigate the mechanisms by which peptone stimulates CCK-RP secretion, acute bilateral subdiaphragmatic vagotomy was performed in the donor rats 30 min before

intraduodenal perfusion of 5% peptone. The administration of concentrated perfusate collected from the acute vagotomized donor rats caused a $64 \pm 12\%$ increase in pancreatic protein output over basal and elevated plasma CCK levels to 7.3 ± 1.1 pM (Fig. 3, A and B). These values were similar to those observed in control experiments indicating that the vagus nerves do not play a role in the mediation of CCK-RP secretion. Similarly, administration of concentrated perfusate collected from donor rats pretreated with guanethidine increased plasma CCK levels to 6.4 ± 0.7 pM and caused a $60 \pm 12\%$ increase in protein output over basal in the recipient rats (Fig. 3, A and B). In contrast, administration of concentrated perfusates collected from donor rats pretreated with atropine or hexamethonium failed to significantly increase pancreatic amylase secretion or plasma CCK levels in the recipient rats (Fig. 3, A and B). These observations indicate that peptone acts at a presyn-

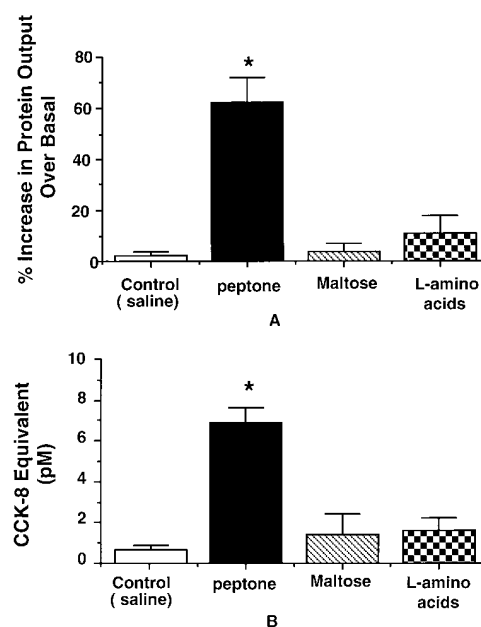


Figure 2. Pancreatic protein outputs (A) and plasma CCK levels (B) in recipient rats in response to intraduodenal administration of concentrated intestinal perfusates obtained from donor rats perfused with various nutrient solutions between the fifth and eighth hour post diversion of bile pancreatic juice. Values are mean $\pm$ SE for eight rats in each group. * $P < 0.01$.

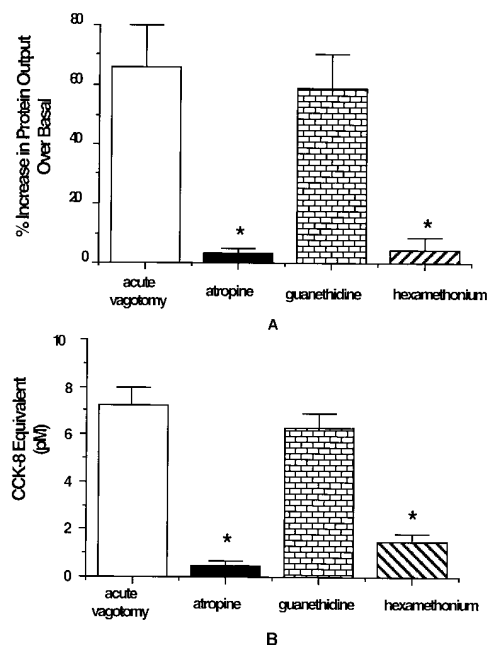


Figure 3. Pancreatic protein secretion (A) and plasma CCK levels (B) in the recipient rats in response to intraduodenal administration of concentrated intestinal perfusates obtained from donor rats perfused with 5% peptone solution. Donor rats were pretreated with acute vagotomy, atropine (100 μ g/kg per hour), guanethidine (5 mg/kg) or hexamethonium (15 mg/kg bolus plus 7.5 mg/kg per hour iv infusion) before peptone infusion. Values are mean $\pm$ SE for eight rats in each group. * $P < 0.01$.

aptic site of the extravagal cholinergic pathway (enteric nervous system) to stimulate CCK-RP secretion.

Effects of mucosal anesthetic and serosal application of benzalkonium chloride (BAC). Administration of concentrated peptone perfusate collected from donor rats pretreated with local anesthetic (lidocaine) failed to increase the plasma CCK levels and pancreatic protein secretion in recipient rats (Fig. 4, A and B). Thus topical application of lidocaine prevented CCK-RP secretion stimulated by 5% peptone. This indicates that a local enteric neural circuitry is involved in mediating the secretion of CCK-RP. In contrast to the observation made with mucosal application of lidocaine, intraduodenal administration of concentrated perfusate collected from donor rats pretreated with serosal benzalkonium chloride which ablates myenteric neurons resulted in a $64 \pm 12\%$ increase in protein secretion and elevated plasma CCK level to 6.0 ± 0.5 pM (Fig. 4, A and B). These values were similar to levels observed in control experiments. Therefore it appears that the submucosal neural plexus but not the myenteric plexus is involved in the mediation of peptone stimulated CCK-RP secretion.

Effects of 5HT and substance P antagonists. To delineate the local neural reflex in the submucosal plexus which mediates CCK-RP secretion, we examined the effects of 5HT and substance P antagonists since both 5HT and substance P are found in abundance in the enterochromaffin cells and the enteric neural plexuses. Intraduodenal administration of concentrated perfusates collected from donor rats pretreated with ketanserin (5HT₂ antagonist) or ICS 205-930 (5HT₃ antagonist) resulted in partial or no increase in pancreatic protein output and plasma CCK levels in the recipient rats (Fig. 5, A and B).

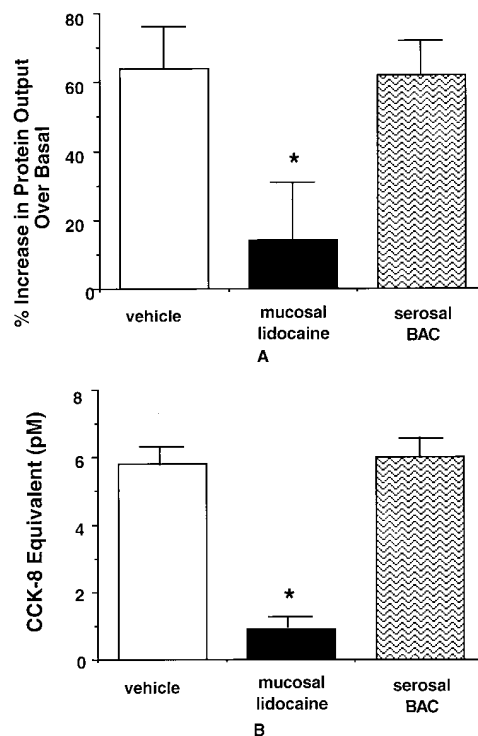


Figure 4. Pancreatic protein secretion (A) and plasma CCK levels (B) in recipient rats in response to intraduodenal administration of concentrated intestinal perfusates obtained from donor rats perfused with 5% peptone solution. Donor rats were pretreated with topical application of lidocaine or serosal application of benzalkonium chloride (BAC). Values are mean $\pm$ SE for eight rats in each group. * $P < 0.01$.

On the other hand, infusion of intestinal perfusates obtained from donor rats pretreated with 5HTP-DP-acetyl (5HT_{1p} antagonist) resulted in normal increases in pancreatic protein secretion and plasma CCK levels in the recipient rats (Fig. 5, A and B). This suggests that 5HT₃ and possibly 5HT₂, but not 5HT_{1p} receptors are involved in the mediation of CCK-RP secretion.

In separate studies, we showed that intestinal perfusates collected from donor rats pretreated with the substance P antagonist (CP-96,345) but not its inactive enantiomer (CP-96,344), when infused into the recipient rats resulted in no increase in pancreatic protein secretion (Fig. 6). This suggests that substance P containing neurons are involved in the local neural reflex mediating the secretion of CCK-RP.

Discussion

It has been previously demonstrated that protein is the major dietary factor stimulating CCK release in the rats (1), although the responsible mechanism(s) remains to be determined. Recent studies suggest that protein does not act directly on CCK containing cells (19) but is likely to involve some intermediary luminal factor(s). In species such as rats where feedback inhibition of pancreatic secretion occurs, CCK release appears to be controlled by active intraluminal proteases (4). Since proteins may bind and thus inhibit intraluminal endopeptidases, it has been proposed that dietary protein in the intestine by com-

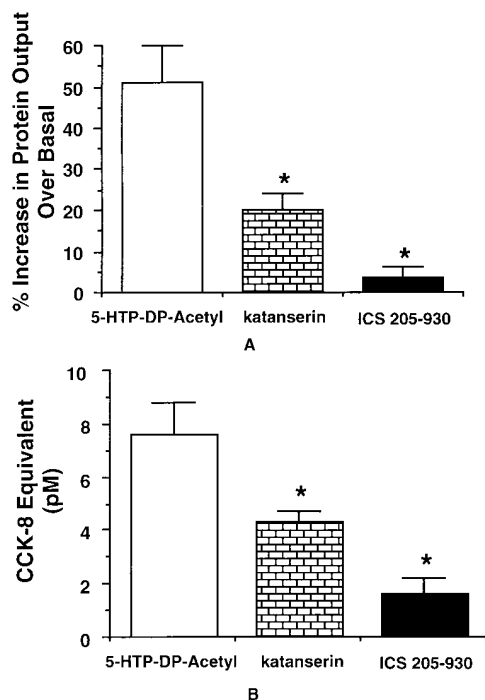


Figure 5. Pancreatic protein outputs (A) and plasma CCK levels (B) in recipient rats in response to intraduodenal administration of concentrated intestinal perfusates obtained from donor rats perfused with 5% peptone. Donor rats were pretreated with various 5HT receptor antagonists, 5HTP-DP-acetyl (1 mg/kg iv), katanserin (200 μ g/kg iv) and ICS 205-930 (200 μ g/kg iv). Values are mean $\pm$ SE for six rats in each group. * $P < .005$.

peting for trypsin which would otherwise inactivate the CCK-RP, causes an increase in luminal CCK-RP which, in turn, releases CCK and stimulates pancreatic secretion (4). This represents the major mechanism by which intact proteins such as casein stimulate CCK release. Our current study clearly showed that in contrast to intact protein which binds proteolytic enzymes but does not directly stimulate CCK-RP secretion, peptone stimulates CCK-RP release into the lumen. In contrast, maltose and L-amino acids failed to evoke secretion of CCK-RP into the lumen. These findings are consistent with the observations that protein and peptides but not carbohydrates (1) or amino acids (1) elevate plasma CCK levels in the rats.

Our studies showed that in rats with diversion of bile pancreatic juice, casein was ineffective to stimulate CCK release and pancreatic secretion. This supports the proposal that casein stimulates CCK release by protecting the intraluminal CCK-releasing peptides from inactivation (2, 20). It is interesting to note that Hara et al. (21) who reported that casein stimulated pancreatic secretion in rats with chronic (7 d) division of bile pancreatic juice. This unusual observation requires confirmation since it differs from the earlier studies by Schneeman and Lyman (22) who showed that the pancreas failed to respond to trypsin inhibitors under conditions of chronic bile pancreatic juice diversion. Hara et al. proposed that the longer period of bile pancreatic juice diversion in their model may restore the responsiveness of acinar cells to CCK (21). In their studies however neither CCK nor CCK-RP were measured making it difficult to explain their observation.

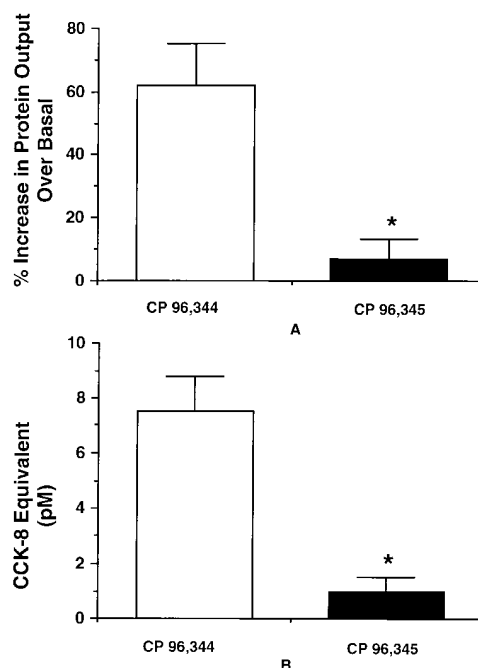


Figure 6. Pancreatic protein outputs (A) and plasma CCK levels (B) in recipient rats in response to intraduodenal administration of concentrated intestinal perfusates obtained from donor rats perfused with 5% peptone solution. Donor rats were pretreated with the sub P antagonist CP-96,345 (2.5 mg/kg, ip) or its inactive enantiomer CP-936,344 (2.5 mg/kg, ip). Values are mean $\pm$ SE for six rats in each group. * $P < 0.05$.

Similar to the observations of Spannagel and Green (23), and Levan et al. (24), we found that peptone could stimulate CCK secretion in rats with diversion of bile pancreatic juice. Peptides appear to mediate the effect of peptone because mixed amino acids were ineffective to evoke pancreatic secretion or plasma CCK release. It has been proposed that luminal nutrients may be necessary to maintain normal CCK synthetic activity and that the release of CCK occurs spontaneously in the absence of the suppressive effects of pancreatic proteases (24). Our studies clearly demonstrate that peptone in the duodenum acts by stimulating the luminal secretion of CCK-RP and this in turn stimulates the release of CCK. This may explain the observation that peptide digests of lactalbumin not intact lactalbumin were potent stimuli of pancreatic enzyme secretion (5). Therefore it appears that intact protein such as casein acts by competing for proteolytic enzymes which would otherwise inactivate the CCK-RP. The resulting increase of active CCK-RP in the intestine releases CCK and stimulates pancreatic secretion. On the other hand, peptone, a pancreatic protease digest of protein is capable of stimulating the secretion of CCK-RP. These two mechanisms acting in concert may be important in the postprandial increase of CCK secretion in rats.

Extrinsic innervation of the gut does not appear to play a role in controlling the release of CCK. Truncal vagotomy had no effect on basal or tryptophan stimulated CCK release in dogs (25). In anesthetized rats, vagotomy also did not affect endogenous CCK release in response to the diversion of bile pancreatic juice or casein feeding (26). Similarly, the basal CCK release was not controlled by the vagus and vagal stimu-

lation did not augment or diminish protein stimulated CCK release in conscious rats (27). The demonstration that vagotomy did not affect the secretion of CCK-RP in the present study is consistent with the above observations. In addition we also demonstrated that adrenergic blockade also did not influence the secretion of CCK-RP in response to protein stimulation.

In contrast to vagotomy, the administration of atropine and hexamethonium completely abolished the secretion of CCK-RP. This suggests that the secretion of CCK-RP may involve a nicotinic synapse and a cholinergic secretomotor neuron. We have also shown previously that atropine inhibited the rise in plasma CCK and pancreatic secretion evoked by diversion of bile pancreatic juice in anesthetized rats (18). The effect of atropine on feedback regulation of pancreatic secretion however is controversial. Other investigators showed that atropine has no effect in conscious rats (28). Experimental design may account for these differences. In the studies by Levan and Green (28), conscious rats were used and bile pancreatic juice was returned to the duodenum. Return of the bile pancreatic juice, in addition to the infusion of trypsin inhibitor, may contribute to the lack of effects of atropine in conscious rats observed by these investigators. Other factors generated by changes in the state of consciousness may also be responsible for these differences.

We have previously demonstrated that intramural neural pathways are involved in mediating the secretion of CCK-RP, since infusion of tetrodotoxin into the superior mesenteric artery inhibited the rise in plasma CCK and pancreatic secretion evoked by the diversion of bile pancreatic juice (18). The enteric nervous system is composed of the myenteric and submucous plexuses. To identify the local neural plexus involved, we examined the effect of a topical anesthetic, lidocaine, applied directly to the duodenal mucosa. This technique has been shown to abolish the rise in plasma CCK and pancreatic secretion evoked by the diversion of bile pancreatic juice (18) but did not affect the functioning of the myenteric plexus since it did not inhibit duodenal contraction in response to vagal stimulation (unpublished data). In our study we showed that the intraduodenal application of lidocaine completely abolished the secretion of CCK-RP evoked by peptone. In contrast, serosal application of the surfactant, benzalkonium chloride, to the duodenum, which has been shown to ablate > 90% of the ganglia in the myenteric plexus without affecting the submucous plexus (12), did not influence the secretion of the CCK-RP. To demonstrate that we have successfully destroyed most of the myenteric plexus, we showed no contraction in the benzalkonium chloride treated intestinal segment in response to electrical field stimulation (unpublished data). These observations indicate that the enteric circuitry controlling CCK-RP secretion is present in the submucous not myenteric plexus. Similar studies with intestinal perfusion of lidocaine and intraarterial infusion of tetrodotoxin showed that the submucous nervous system is involved in mediating intestinal secretion induced by cholera toxin (29) or bile salts (30).

To delineate the local neural reflex in the submucous plexus which mediates CCK-RP secretion, we examined the effect of the 5HT₃ receptor antagonist, ICS 205-930 which has been shown to prevent 5HT-induced vagal afferent mediated inhibition of the nociceptive tailflick reflex and a complex series of cardiovascular responses (13). Administration of ICS 205-930 markedly inhibited the secretion of CCK-RP in response to peptone stimulation, suggesting that 5HT is involved

in activating the neural reflex responsible for the secretion of CCK-RP. Others have shown that mucosal stroking or cholera toxin also released 5HT, which in turn activates a reflex cascade to induce secretion via an atropine-sensitive pathway (31–33). Myenteric neurons contain 5HT (34, 35), but none of the neurons in the submucous plexus are thought to be serotonergic (34–36). Since we have ruled out the involvement of the myenteric plexus, the most obvious source of 5HT is the mucosal enterochromaffin cells which are well situated for sensing intraluminal chemical or mechanical events. The receptors responsible for the 5HT mediated reflex appears to be the 5HT₃ receptor since administration of 5HT_{1p} receptor antagonists failed to affect the secretion of CCK-RP. The dose of 5HTP-DP-acetyl (5HT_{1p} antagonist) used has been previously shown to significantly increase the rate of gastric emptying (37). It is interesting to note that ketanserin, a 5HT₂ receptor antagonist partially inhibited the secretion of CCK-RP. This suggests that 5HT may concurrently activate both 5HT₂ and 5HT₃ receptor subtypes located in the submucous plexus to stimulate the secretion of CCK-RP. Although activation of two receptor subtypes to elicit a single response may at first seem unusual, there has been a number of reports using receptor-selective agonists and antagonists at α -adrenoreceptor (38), dopamine (39), cholinergic (40), adenosine (41), and histamine (42) receptors that have clearly shown that dual activation of two receptor subtypes may be involved to elicit a single response. In fact, recently it has been shown that the peripheral co-nociceptive actions of 5HT in the viscera require the activation of 5HT₂ and 5HT₃ receptor subtypes located in cardiopulmonary capsaicin-sensitive vagal afferents (43). Further detailed dose response studies are needed to examine if dual activation of 5HT₂ and 5HT₃ receptor subtypes is necessary to evoke the secretion of CCK-RP.

Recently studies demonstrated that primary afferent neurons exist in the submucous plexus. These neurons contain acetylcholine as well as neuropeptides, including substance P and calbinin (32). Their morphological and functional characteristics are consistent with their role as sensory neurons (44, 45). They are multipolar and receive few synaptic inputs indicating they are neither relayed or final neurons in a reflex pathway. Previous studies have demonstrated that administration of the substance P antagonist CP-96,345, markedly reduced the vascular inflammatory response to an allograft (15) and reduced toxin-A-induced secretion and mucosal permeability mediated by substance P (16). These findings indicate that sub P containing afferent neurons in the submucous plexus may mediate various physiological processes of the intestinal mucosa. We showed that administration of the substance P antagonist CP-96,345 not its enantiomer SCP-96,344 inhibited the secretion of CCK-RP. This suggests that substance P containing neurons in the submucous plexus are involved in the local neural reflex mediating the secretion of CCK-RP.

Based on the observations generated in this study, we propose that peptone in the duodenum stimulates 5HT release from enterochromaffin cells which activates 5HT receptors located on sensory substance P neurons in the submucous plexus. Signals are then transmitted to cholinergic interneurons and to epithelial CCK-RP containing cells via cholinergic secretomotor neurons (Fig. 7). This enteric neural circuitry appears to be responsible for the secretion of CCK-RP in response to dietary protein stimulation and plays an important role in the postprandial release of CCK in rats.

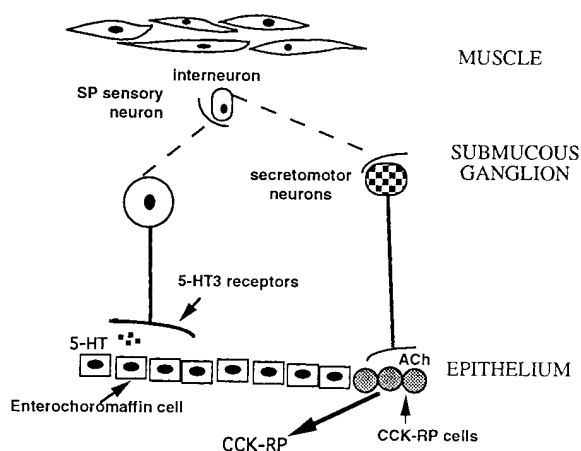


Figure 7. Proposed enteric circuitry controlling CCK-RP secretion. Peptone in the lumen stimulates the release of 5HT from enterochromaffin cells which activates 5HT receptors on sensory substance P neurons in the submucous plexus. Signals are then transmitted to cholinergic interneurons and to epithelial CCK-RP containing cells via cholinergic secretomotor neurons.

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