Effects of peripheral CCK receptor blockade on gastric emptying in rats

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Reidelberger, Roger D., Linda Kelsey, Dean Heimann, and Martin Hulce. Effects of peripheral CCK receptor blockade on gastric emptying in rats. Am J Physiol Regul Integr Comp Physiol 284: R66–R75, 2003. First published October 3, 2002; 10.1152/ajpregu.00484.2002.—Type A CCK receptor (CCKAR) antagonists differing in blood-brain barrier permeability [devazepide penetrates; the dicyclohexylammonium salt of Nα-3-quinalinoyl-d-Glu-V,N-dipentylamide (A-70104) does not] were used to test the hypothesis that duodenal nutrient-induced inhibition of gastric emptying is mediated by CCKARs located peripheral to the blood-brain barrier. Rats received A-70104 (700 or 3,000 nmol kg−1 h−1 iv) or devazepide (2.5 μmol/kg iv) and either a 15-min intravenous infusion of CCK-8 (3 nmol kg−1 h−1) or duodenal infusion of casein, peptone, Intralipid, or maltose. Gastric emptying of saline was measured during the last 5 min of each infusion. A-70104 and devazepide abolished the gastric emptying response to a maximal inhibitory dose of CCK-8. Each of the macronutrients inhibited gastric emptying. A-70104 and devazepide attenuated inhibitory responses to each macronutrient. Intravenous injection of a CCK antibody to immunoneutralize circulating CCK had no effect on peptone or Intralipid-induced responses. Thus endogenous CCK appears to act in part by a paracrine or neurotransmitter mechanism at CCKARs peripheral to the blood-brain barrier to inhibit gastric emptying.

CCK is a peptide that is found throughout the brain and in neurons and endocrine cells of the gastrointestinal tract. Studies demonstrating that systemic administration of type A CCK receptor (CCKAR) antagonists attenuate nutrient-induced inhibition of gastric emptying in a variety of species provide compelling evidence that CCK plays an essential role in the physiological control of gastric emptying (4, 12, 13, 15, 28, 37, 49). The popular hypothesis is that CCK, secreted from enteroendocrine cells in the upper small intestine in response to duodenal delivery of nutrients, acts through paracrine stimulation of intestinal vagal sensory neurons to inhibit gastric emptying. This hypothesis is supported by studies demonstrating the existence of CCK-secreting endocrine cells in the epithelium of the upper small intestine (48), CCKARs within vagal af-

Methods

Subjects. Male Sprague-Dawley rats (Sasco, Madison, WI; ~350 g at the start of the study) were housed individually in hanging wire-mesh cages in a temperature-controlled room with a 12:12-h light-dark cycle (lights off at 1600). The animals were provided ground rat chow (Purina #5001, 3.3 kcal/g) and water ad libitum. The Animal Studies Subcom-

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mittee of the Omaha Veterans Affairs Medical Center approved the experimental protocol. Animal experimentation was conducted in conformity with the guiding principles of the American Physiological Society for research involving animals and human beings (1).

**Surgical procedures.** Gastric, duodenal, and jugular vein cannulas were implanted according to procedures described previously (41, 50). The stainless steel gastric cannula was used to instill saline into the stomach and to withdraw gastric contents. The duodenal cannula was implanted in the aboral direction with its tip located 3 cm distal to the pyloric sphincter. The duodenal cannula was plugged with stainless steel wire and flushed with 0.5 ml of physiological saline every other day to maintain patency. The jugular vein cannula was filled with heparinized saline (60 U/ml), plugged with stainless steel wire, and flushed with 0.5 ml of heparinized saline every other day to maintain patency.

**Effects of devazepide and A-70104 on CCK-8-induced inhibition of gastric emptying.** This series of experiments defined doses of A-70104 and devazepide that can block the effects of a maximal inhibitory dose of CCK-8 (3 nmol·kg⁻¹·h⁻¹) on gastric emptying. The dose-response effects of CCK-8 on gastric emptying were determined in a prior study (39). Five different experiments were performed. The first two experiments determined the effects of a bolus intravenous injection of devazepide (2.5 μmol/kg) on CCK-8-induced inhibition of gastric emptying and on gastric emptying when administered alone. The next three experiments determined the effects of intravenous infusion of 700 and 3,000 nmol·kg⁻¹·h⁻¹ of A-70104 on CCK-8-induced inhibition of gastric emptying, and intravenous infusion of 700 nmol·kg⁻¹·h⁻¹ of A-70104 on gastric emptying when administered alone.

Rats with gastric and jugular vein cannulas were adapted to a 17-h fast, followed by light restraint in a Bollman-type cage, flushing of the stomach with water, and a 15-min intravenous infusion (3.2 ml/h) of 0.15 M NaCl, 0.1% BSA. On experimental days, rats received a bolus intravenous injection of devazepide (2.5 μmol/kg, Merck Sharpe & Dohme Research Laboratories) or vehicle (5% DMSO, 5% Tween 80, 90% 0.15 M NaCl) 10 min before receiving a 15-min intravenous infusion of CCK-8 (3 nmol·kg⁻¹·h⁻¹), Research Plus, Bayonne, NJ) or vehicle (0.15 M NaCl, 0.1% BSA). Ten minutes after onset of CCK-8 infusion, 3 ml of saline containing 60 mg/ml phenol red was instilled into the stomach. Gastric contents were recovered 5 min later through the gastric cannula, the volume was measured, and the concentration of phenol red was determined spectrophotometrically to provide a measure of the amount of saline emptied during the 5-min period. A-70104 experiments were identical in design, except rats received a 25-min intravenous infusion of A-70104 (700 or 3,000 nmol·kg⁻¹·h⁻¹), Abbott Laboratories, Abbott Park, IL) or vehicle (0.15 M NaCl, 0.1% BSA, 1% DMSO) beginning 10 min before onset of CCK-8 or vehicle infusion. Each rat (n = 11 or 12) received each treatment in random order at intervals of at least 48 h.

**Effects of devazepide and A-70104 on gastric emptying responses to duodenal infusions of protein, fat, and carbohydrate.** Seventeen experiments were performed. The first four experiments determined the dose-dependent effects of duodenal infusion of four different nutrients on gastric emptying. Nutrient doses were casein (0.1, 0.2, 0.4, 0.9 g/h; casein-Hammersten, United States Biochemical), peptone (0.7, 1.4, 2.1, 2.8 g/h; EZMix tryptone, Sigma), Intralipid (0.2, 0.4, 0.9, 1.8 g/h; Baxter Healthcare), and maltose (0.7, 1.3, 2.6, 5.2 g/h; Sigma). The next four experiments determined the effects of devazepide (2.5 μmol/kg) and A-70104 (700 nmol·kg⁻¹·h⁻¹) on gastric emptying responses to casein (0.9 g/h) and peptone (0.9 and 1.4 g/h). The next six experiments determined the effects of devazepide (2.5 μmol/kg) and A-70104 (700 nmol·kg⁻¹·h⁻¹) on gastric emptying responses to Intralipid (0.2, 0.4, and 1.6 g/h). The final three experiments determined the effects of devazepide (2.5 μmol/kg) and A-70104 (700 or 3,000 nmol·kg⁻¹·h⁻¹) on gastric emptying responses to maltose (1.1 and 1.3 g/h). Treatments were administered to groups of 8–12 rats as above for the CCK-8 experiments, with the exception that nutrient doses were infused into the duodenum at a rate of 8.9 ml/h for 15 min. Water was used as vehicle and diluent for each nutrient dose.

**Effects of immunoneutralization of circulating CCK on gastric emptying responses to intravenous infusion of CCK-8 and duodenal infusions of peptone and Intralipid.** Four experiments were performed using procedures similar to those described above for determining the effects of devazepide and A-70104 on CCK-8- and duodenal nutrient-induced inhibition of gastric emptying. The first two experiments determined the effects of intravenous injections (0.1 ml) of the CCK monoclonal antibody (0.1 mg CCK MAb 93) and control keyhole limpet hemocyanin monoclonal antibody (0.1 mg KLH MAb) on the gastric emptying response to intravenous infusion of CCK-8 (1 nmol·kg⁻¹·h⁻¹). The final two experiments determined the effects of intravenous injection (0.1 ml) of CCK MAb 93 (0.1 mg) on gastric emptying responses to duodenal infusions of peptone (1 g/h) and Intralipid (0.2 g/h). CCK MAb was not tested with maltose because carbohydrates produce little if any increase in plasma CCK (5, 8, 24) and thus are not likely to inhibit gastric emptying by an endocrine mechanism. Antibodies were diluted separately in 0.15 M NaCl before intravenous administration. CCK MAb 93 and KLH MAb were obtained from the Antibody Core of the CURE Gastroenteric Biology Center, West Los Angeles VA Medical Center. The procedures used to produce, purify, and characterize these antibodies were described previously (21). CCK MAb 93 has high binding affinity and specificity for a full range of biologically active CCK and gastrin molecules that contain the common COOH-terminal pentapeptide region. We used these same antibodies in a previous study investigating the role of circulating CCK in control of food intake and pancreatic secretion (42).

**Statistical analyses.** Values are presented as means ± SE. Data from each experiment were analyzed separately. Effects of devazepide, A-70104, and CCK MAb 93 on gastric emptying, CCK-8-induced inhibition of gastric emptying, and duodenal nutrient-induced inhibition of gastric emptying were evaluated using either a paired t-test or a repeated-measures ANOVA depending on the design of a specific experiment. In each analysis, planned comparisons of treatment means were evaluated by direct contrasts of means using the computer program SYSTAT. Differences between means were considered significant when P < 0.05. A one-tailed test was used for postulated unidirectional effects.

**RESULTS**

Effects of devazepide and A-70104 on CCK-8-induced inhibition of gastric emptying. Figure 1A shows the effects of devazepide (2.5 μmol/kg iv) on the gastric emptying response to a maximal inhibitory dose of CCK-8 (3 nmol·kg⁻¹·h⁻¹ iv). CCK-8 significantly inhibited gastric emptying by 49% (P < 0.001). Devazepide injection before CCK-8 infusion resulted in a gastric emptying response that was not different from
that observed after vehicle administration alone (P > 0.05). In a subsequent experiment, devazepide (2.5 μmol/kg iv) administration alone did not affect gastric emptying [2.0 ± 0.13 ml emptied after devazepide injection vs. 2.3 ± 0.06 ml after vehicle injection (P > 0.05)].

Figure 1B shows that a maximal inhibitory dose of CCK-8 (3 nmol·kg⁻¹·h⁻¹ iv) reduced gastric emptying by 55% (P < 0.001), and A-70104 (700 nmol·kg⁻¹·h⁻¹ iv) significantly attenuated this response by 46% (P < 0.01). A larger dose of A-70104 (3,000 nmol·kg⁻¹·h⁻¹ iv) completely blocked the CCK-8-induced response (Fig. 1C). In a separate experiment, A-70104 (700 nmol·kg⁻¹·h⁻¹ iv) administration alone had no effect on gastric emptying [1.9 ± 0.1 ml emptied after A-70104 vs. 1.9 ± 0.09 ml after vehicle injection (P > 0.05)].

Effects of devazepide and A-70104 on gastric emptying responses to duodenal infusions of protein, fat, and carbohydrate. Figure 2A shows that duodenal infusion of casein dose dependently reduced the volume of saline emptied from the stomach (F(3,32) = 31.9, P < 0.001). The minimal effective dose (0.2 g/h) inhibited gastric emptying by 15% (P < 0.05); the maximal effective dose (0.9 g/h; the largest dose given) inhibited emptying by 50% (P < 0.001). Figure 3A shows the effects of devazepide and A-70104 on casein-induced inhibition of gastric emptying. Casein (0.9 g/h) inhibited gastric emptying by 52% (P < 0.001) and devazepide (2.5 μmol/kg iv) significantly attenuated this response by 66% (P < 0.05). In the same experiment, A-70104 (700 nmol·kg⁻¹·h⁻¹ iv) significantly attenuated the casein-induced response by 32% (P < 0.05).

Figure 2B shows that duodenal infusion of peptone dose dependently reduced the volume of saline emptied from the stomach (F(3,46) = 8.0, P < 0.001). The minimal effective dose (0.7 g/h; the lowest dose given) inhibited gastric emptying by 23% (P < 0.05); the maximal effective dose (2.8 g/h; the largest dose given) inhibited emptying by 49% (P < 0.001). Figure 3B shows the effects of devazepide and A-70104 on peptone-induced inhibition of gastric emptying. Figure 3B shows that peptone (0.9 g/h) inhibited gastric emptying by 46% (P < 0.01) and that devazepide (2.5 μmol/kg iv) significantly attenuated the response by 64% (P < 0.001). In the same experiment, A-70104 (700 nmol·kg⁻¹·h⁻¹ iv) significantly attenuated the peptone-induced response by 51% (P < 0.05). Figure 3C shows that duodenal infusion of a higher dose of peptone (1.4 g/h) inhibited gastric emptying by 41% (P < 0.01) and that devazepide (2.5 μmol/kg iv) significantly attenuated the response by 57% (P < 0.05). In a separate experiment, this same dose of peptone (1.4 g/h) inhibited gastric emptying by 46% (P < 0.001), Fig. 3D) and A-70104 (700 nmol·kg⁻¹·h⁻¹ iv) significantly attenuated the response by 46% (P < 0.05).

Figure 2C shows that duodenal infusion of maltose dose dependently reduced the volume of saline emptied from the stomach (F(3,36) = 36.3, P < 0.001). The minimal effective dose (1.3 g/h) inhibited gastric emptying by 52% (P < 0.001); the maximal effective dose (5.2 g/h; the largest dose given) inhibited emptying by 65% (P < 0.001). Figure 4, A-C, shows the effects of devazepide and A-70104 on maltose-induced inhibition of gastric emptying. Figure 4A shows that maltose (1.3 g/h) in-
hibited gastric emptying by 40% \((P < 0.001)\) and that devazepide (2.5 \(\mu\)mol/kg iv) significantly attenuated the response by 64% \((P < 0.001)\). In the same experiment, A-70104 (700 \(\text{nmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\) iv) did not attenuate the maltose-induced response \((P > 0.05)\). Figure 4B shows that duodenal infusion of a lower dose of maltose (1.1 g/h) inhibited gastric emptying by 55% \((P < 0.001)\) and that devazepide (2.5 \(\mu\)mol/kg iv) significantly attenuated the response by 43% \((P < 0.05)\). In a separate experiment, this same dose of maltose (1.1 g/h) inhibited gastric emptying by 61% \((P < 0.001, \text{Fig. 4C})\) and a higher dose of A-70104 (3,000 \(\text{nmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\) iv) significantly attenuated this response by 27% \((P < 0.01)\).

Figure 2D shows that duodenal infusion of Intralipid reduced the volume of saline emptied from the stomach \((F_{4,44} = 4.5, P < 0.01)\). The minimal effective dose (0.2 g/h; the lowest dose given) inhibited gastric emptying by 32% \((P < 0.01)\); the maximal effective dose (1.8 g/h;...
the largest dose given) inhibited emptying by a similar amount (44%, $P < 0.01$). Figure 5, A–F, shows the effects of devazepide and A-70104 on Intralipid-induced inhibition of gastric emptying. Figure 5A shows that Intralipid (0.2 g/h) inhibited gastric emptying by 47% ($P < 0.001$) and that devazepide (2.5 $\mu$mol/kg iv) significantly attenuated the response by 49% ($P < 0.01$). Figure 5B shows that this same dose of Intralipid (0.2 g/h) inhibited gastric emptying by 52% ($P < 0.001$) and that A-70104 (700 nmol·kg$^{-1}$·h$^{-1}$ iv) significantly attenuated the response by 42% ($P < 0.05$). Figure 5C shows that duodenal infusion of a higher dose of Intralipid (0.4 g/h) inhibited gastric emptying by 65% ($P < 0.001$) and that devazepide (2.5 $\mu$mol/kg iv) significantly attenuated the response by 42% ($P < 0.05$). Figure 5D shows that this same dose of Intralipid (0.4 g/h) inhibited gastric emptying by 51% ($P < 0.01$) and that A-70104 (700 nmol·kg$^{-1}$·h$^{-1}$ iv) did not attenuate the response ($P > 0.05$). Figure 5E shows that duodenal infusion of a higher dose of Intralipid (1.6 g/h) inhibited gastric emptying by 54% ($P < 0.001$) and that devazepide (2.5 $\mu$mol/kg iv) did not attenuate the response ($P > 0.05$). Figure 5F shows that this same dose of Intralipid (1.6 g/h) inhibited gastric emptying by 66% ($P < 0.001$) and that A-70104 (700 nmol·kg$^{-1}$·h$^{-1}$ iv) also did not attenuate this response ($P > 0.05$).

Effects of immunoneutralization of circulating CCK on gastric emptying responses to intravenous infusion of CCK-8 and duodenal infusions of peptone and Intralipid. Figure 6A shows that intravenous infusion of CCK-8 (1 nmol·kg$^{-1}$·h$^{-1}$) significantly inhibited gastric emptying by 36% ($P < 0.01$) and that CCK MAb (0.1 mg iv) completely reversed this response ($P < 0.01$). In the same experiment, the CCK MAb did not affect gastric emptying when administered alone ($2.03 \pm 0.11$ ml emptied after CCK MAb vs. $2.06 \pm 0.12$ ml after vehicle ($P > 0.05$)). Figure 6B shows that CCK-8 (1 nmol·kg$^{-1}$·h$^{-1}$) significantly inhibited gastric emptying by 58% ($P < 0.001$) and that intravenous injection of the control monoclonal antibody KLH MAb did not affect this response ($P > 0.05$).

Figure 7, A and B, shows the effects of CCK MAb on gastric emptying responses to duodenal infusions of peptone and Intralipid. Peptone (1 g/h) significantly inhibited gastric emptying by 29% (Fig. 7A, $P < 0.01$) and CCK MAb (0.1 mg iv) did not attenuate this response ($P > 0.05$). Intralipid (0.2 g/h) also inhibited gastric emptying by 29% (Fig. 7B, $P < 0.01$) and CCK MAb (0.1 mg iv) had no effect on this response ($P > 0.05$).

DISCUSSION

CCKAR antagonists with different blood-brain barrier permeabilities [devazepide penetrates (34, 53); A-70104 does not (53)] were used to test the hypothesis that the inhibitory effects of duodenal delivery of carbohydrate, protein, and fat on gastric emptying are mediated in part by endogenous CCK action at CCK-ARs located peripheral to the blood-brain barrier. If this hypothesis were true, intravenous administration of either antagonist should attenuate inhibitory responses to duodenal infusions of each macronutrient. The present study demonstrated that 1) duodenal infusions of maltose, casein, peptone, and Intralipid inhibited gastric emptying of saline; 2) inhibitory responses to each macronutrient were attenuated by both devazepide and A-70104; and 3) intravenous injection of a CCK antibody to immunoneutralize circulating CCK had no effect on peptone- or Intralipid-
induced inhibition of gastric emptying. Together, these results suggest that endogenous CCK acts by a paracrine and (or) neurocrine mechanism at CCKARs peripheral to the blood-brain barrier to inhibit gastric emptying.

We chose to test the effects of A-70104 on gastric-emptying responses to duodenal infusion of maltose, peptone, casein, and Intralipid because devazepide had previously been shown to attenuate the inhibitory effects of each of these macronutrients, except for casein, on gastric emptying in rats (13, 15, 37). Rates of infusion of macronutrients into the duodenum in our study also appear to be within the physiological range of gastric emptying rates for these nutrients. Caloric rates of infusion in the present study were 0.11–0.17 kcal·kg$^{-2/3}$·min$^{-1}$ for casein, peptone, and maltose, and 0.06–0.44 kcal·kg$^{-2/3}$·min$^{-1}$ for Intralipid. Physiological rates of gastric emptying have been reported to be in the range of 0.17–0.53 kcal·kg$^{-2/3}$·min$^{-1}$ for rats, 0.15–0.43 kcal·kg$^{-2/3}$·min$^{-1}$ for monkeys, and 0.11–0.48 kcal·kg$^{-2/3}$·min$^{-1}$ for humans (19). Results were normalized to metabolic body size to facilitate interspecific comparisons (14).

It was not the intent of the present study to compare the effects of A-70104 across nutrients or doses of a specific nutrient or to compare the effects of A-70104 and devazepide. Only single doses of A-70104 and devazepide were tested with most nutrients. We believe that a meaningful comparison of the effects of A-70104 and devazepide would require that multiple doses of each antagonist be tested with each nutrient. Only then could A-70104 and devazepide potencies and efficacies be determined and compared in a statistically rigorous manner. This was not the intent of the present study, although we have used this approach in comparing the effects of CCK, amylin, and other amylin-related peptides on gastric emptying and food intake (39, 40).

We previously observed that devazepide’s ability to attenuate anorexic responses to duodenal nutrient infusions appears to diminish as nutrient doses are increased (50–52). We speculated that an apparent lower
effectiveness of devazepide at the higher nutrient doses may be due to a greater stimulation of redundant satiety mechanisms. This is the reason why in the present study, we first characterized the dose-response effects of duodenal infusion of each macronutrient on gastric emptying and then tested the effects of A-70104 on gastric emptying responses to low and high effective doses of each macronutrient. Devazepide, the gold standard CCKAR antagonist, which readily penetrates the blood-brain barrier, was used as a positive control, in that devazepide would be expected to attenuate those nutrient-induced responses that are attenuated by A-70104, which it did.

Numerous studies have previously demonstrated that systemic administration of the CCKAR antagonists devazepide and loxiglumide attenuates the inhibitory effects of oral, intragastric, and duodenal delivery of carbohydrate, peptone, and fat on gastric emptying in a variety of species (4, 12, 13, 28, 37, 49). It is not clear from these studies, however, whether the antagonists blocked central and (or) peripheral sites of endogenous CCK action to affect gastric emptying, because the antagonists may have penetrated the blood-brain barrier to block central as well as peripheral CCKARs. Devazepide clearly can penetrate the blood-brain barrier (34, 53). It is less certain whether this is also true for loxiglumide, a proglumide derivative, although there is some evidence to suggest that proglumide and the proglumide derivative CR1409 can penetrate the blood-brain barrier (17). The present study confirms and extends these earlier gastric emptying studies by showing that peripheral administration of A-70104, a CCKAR antagonist that does not readily penetrate the blood-brain barrier (53), attenuates the inhibitory effects of duodenal infusions of carbohydrate, peptone, and fat on gastric emptying.

There is strong evidence that exogenous CCK inhibits gastric emptying by stimulating an intestinal vagovagal mechanism (36). It remains to be established that endogenous CCK acts by the same pathway to inhibit gastric emptying. The popular hypothesis is that duodenal delivery of each of the major macronutrients stimulates the secretion of CCK from epithelial cells in the mucosa of the upper intestine, which acts locally at CCKAR receptors on vagal sensory nerves to stimulate the vago-vagal mechanism. If this hypothesis were true, then it would be important to show that 1) duodenal administration of each of the major macronutrients increases intestinal vagal afferent nerve activity, and CCKAR blockade attenuates this response; 2) duodenal administration of the various macronutri-
ents inhibits gastric emptying, CCKAR blockade attenuates this response, and blockade of intestinal vagal afferent transmission abolishes the CCKAR antagonist-induced response; and 3) vagal afferent fibers with CCKARs on their surface membrane are located near intestinal CCK-secreting cells.

Each of the major macronutrients has been reported to increase intestinal vagal afferent fiber activity (9, 18, 22, 26, 27, 35). Grundy and coworkers (9, 22) showed that peripheral administration of the CCKAR antagonist devazepide abolishes the stimulatory effect of luminal oleate and casein hydrolysate on intestinal vagal afferent nerve activity. Numerous other studies have shown that vagal denervation, as well as CCKAR blockade, attenuates the inhibitory effects of each of the major macronutrients on gastric emptying (11, 15, 37). The present study demonstrates that blockade of CCKARs peripheral to the blood-brain barrier also attenuates the inhibitory effects of duodenal nutrient administration on gastric emptying. It remains to be determined whether selective denervation of intestinal vagal sensory nerves abolishes the stimulatory effect of CCKAR blockade on gastric emptying. Nevertheless, these studies provide strong support for the hypothesis that endogenous CCK inhibits gastric emptying primarily by binding to CCKARs on intestinal vagal sensory nerves.

Berthoud and Patterson (2) used light microscopy to assess the anatomic relationship between intestinal CCK-secreting endocrine cells and vagal sensory neurons in rat intestine. They found no close anatomic association between these cell types, although CCK-immunoreactive cells were located ~10 to >100 μm from the axons, suggesting a possible paracrine mechanism of CCK action to stimulate vagal activity. Initial attempts at using immunohistochemistry to identify CCKARs on the surface of vagal afferent fibers have not been successful, although in the same studies, CCKAR immunoreactivity was found on interstitial cells of Cajal, smooth muscle, and enteric neurons in rat pylorus (33) and within enteric neurons in rat intestine (46). The discovery by Sternini et al. (46) of CCKAR-like immunoreactivity in a discrete population of gastric myenteric neurons suggests the possibility that CCK may induce inhibition of gastric motility by acting at CCKARs on myenteric neurons in the stomach. CCK-immunoreactivity has also been detected within intrinsic neurons of the enteric nervous system of the small intestine and stomach (16, 20, 43). These findings suggest that CCK may act in part by a neurocrine mechanism at nonvagal CCKARs within the stomach and (or) small intestine to affect gastric emptying.

There is some evidence to suggest that intestinal CCK may enter the bloodstream to act as a hormonal signal at distant CCKARs to inhibit gastric emptying. Liddle et al. (25) showed that exogenous CCK inhibits gastric emptying in humans at doses that reproduce postprandial plasma levels of CCK. Putative sites of action include CCKARs on vagal afferent neurons (44, 45) and myenteric neurons (46) in the stomach, interstitial cells of Cajal, enteric neurons, and smooth muscle in the pylorus (33), and enteric neurons (46) and vagal afferent neurons in the small intestine (9, 22). Results of the present study suggest that an endocrine mechanism of CCK action to inhibit gastric emptying is not essential because immunoneutralization of circulating CCK had no effect on gastric emptying responses to duodenal infusion of peptone and fat. Carbohydrate-induced inhibition of gastric emptying also appears to be mediated in part by a nonendocrine mechanism of CCK action, because CCKAR blockade attenuates carbohydrate-induced inhibition of gastric emptying (37, present study), yet delivery of carbohydrate to the gastrointestinal tract produces little if any increase in plasma CCK (5, 8, 24).

The lack of an effect in the present study of intravenous administration of the CCK MAb on gastric emptying responses to peptone and fat was not due to the use of an insufficient dose of antibody, because the antibody dose was able to block the gastric emptying response to a supraphysiological dose of CCK-8 (1 nmol·kg⁻¹·h⁻¹ iv). We previously reported that a 1 nmol·kg⁻¹·h⁻¹ iv dose of CCK-8 increases plasma CCK levels in rats by >150 pM (3). In contrast, numerous studies have demonstrated that gastrointestinal delivery of various macronutrients increases plasma CCK levels by at least 10–15 pM. For example, Brenner et al. (5) reported that in rats, 10-min duodenal infusions of oleate (0.9 g/h) and casein (2 g/h), the most potent macronutrient stimulators of CCK secretion into the bloodstream of rats, increased plasma CCK levels to 13 and 10 pM, respectively. In a similar study in rats, Raybould et al. (38) reported that a 10-min duodenal infusion of Intralipid (0.6 g/h) increased plasma CCK to 6.5 pM. Intralipid and peptone were administered into the duodenum at comparable rates in the present study. Thus our intravenous dose of CCK antibody should have been sufficient to attenuate gastric emptying responses to Intralipid and peptone if the plasma CCK responses to these nutrients play an essential role in controlling gastric emptying.

There is some evidence to suggest that CCK may also act as a neurotransmitter or neuromodulator within the brain to inhibit gastric emptying. Intracerebroventricular administration of CCK has been reported to inhibit (10), stimulate (32), and to have no effect (7) on gastric emptying. Injection of CCK directly into the nucleus of the solitary tract has been shown to decrease gastric tonic and phasic pressures (47). It remains to be determined whether blockade of CCKARs in specific brain regions affects gastric emptying.

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