Endogenous cholecystokinin reduces food intake and increases Fos-like immunoreactivity in the dorsal vagal complex but not in the myenteric plexus by CCK₁ receptor in the adult rat

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IN 1994, ROBERT C. RITTER and colleagues (39) put forth a hypothesis stating that endogenous CCK secreted by the I cells of the small intestine may decrease food intake by activating first-order neurons in the enteric nervous system (ENS) of the gastrointestinal tract. The ENS consists of two ganglionated plexuses, myenteric and submucosal. The myenteric plexus is located between the outer longitudinal and the inner circular plexuses, myenteric and submucosal. The myenteric plexus is known vagal afferents, all of which may be activated by CCK, and participates in the reduction of food intake by this hormone.

The goal of this work is to examine the role of the myenteric neurons and the DVC in the reduction of food intake by endogenous CCK, by quantifying Fos-like immunoreactivity (Fos-LI), a marker for neuronal activation, in these peripheral and central neurons.

The above and other hypotheses were previously tested using exogenous CCK-8, with the following results. Exogenous CCK-8 decreased food intake through CCK₁ receptors acting through an intact vagus nerve (14, 46). Exogenous CCK-8 increased Fos-LI in the DVC (12) and the myenteric plexus of the duodenum and jejunum through CCK₁ receptors (22, 41, 49). Furthermore, bilateral subdiaphragmatic vagotomy attenuated Fos expression only in the DVC but not in the myenteric plexus (42).

Similar to exogenous CCK-8, Yox et al. (53–55) demonstrated that intraduodenal infusion of oleate, a long-chain fatty acid and a stimulant of endogenous CCK release, reduces food intake via CCK₁ receptors and the vagus nerve. In addition, intraduodenal infusion of fat also increased Fos-LI in the DVC, via CCK₁ receptors and the vagus nerve (24).

Recently, we have shown that oleate infusions increased Fos-LI in the myenteric neurons via CCK₁ receptors (19). However, the latter studies do not answer the question of whether activation of the myenteric neurons via CCK₁ receptors is obligatory for endogenous CCK to inhibit food intake.

Although fatty acids, e.g., oleate, release endogenous CCK, as nutrients, they have multiple effects in addition to releasing CCK. For example, infusions of oleic acid stimulate secretion of PYY and GLP-1 (40), which have also been shown to reduce food intake and increase central Fos-LI (3, 4). The actions of PYY on food intake (1), as with CCK-8, require an intact vagus. Furthermore, we have shown that duodenal infusions of oleate increased myenteric Fos-LI (19) to approximately twice the increase caused by a supraphysiological dose of exogenous CCK-8 (50), suggesting that factors other than CCK are involved in these peripheral Fos-LI increases. In addition, although devazepide, a specific CCK₁ receptor antagonist, significantly attenuated myenteric and DVC Fos-LI in response to oleate, it did not abolish it (19). Therefore, it is clear that oleate has effects that are unrelated to the release of CCK.

The vagus is a complex nerve that can be stimulated by CCK via inputs from several regions of the intestine. There are three known vagal afferents, all of which may be activated by CCK,
intramuscular arrays (IMA), intraganglionic laminar endings (IGLE), and intravillous arbors (IVAs) (7, 17, 18). The IMAs synapse mainly on the various muscle layers of the gut and function as mechanoreceptors and tension receptors. The IGLEs are basketlike structures that surround the myenteric ganglia and function as mechanosensory receptors (muscle stretch or contraction). Besides having mechanoreceptors and tension receptors functions, there is evidence to suggest that both IMAs and IGLEs can be activated by CCK-8 and may have a role in the reduction of food intake by the same peptide (9, 10). The IVAs reach to the enteroendocrine cells and function as chemoreceptors.

To stimulate endogenous CCK release without the confounding effects of intraluminal nutrients, we carried out studies using camostat, a nonnutrient, nonproteinaceous synthetic trypsin inhibitor that causes release of endogenous CCK (15, 27, 30, 36, 48). To the best of our knowledge, this is the first report that examines reduction of food intake and Fos-LI in the DVC and the myenteric neurons in response to a nonnutrient, strong releaser of endogenous CCK. In addition, this is the first work that investigates whether the myenteric neurons play an essential role in the reduction of food intake by endogenous CCK in the same way as exogenous CCK-8. An additional reason for comparing the effects of endogenous CCK to the previously described effects of CCK-8 is because the only detectible endocrine form of CCK released by camostat is CCK-58 (36), which has several physiological effects that differ markedly from CCK-8 (52).

MATERIALS AND METHODS

Experimental Procedures

The Tuskegee University Institutional Animal Care and Use Committee approved all of the animal protocols. Sprague-Dawley rats (250–350 g) were housed individually in wire-mesh cages, in a controlled environment (lights were on from 0600 to 1800, and temperature was maintained at 21.5°C), and given free access to water and pelleted rodent chow (Teklad, Madison, WI).

To adapt the rats to our experimental protocol and laboratory environment, each rat was handled daily for 10 min, and orogastrically with 3.5 ml of double-distilled water (ddH2O). All rats were fasted beginning at 1800 on the day prior to all of the experiments. All injections were made in a volume of 0.5 ml of vehicle (saline or DMSO) and all gavages were made in a volume of 3.5 ml of ddH2O.

Effect of camostat on myenteric and DVC Fos-LI. For this experiment, we used a total of 48 adult, male Sprague-Dawley rats divided to 12 treatment groups (n = 4 rats per group). Two groups received CCK-8 (Bachem, Torrance, CA; 40 μg/kg) or vehicle intraperitoneally, and 10 groups received camostat [Camostat mesilate, (FOIPAN: N,N-dimethylcarbamoylmethyl-4-(4-guanidinobenzoyloxy) phenylacetate monomethanesulfonate)], 200 mg/kg; Ono Pharmaceuticals, Osaka, Japan) or ddH2O by orogastric gavage. On the basis of our previous experience (19–22, 33–35, 49, 50), we killed the two groups of rats that received CCK-8 or vehicle at 90 min postinjection. This is the time point for producing maximum Fos expression in response to exogenous CCK-8. On the other hand, to evaluate the time point for producing maximum Fos expression in response to camostat, the remaining 10 groups that received this treatment or ddH2O were killed at five different time points post gavage: 90, 105, 120, 135, and 150 min. After the injection or the gavage, all rats were euthanized with an overdose of pentobarbital sodium (10 mg/kg ip).

Effect of devazepide, a CCK1 receptor antagonist, on DVC Fos-LI induced by camostat. Thirty-two adult, male Sprague-Dawley rats were assigned to eight treatment groups (n = 4 rats/group). Four groups received the CCK1 receptor antagonist, devazepide (1,000 μg/kg; ML Laboratories, Leicester, United Kingdom) or DMSO ip, followed 15 min later by exogenous CCK-8 (40 μg/kg ip) or saline. The remaining four groups received devazepide (1,000 μg/kg ip) or DMSO, followed 15 min later by an orogastric gavage of camostat (200 mg/kg) or ddH2O. As we stated previously with regard to the time point of maximum Fos expression in response to CCK-8 or camostat, 90 min after the CCK-8 or vehicle injection, and—based on the results of the previous experiment—105 min after the gavage of camostat or ddH2O, all rats were euthanized with an overdose of pentobarbital sodium (10 mg/kg ip).

Effect of vagotomy on DVC Fos-LI induced by camostat. Eight adult, male Sprague-Dawley rats underwent bilateral surgical sectioning of the vagus nerve (2 groups, n = 4 rats per group). Another group of eight rats underwent sham vagotomy (2 groups, n = 4 rats per group) surgery, as previously described (42). Briefly, we exposed the right and left vagi along the sides of the esophagus through a ventral midline celiotomy incision and severed them under the guidance of a surgical microscope. Sham surgeries were performed by manipulating the vagi without severing them. All rats were allowed 2 wk recovery time from the surgery during which they were observed clinically for any abnormal behavior or pain. Vagotomy was verified twice, first by measuring reduction of 10% sucrose intake in response to CCK-8 10 μg/kg ip for 30 min, and second, by inspection during postmortem examination. During death, all vagotomized rats showed severely distended stomachs, which is an accepted verification of vagotomy.

The vagotomy groups received camostat (200 mg/kg) or ddH2O and the sham groups received the same treatments, all through orogastric gavage. Again, on the basis of the results of the first experiment, all of the rats were euthanized 105 min postgavage of camostat or ddH2O with an overdose of pentobarbital sodium (10 mg/kg ip).

Immediately after the euthanasia, all rats were perfused transcardially in two stages. First, with 500 ml of Krebs solution (Krebs saline formula in mM: 119 NaCl, 4.7 KCl, 1.2 NaH2PO4·H2O, 25 NaHCO3, 1.2 MgSO4, and 11.1 glucose, and 1 M CaCl2) to collect the intestine, and second, with 500 ml of 0.4% formaldehyde solution in 0.1 M phosphate buffer saline (PBS) to fix the brains in situ.

The small intestine was exposed through a ventral midline celiotomy incision, and the duodenum and jejunum were collected. On the basis of our previous experiments (20–22, 33, 49, 50), the duodenal sample was 5–10 cm aboral from the pylorus and the jejunal sample was 20–25 cm aboral from the pylorus. The lumens of the removed segments were rinsed with Krebs solution, and the segments were placed in Sylgard-coated Petri dishes (World Precision Instruments, Sarasota, FL), opened along the mesenteric attachment, stretched, and pinned with the mucosal side up, and stored overnight in Zamboni’s fixative at 4°C. On the next day, the tissues were unpinned and cleared three times in 100% DMSO, 10 min each time, followed by three 10-min rinses with 0.1 M PBS, pH 7.4. Whole mounts (~1 cm²) of longitudinal smooth muscle with adhering myenteric plexus from the duodenum and jejunum were prepared using a dissecting microscope and processed for immunohistochemical detection of Fos-LI.

After collection of the intestinal tissues, the brain stems were collected and postfixed with 0.4% formaldehyde for 2 h at 4°C, followed by overnight placement in a 25% sucrose solution at room temperature. The next day, they were sectioned at 40 μm on a cryostat at −20°C and blocked with 50% ethyl alcohol for 30 min, to reduce the production of free radicals and improve background staining. Consistent with our previous work (19, 20, 33–35, 49, 50), the areas cut included the following levels of the DVC, according to the rat brain atlas of Paxinos and Watson (32): the AP (~4.5 mm caudal to interaural plane), the NTS (~4.5 and ~4.8 mm caudal to interaural plane), and the DMV (~4.5 and ~4.8 mm caudal to interaural plane) (please see Fig. 1). Sections were taken at multiple levels of the NTS to ensure sampling from sites receiving both gastric and intestinal vagal afferent innervation.
Effect of Camostat on DVC and Myenteric Fos-LI

Figure 1 shows a photomicrographs of three nuclei in the DVC: AP, NTS, and the DMV captured from a camostat mesilate-treated animal (A) and a double-distilled water (ddH2O)-treated animal (A'). Camostat increased Fos-like immunoreactivity, shown by the black dots and indicated by the arrow in A. cc, central canal. Scale bar = 100 μm.

RESULTS

Effect of Camostat on food intake and and activates the DVC

Four groups of rats (250–350 g each, n = 5 rats per group) were assigned to this experiment and deprived from food but not water overnight. On the following day, the rats were deprived of food but not water overnight. After the gavage, intakes of 10% sucrose were recorded every 5 min for 120 min. The rats were deprived of regular food for the entire test period.

Statistical Analysis

Data were analyzed using two-way ANOVA (treatment and time are the two independent variables), and multiple comparisons were performed using Bonferroni’s t-test. Results are displayed as means ± SE. The food intake data were analyzed by a one-way ANOVA and Bonferroni’s t-test for multiple comparisons. Data were considered statistically significant if P < 0.05.

Effect of Camostat on DVC and Myenteric Fos-LI

Effect of Camostat on DVC and Myenteric Fos-LI

Immunohistochemistry

The intestinal whole mounts and the brain sections were incubated for 24 h at room temperature in a primary antiseraum raised in rabbit against a peptide consisting of amino acids 4–17 of human Fos (Ab-5, 1:12,000 dilution; Oncogene, San Diego, CA). The next day, the tissues were rinsed with 0.01 M Tris-phosphate-buffered saline (TPBS) and incubated overnight in biotinylated donkey anti-rabbit serum (1:500 dilution; Jackson ImmunoResearch Laboratories, West Grove, PA). Then, the tissues were incubated for 3 h in avidin conjugated to horseradish peroxidase, washed with 0.01 M TPBS, and reacted with glucose oxidase and DAB (Sigma, St. Louis, MO) intensified with nickel (5, 11).

Counting Procedures

Two independent examiners, blinded to the treatments of this study, counted Fos-positive neurons in the myenteric neurons. For each intestinal whole mount 10 non-overlapping, ×40 microscopic fields were counted and averaged. In the brain stem sections, Fos-positive cells were counted by an automated computer software (ImagePro Plus; Media Cybernetics, Baltimore, MD) in the AP, NTS, and DMV at planes −4.5 mm and −4.8 mm caudal to the interaural plane, according to the rat brain atlas (32) (Fig. 1).

Effect of camostat on food intake. To establish a baseline for food intake, we measured the intake of 10% sucrose in 12, overnight food-deprived, Sprague-Dawley male rats (250–350 g each), six times during a 16-18-day period following orogastric gavage of 3.5 ml ddH2O or an intraperitoneal injection of saline. On the experiment day, the rats were deprived of food but not water overnight. On the following day, the rats were divided into four groups (n = 5 rats per group), two of which received orogastric gavage of camostat (200 mg/kg) or ddH2O, and the remaining two groups of rats received CCK-8 (10 μg/kg ip) or saline. The intake of 10% sucrose was recorded every 5 min for 120 min. The rats were deprived of regular food for the entire test period.

Effect of devazepide, a CCK1 receptor antagonist, and L365,260, a specific CCK2 receptor antagonist on reduction of food intake by camostat. Four groups of rats (250–350 g each, n = 5 rats per group) were assigned to this experiment and deprived from food but not water overnight. The following day, each group received two treatments, an intraperitoneal injection of devazepide (1 mg/kg dissolved in 0.5 ml of 0.01% DMSO) or placebo, followed by orogastric gavage of camostat mesilate (200 mg/kg dissolved in 3.5 ml of ddH2O) or ddH2O (3.5 ml). After the gavage, intake of 10% sucrose was recorded every 5 min for 120 min. The rats were deprived of regular food for the entire test period.

Effect of L365,260, a specific CCK2 receptor antagonist on reduction of food intake by camostat. Four groups of rats (250–350 g each, n = 3 rats per group) were deprived from food but not water overnight. The following day, each group received two treatments, an IP injection of L365,260 (1 mg/kg dissolved in 0.5 ml of 0.01% DMSO) or 0.01% DMSO (0.5 ml), followed by orogastric gavage of camostat mesilate (200 mg/kg dissolved in 3.5 ml of ddH2O) or ddH2O (3.5 ml). After the gavage, intake of 10% sucrose was recorded every 5 min for 120 min. The rats were deprived of regular food for the entire test period.

Statistical Analysis

Data were analyzed using two-way ANOVA (treatment and time are the two independent variables), and multiple comparisons were performed using Bonferroni’s t-test. Results are displayed as means ± SE. The food intake data were analyzed by a one-way ANOVA and Bonferroni’s t-test for multiple comparisons. Data were considered statistically significant if P < 0.05.
camostat increased Fos-LI significantly more than ddH₂O only at the 105 and 135 min time points (Fig. 3, A and B). In the DMV/NTS (−4.8 mm caudal to the interaural plane), orogastric gavage of camostat increased Fos-LI significantly more than ddH₂O only at the 105-, 120-, and 135-min time points (Figs. 4, A and B).

Figure 5 depicts Fos-like immunoreactivity in the myenteric plexus of the duodenum in response to camostat, exogenous CCK-8, and saline. Two-way ANOVA revealed no difference in the counts of Fos-positive neurons in the myenteric neurons between camostat and ddH₂O in the duodenum (Fig. 6) and jejunum (Fig. 7). However, as previously reported, the counts of Fos-positive neurons in response to exogenous CCK-8 were significantly more than saline (Figs. 6 and 7).

Effect of Devazepide, a CCK₁ Receptor Antagonist, on DVC Fos-LI

In the AP, NTS/DMV at planes −4.5 mm and −4.8 mm caudal to the interaural plane, devazepide significantly attenuated camostat-induced Fos expression (Fig. 8, A and B).

Effect of Vagotomy on DVC Fos-LI

In the AP, NTS/DMV at planes −4.5 mm and −4.8 mm caudal to the interaural plane, vagotomy significantly attenuated Fos expression induced by camostat (Fig. 9).
As a positive control, we also compared the results of the previous three experiments with exogenous CCK-8. As expected and documented (19–22, 33–35, 49, 50), CCK-8 increased Fos-LI in all of the DVC nuclei, and devazepide and vagotomy significantly attenuated it.

**Effect of Camostat on Food Intake**

One-way ANOVA revealed significant reduction of 10% sucrose intake for 120 min in response to orogastric gavage of camostat compared with ddH₂O. As expected, exogenous CCK-8 also reduced the intake of 10% sucrose more than saline (Fig. 10).

**Effect of CCK₁ and CCK₂ Receptor Antagonists on Camostat Reduction of Food Intake**

Devazepide, a CCK₁ receptor antagonist (Fig. 11A) but not L365,260, a CCK₂ receptor antagonist (Fig. 11B), reversed the reduction of food intake by camostat (Fig. 11, A and B).
DISCUSSION

We have shown three important results: 1) endogenous CCK released by camostat increases Fos-LI in the DVC, which requires an intact vagus; 2) reduces food intake by CCK-1 receptors in adult rats, and 3) the pathway by which this reduction is taking place does not appear to involve the myenteric neurons.

To explain the pathway by which CCK decreases food intake, Ritter et al. (39) hypothesized that nutrients that stimulate the release of endogenous CCK from the I cells of the upper small intestine (duodenum and jejunum) e.g., fat and protein, activate the gut enteric neurons, which, in turn, activate satiety centers in the DVC, mainly through the vagus nerve and by activating CCK1 receptors.

On the basis of the above hypothesis, we began to test the role for the myenteric neurons in the reduction of food intake by CCK, supported by the following data. First, there is electrophysiological (29, 43) and immunohistochemical (21, 22, 33, 34, 49, 50) evidence demonstrating activation of

Fig. 8. A: effect of devazepide, a CCK1 receptor antagonist, on camostat-induced Fos expression in the dorsal vagal complex. Four groups of rats (n = 4 rats per group) received two treatments each, 15 min apart. DMSO (0.5 ml)/camostat (Cam; 200 mg/kg in 3.5 ml of ddH2O), DMSO/ddH2O (3.5 ml), Devazepide (Dev; 500 µg/kg in 0.5 ml of DMSO)/camostat, and Dev/ddH2O. Dev and DMSO were given intraperitoneally in a volume of 0.5 ml, and camostat and ddH2O were given by orogastric gavage in a volume of 3.5 ml. Fos-positive neurons were quantified in the AP, NTS, and DMV in planes −4.5 and −4.8 mm caudal to the interaural plane (32). *Significant difference compared with vehicle. †Significant difference between Dev/Cam and DMSO/Cam. B: effect of devazepide, a CCK1 receptor antagonist, on CCK-8-induced Fos expression in the dorsal vagal complex. Four groups of rats (n = 4 rats per group) received two treatments each, 15 min apart. DMSO (0.5 ml)/CCK-8 (10 µg/kg), DMSO/saline, Dev (500 µg/kg in 0.5 ml of DMSO)/CCK-8, and Dev/saline. All injections were given intraperitoneally in a volume of 0.5 ml. Fos-positive neurons were quantified in the AP, NTS, and DMV in planes −4.5 and −4.8 mm caudal to the interaural plane (32). *Significant difference compared with saline. †Significant difference between Dev/CCK-8 and DMSO/CCK-8.

Fig. 9. Effect of vagotomy on camostat-induced Fos expression in the dorsal vagal complex. Four groups of rats (n = 4 rats per group) underwent bilateral subdiaphragmatic vagotomy surgery (VGX) or sham vagotomy. Two groups received orogastric gavage of camostat (200 mg/kg in 3.5 ml ddH2O) and the other two received ddH2O (3.5 ml VEH). Fos-positive neurons were quantified in the AP, NTS, and DMV in planes −4.5 and −4.8 mm caudal to the interaural plane (32). *Significant difference compared with vehicle. †Significant difference between VGX-CM and sham-CM. CM, camostat.

Fig. 10. Intake of 10% sucrose in response to orogastric gavage of camostat or intraperitoneal injection of exogenous CCK-8 in the adult rat. The intake was measured for 120 min in rats gavaged with camostat (200 mg/kg dissolved in 3.5 ml water) or CCK-8 (10 µg/kg ip). *Significant difference between CCK-8 and saline. †Significant difference between camostat and ddH2O.
myenteric neurons by exogenous CCK-8 and endogenous CCK released in response to nutrients (19), through CCK₁ receptors (19, 42). Second, myenteric neurons that synapse with vagal afferents that supply the gut contain CCK₁ receptors. (6, 8, 21, 31, 38). Third, this neuronal connection links the gut with important central satiety control nuclei in the DVC, e.g., DMV, NTS, and AP (2, 44, 45). Furthermore, the three components of this circuit, that is, myenteric neurons, vagus nerve, and DVC transmit CCK₁ receptor signals (25, 26) and can be activated by both CCK-8 and endogenous CCK. In addition, vagal sensory neurons that contain CCK₁ receptors are in close proximity to the CCK secreting I cells, suggesting that direct activation of the vagus by endogenous CCK is also possible. Our current work provides behavioral, immunohistochemical, pharmacological, and surgical evidence that camostat, a nonnutrient, synthetic trypsin inhibitor and a strong releaser of endogenous CCK, reduces food intake and increases Fos-LI, a marker of neuronal activation, by CCK₁ receptors and in the central nervous system, that is, DVC, but not in the myenteric neurons of the duodenum and jejunum. This reduction and activation were dependent on CCK₁ receptors, and the vagus nerve, that is, the same requirements for reduction of food intake caused by exogenous CCK-8. It should be noted that the enteric nervous system consists of two ganglionated nerve plexuses: myenteric and submucosal. This work tested only the role of the myenteric plexus in the reduction of food intake caused by endogenous CCK.

Camostat increases circulating endogenous CCK, in rats and mice (15, 27, 30). In addition, it increases pancreatic endocrine and exocrine secretions and delays gastric emptying, all by CCK₁ receptor (15, 27, 30). In the adult rat, Voigt et al. (48) demonstrated that camostat reduces food intake. However, they failed to reverse this response using 100 μg/kg devazepide compared with the 1,000 μg/kg used in the present study. Devazepide at 1,000 μg/kg has been reported to not interact with the CCK₂ receptor (30). Furthermore, the lack of influence of the CCK₂ receptor antagonist in the present work suggests that the actions of endogenous CCK are mediated by the CCK₁ receptor but not the CCK₂ receptor. Therefore, the current work provides the first evidence in adult rats that CCK released by a nonnutrient, camostat, causes reduction of food intake, which was mediated by CCK₁ receptors. Only one other study reported reduction of food intake by endogenous CCK released by a nonnutrient (soybean trypsin inhibitor) through CCK₁ receptors, and this was in neonatal rats (51).

The results of the current work also provide the first immunohistochemical evidence that demonstrates that camostat increases Fos-LI in the DVC. This increase was due to release of endogenous CCK, because a specific CCK₁ receptor antagonist, that is, devazepide, attenuated this response.

In a majority of measurements, Fos-LI differed substantially between CCK-8 treatments compared with camostat treatment. In some cases, the differences were quantitative, for example, Fos-LI in the area postrema was threefold higher in CCK-8 treated rats compared with camostat, although both increases were statistically significant (Fig. 2). Likewise, CCK-58 increased Fos-LI more than twofold higher in the NTS (Fig. 3B). However, although CCK-8 markedly increased Fos-LI in the myenteric plexus of the duodenum (Fig. 6) and jejunum (Fig. 7), camostat had a very small, nonsignificant effect in these neurons. These differences between responses to CCK-8 vs. endogenous CCK may be due to differences in dose of CCK-8 received (50), or to differences in biological actions between CCK-8 vs. endogenous CCK. CCK-8 was injected at 40 μg/kg, which produces much greater than physiological levels of CCK in

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**Fig. 11.** A: effect of devazepide, a CCK₁ receptor antagonist, on the reduction of food intake by camostat (Cam) mesilate. Four groups of rats (n = 5 per group) were injected intraperitoneally with Dev (1 mg/kg in 0.5 ml of 0.01% DMSO) or 0.01% of DMSO (0.5 ml) followed 15 min later by an orogastric gavage of Cam or ddH₂O. The intake of 10% sucrose solution was measured for 120 min. *Significant difference between DMSO/Cam and DMSO/ddH₂O. †Significant difference between Dev/Cam and DMSO/Cam. Cam reduced food intake significantly (*), and devazepide blocked this attenuation (†). B: effect of L365,260, a CCK₂ receptor antagonist, on the reduction of food intake by Cam mesilate. Four groups of rats (n = 3 per group) were injected intraperitoneally with L365,260 (1 mg/kg in 0.5 ml of 0.01% DMSO) or 0.01% of DMSO (0.5 ml) followed 15 min later by an orogastric gavage of Cam or ddH₂O. The intake of 10% sucrose solution was measured for 120 min. *Significant difference between DMSO/Cam and DMSO/ddH₂O. **Significant difference between L365,260/Cam and L365,260/ddH₂O and not significantly different from DMSO/Cam. Cam reduced food intake significantly (*), and L365,260 failed to block this reduction.
the circulation, and such doses consistently produce acute edematous pancreatitis in rats (16, 28). In contrast, gavage of camostat at 100 mg/kg in rats increased circulating CCK to physiological levels of 17 pM (36), nearly identical to that (15 pM), caused by the dietary protein casein (37). Therefore, the quantitative differences in Fos-LI between CCK-8 and camostat might be due to the much greater amounts of CCK bioactivity in rats given CCK-8. On the other hand, the near absence of an increase in Fos-LI, compared with high and significant Fos-LI in the myenteric plexuses of the duodenum and jejunum could also suggest qualitative differences between CCK-8 and endogenous CCK in inducing Fos-LI. At this time, we cannot determine whether the differences in Fos-LI results from quantitative, qualitative, or both differences between CCK-8 and endogenous CCK. This is consistent with several studies showing marked qualitative, as well as quantitative differences, in biological activity between CCK-8 and CCK-58 (13, 23, 47, 52), the endogenous form of CCK. Because CCK-8 is apparently not a physiological endocrine form of CCK in the rat, the differential effect of CCK-8 vs. camostat on Fos-LI in the myenteric plexus may provide another example of the marked differences in pattern of bioactivity between CCK-8 and CCK-58.

Figure 12 illustrates how exogenous CCK-8 or endogenous CCK could activate the vagus. The only route for intraperitoneal CCK-8 to reach vagal afferent fibers is through the circulation. Therefore, all three vagal afferent fibers, IMA, IGLE, and IVA (17, 18), should receive similar concentrations of CCK-8 after intraperitoneal injection. However, these three vagal afferent fibers may be exposed to different concentrations of endogenous CCK. The IVA and the IGLE are close enough to the CCK-secreting I cell for the endogenous peptide to activate these fibers by a paracrine route. However, the IMA may be exposed only to circulating CCK and therefore may be at much lower concentrations of CCK than encountered by fibers activated by the paracrine route. All threeafferent fiber types are connected to the central nervous system, at the level of the DVC, through the vagus or the 10th cranial nerve. Alternatively, endogenous CCK may directly activate the DVC by an endocrine route, causing reduction of food intake. In conclusion, our data show for the first time that endogenous, peripherally produced CCK reduces food intake by a central mechanism that involves an intact vagus nerve and functional CCK1 receptors. Furthermore, these data demonstrate that there is not a clear role for the myenteric plexus in the reduction of food intake caused by endogenous CCK. Finally, a possible role for the submucosal plexus in mediating CCK reduction of food intake remains to be determined.

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