Abdominal vagal mediation of the satiety effects of CCK in rats

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Reidelberger, Roger D., Jessica Hernandez, Bernd Fritzsch, and Martin Hulce. Abdominal vagal mediation of the satiety effects of CCK in rats. Am J Physiol Regul Integr Comp Physiol 286: R1005–R1012, 2004. First published December 30, 2003; 10.1152/ajpregu.00646.2003.—CCK type 1 (CCK1) receptor antagonists differing in blood-brain barrier permeability were used to test the hypothesis that satiety is mediated in part by CCK action at CCK1 receptors on vagal sensory nerves innervating the small intestine. Devazepide penetrates the blood-brain barrier; A-70104, the dicyclohexylammonium salt of Na-3-quinolinoyl-o-Glu-N,N-dipentylamide, does not. At dark onset, non-food-deprived control rats and rats with subdiaphragmatic vagotomies received a bolus injection of devazepide (2.5 μmol/kg iv) or a 3-h infusion of A-70104 (3 μmol·kg–1·h–1 iv) either alone or coadministered with a 2-h intragastric infusion of peptone (0.75 or 1 g/h). Food intake was determined from continuous computer recordings of changes in food bowl weight. In control rats both antagonists stimulated food intake and attenuated the anorectic response to intragastric infusion of peptone. In contrast, only devazepide was effective in stimulating food intake in vagotomized rats. Thus endogenous CCK appears to act both at CCK1 receptors beyond the blood-brain barrier and by a CCK1 receptor-mediated mechanism involving abdominal vagal nerves to inhibit food intake.

METHODS

Subjects. Male rats (Sasco Sprague-Dawley, Charles Rivers Lab, Kingston, NY; 300–500 g at the time of surgery) 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 rat chow (Purina no. 5001, 3.3 kcal/g) and water ad libitum. The Animal Studies Subcommittees of the Omaha Veterans Affairs Medical Center approved the experimental protocol. Animal experimentation was conducted in conformity with the “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society (1).

Surgical procedures. For each animal, bilateral cutting of the subdiaphragmatic vagal nerves and implantation of gastric and jugular vein cannulas were accomplished within a single surgery as described previously (36, 50). Gastric and jugular vein cannulas were filled with water and heparinized saline (40 U/ml), respectively, plugged with stainless steel wire, and flushed every other day to maintain patency. Cannulas were connected to 40-cm lengths of tubing passed through a protective spring coil connected between a light-weight saddle (ITC, Woodland Hills, CA) worn by the rat and either a single- or double-channel infusion swivel (Instech Laboratories, Plymouth

CCK is a peptide that is found throughout the brain and in neurons and endocrine cells of the gastrointestinal tract. Studies demonstrating that CCK type 1 (CCK1) receptor antagonists stimulate food intake in a variety of species provide compelling evidence that CCK plays an essential role in producing the satiation that occurs with ingestion of a meal (4, 11, 13, 20, 28, 40). The popular hypothesis is that CCK, secreted from endocrine cells in the upper small intestine in response to duodenal delivery of nutrients, acts through local, paracrine stimulation of intestinal vagal sensory neurons to inhibit food intake. This hypothesis is supported by studies demonstrating the existence of CCK-secreting endocrine cells in the epithelium of the upper small intestine (5, 47). CCK1 receptors within vagal afferent nerves (30, 53), activation of intestinal vagal afferent neurons by exogenous and endogenous CCK (14, 18, 24), similar attenuation by CCK1 receptor antagonists and vagal neural lesions of anorexic responses to exogenous CCK and nutrient administration (41), and stimulation of food intake by a CCK1 receptor antagonist that does not penetrate the blood-brain barrier (37, 38).

There are several lines of evidence suggesting that this mechanism is not the only one by which CCK produces satiety. We and others have demonstrated that systemic administration of the CCK1 receptor antagonist devazepide can increase food intake in rats whether or not they are vagotomized (36) or pretreated with capsaicin to lesion visceral sensory nerves (42). Other evidence suggests that CCK acts as a neurotransmitter or neuromodulator within two different brain regions to produce satiety: one region that includes the nucleus tractus solitarius in the hindbrain, and another more distributed region within the medial-basal hypothalamus. This conclusion is supported by studies showing that food intake releases hypothalamic CCK (15, 43), site-specific injections of CCK into the medial-basal hypothalamus and the caudal brain stem inhibit food intake (7), and brain injections of CCK antisera (16) and CCK receptor antagonists (12, 17, 19, 44) stimulate food intake.

We recently provided evidence using CCK1 receptor antagonists differing in blood-brain barrier permeability (devazepide and A-70104) that endogenous CCK acts at CCK1 receptors peripheral to the blood-brain barrier to inhibit food intake, sham feeding, and gastric emptying in rats (37–39). In the present study the same antagonists were used to assess whether abdominal vagal nerves mediate the peripheral action of endogenous CCK to inhibit food intake in rats. An initial series of experiments determined the effects of subdiaphragmatic vagotomy on anorexic responses to acute intraperitoneal injection and continuous intravenous infusion of CCK-8. A second series of experiments determined the effects of intravenous administration of devazepide and A-70104, either alone or coadministered with an intragastric infusion of peptone, a potent stimulator of intestinal CCK release, on food intake in vagotomized animals.
The double-channel swivel permitted simultaneous administration of CCK1 receptor antagonist intravenously and pentone intragastrically.

Effects of vagotomy on CCK-induced inhibition of food intake. The first series of experiments determined the dose-response effects of intraperitoneal injections of CCK-8 (1, 2, 4, and 8 nmol/kg) during the early dark period on food intake in 4-h fasted rats that had received either a bilateral subdiaphragmatic vagotomy or a sham vagotomy procedure. Animals were adapted to a 12:12-light-dark cycle (lights off at 1300) and a 4-h fast from 1100 to 1500. Excess amounts of fresh ground rat chow were provided each day at 1500. Animals were adapted to experimental conditions for at least 1 wk before the start of experiments. In the initial experiment, control (n = 16) and vagotomized (n = 16) rats received a bolus intraperitoneal injection of CCK-8 (8 nmol/kg, 1 ml/kg; Peninsula Laboratories, San Carlos, CA) or vehicle (0.15 M NaCl, 0.1% BSA) 10 min before providing access to food. Food intake during the ensuing 30 min was determined, as described previously, from continuous computer recordings of changes in food bowl weight (50). Each rat received each treatment in random order on different days separated by at least 48 h. Three subsequent experiments of identical design determined the feeding effects of 4 nmol/kg of CCK-8, then 1 and 2 nmol/kg of CCK-8, on food intake in groups of control (n = 16) and vagotomized (n = 16) rats.

A second series of experiments determined the dose-response effects of 3-h intravenous infusions of CCK-8 (0.3, 1, and 10 nmol·kg⁻¹·h⁻¹) during the early dark period on food intake in non-food-deprived control and vagotomized rats adapted to a 12:12-light-dark cycle (lights off at 1600). Animals were permitted at least 1 wk to recover from surgery. They were then tethered to infusion swivels and adapted to experimental conditions for at least 1 wk before the start of experiments. Excess amounts of fresh ground rat chow were provided each day at 1300. In an initial experiment, control (n = 16) and vagotomized (n = 16) rats received a 3-h intravenous infusion of CCK-8 (1 or 10 nmol·kg⁻¹·h⁻¹, 3 ml/h) or vehicle (0.15 M NaCl, 0.1% BSA) beginning 15 min before dark onset. Food intake during the ensuing 17 h after dark onset was determined as before. Each rat received each treatment in random order on different days separated by at least 48 h. A subsequent experiment of identical design determined the feeding effects of intravenous infusion of CCK-8 (0 and 0.3 nmol·kg⁻¹·h⁻¹) in groups of control (n = 16) and vagotomized (n = 16) rats. At the end of an experiment, data from a rat were excluded if its jugular vein catheter was not patent. A catheter was deemed patent if the rat lost consciousness within 10 s of a bolus injection of the short-acting anesthetic Brevital into the catheter.

Effects of vagotomy on orexigenic responses to devazepide and A-70104. Experiments were similar to those described for the effects of vagotomy on the anorectic response to intravenous infusion of CCK. Three experiments were performed. In the first experiment, non-food-deprived control (n = 16) and vagotomized rats (n = 16) received a bolus intravenous injection of devazepide (2.5 μmol/kg = 1 mg/kg; ML Laboratories, St Albans, Herts, UK) or vehicle (5% DMSO, 5% Tween 80, 90% 0.15 M NaCl) 15 min before receiving a 2-h intragastric infusion of peptone (0.75 and 1 g/h, vagotomized and control rats, respectively; EZMix tryptone, Sigma) that began 15 min before dark onset. Food intake during the ensuing 17 h after dark onset was determined by 10.220.33.1 on July 9, 2017 http://ajpregu.physiology.org/ Downloaded from
was classified as indeterminate. Data from this rat were included in the experimental analyses. Brain sections of 14 of the 15 vagotomized rats selected for evaluation exhibited fluorescent cell labeling in the area postrema but virtually no labeled cells in the DMN, which is consistent with total subdiaphragmatic vagotomy. The other rat in this group exhibited some cell labeling in the right DMN, which is consistent with partial subdiaphragmatic vagotomy. These results, together with those obtained in our prior study (30), indicate that our vagotomy procedure is effective and highly reproducible. Thus we decided not to assess vagal integrity in the remaining animals.

**Effects of vagotomy on CCK-induced inhibition of food intake.** Figure 1, A and B, shows the effects of intraperitoneal injections of different doses of CCK-8 (1, 2, 4, and 8 nmol/kg) on 30-min food intake in 4-h food-deprived rats with sham and subdiaphragmatic vagotomy. Each CCK-8 dose was tested in a separate experiment. Vagotomy completely blocked the anorexic response to 1 and 2 nmol/kg of CCK-8 and attenuated the response to 8 nmol/kg of CCK-8. For the 1, 2, and 8 nmol/kg CCK-8 doses, ANOVA demonstrated a significant main effect of CCK-8 [F(1,30) = 9.4, *P < 0.01; *F(1,29) = 18.4, *P < 0.001; and F(1,28) = 43.8, *P < 0.001, respectively], a nonsignificant main effect of vagotomy [F(1,30) = 0.9, *P > 0.05; *F(1,29) = 0.0005, *P > 0.05; and *F(1,28) = 0.4, *P > 0.05, respectively], and a significant interaction between CCK-8 and vagotomy [F(1,30) = 4.7, *P < 0.05; *F(1,29) = 7.6, *P < 0.01; and F(1,28) = 4.3, *P < 0.05, respectively]. For the 4 nmol/kg CCK-8 dose, ANOVA demonstrated a significant main effect of CCK-8 [F(1,27) = 14.5, *P < 0.001], a nonsignificant main effect of vagotomy [F(1.27) = 1.6, *P > 0.05], and a nonsignificant interaction between CCK-8 and vagotomy [F(1,27) = 0.28, *P > 0.05].

Figure 2, A–C, shows the effects of 3-h intravenous infusions of different doses of CCK-8 (0.3, 1, and 10 nmol·kg⁻¹·h⁻¹) at dark onset on cumulative food intake in non-food-deprived rats with sham and subdiaphragmatic vagotomy. Vagotomy completely blocked the anorexic response to 0.3 nmol·kg⁻¹·h⁻¹ of CCK-8, yet had no significant effect on anorexic responses to the 1 and 10 nmol·kg⁻¹·h⁻¹ doses. The effects of each dose of CCK-8 on food intake in control and vagotomized rats were analyzed separately.

For the 0.3 nmol·kg⁻¹·h⁻¹ dose of CCK-8, repeated-measures ANOVA demonstrated a significant main effect of CCK-8, a significant main effect of vagotomy, and a significant interaction between CCK-8 and vagotomy on 3-h cumulative intake [F(1,25) = 18.1, *P < 0.001; *F(1,25) = 22.2, *P < 0.001; and F(1,25) = 10.1, *P < 0.01, respectively]. In the sham-vagotomized rats, the 0.3 nmol·kg⁻¹·h⁻¹ dose of CCK produced a significant, sustained reduction in cumulative food intake during the 3-h infusion period (Fig. 2A), with a peak inhibition of 56% at 1 h (*P < 0.01), decreasing to 30% inhibition by 3 h (*P < 0.001). Vagotomy alone significantly reduced cumulative food intake with a peak inhibition of 71% at 1 h (*P < 0.01), decreasing to 28% inhibition by 17 h (*P < 0.001). Vagotomy completely abolished the anorexic response to the 0.3 nmol·kg⁻¹·h⁻¹ dose of CCK-8. Cumulative intakes at all time points in vagotomized animals receiving this dose of CCK-8 were not different from those observed at the same time points in the same animals receiving vehicle.

For the 1 nmol·kg⁻¹·h⁻¹ dose of CCK-8, repeated-measures ANOVA demonstrated a significant main effect of CCK-8, a significant main effect of vagotomy, and a nonsignificant interaction between CCK-8 and vagotomy on 3-h cumulative intake [F(1,27) = 20.1, *P < 0.001; *F(1,27) = 14.4, *P < 0.001; and F(1,27) = 1.9, *P > 0.05, respectively]. In the sham-vagotomized rats, the 1 nmol·kg⁻¹·h⁻¹ dose of CCK-8 produced a significant, sustained reduction in cumulative food intake during the infusion period (Fig. 2B), with a peak inhibition of 66% at 1 h (*P < 0.001), decreasing to 30% inhibition by 3 h (*P < 0.001). Vagotomy alone significantly reduced cumulative food intake with a peak inhibition of 41% at 2 h (*P < 0.01), decreasing to 23% inhibition by 17 h (*P < 0.01). Vagotomy did not attenuate the anorexic response to the 1 nmol·kg⁻¹·h⁻¹ dose of CCK-8. In the vagotomized rats, this dose of CCK-8 produced a significant, sustained reduction in cumula-
tive food intake for 6 h, with a peak inhibition of 69% at 1 h ($P < 0.01$), decreasing to 22% inhibition by 6 h ($P < 0.05$).

For the 10 nmol·kg$^{-1}$·h$^{-1}$ dose of CCK-8, repeated-measures ANOVA demonstrated a significant main effect of CCK-8, a significant main effect of vagotomy, and a significant interaction between CCK-8 and vagotomy on 3-h cumulative intake [$F(1,24) = 157$, $P < 0.001$; $F(1,24) = 10.8$, $P < 0.01$; and $F(1,24) = 13.3$, $P < 0.01$, respectively]. In the sham-vagotomized rats, the 10 nmol·kg$^{-1}$·h$^{-1}$ dose of CCK-8 produced a significant, sustained reduction in cumulative food intake during the 17-h experimental period (Fig. 2C), with a peak inhibition of 97% at 3 h ($P < 0.001$), decreasing to 23% inhibition by 17 h ($P < 0.001$). Vagotomy alone significantly reduced cumulative food intake with a peak inhibition of 48% at 1 h ($P < 0.05$), decreasing to 23% inhibition by 17 h ($P < 0.01$). Vagotomy did not attenuate the anorexic response to the 10 nmol·kg$^{-1}$·h$^{-1}$ dose of CCK-8 during the infusion period. In the vagotomized rats, this dose of CCK-8 produced a significant, sustained reduction in cumulative food intake for 10 h, with a peak inhibition of 99% at 2 h ($P < 0.001$), decreasing to 22% inhibition by 10 h ($P < 0.05$).

**Effects of vagotomy on orexigenic responses to devazepide and A-70104.** Figure 3A shows the effects of an intravenous injection of devazepide (2.5 μmol/kg) at dark onset on food intake in non-food-deprived control and vagotomized rats receiving a 2-h intragastric infusion of peptone (1 g/h) at dark onset to stimulate endogenous CCK secretion and to decrease voluntary intake. Repeated-measures ANOVA demonstrated a significant main effect of devazepide, a significant main effect of vagotomy, and a nonsignificant interaction between devazepide and vagotomy on 3-h cumulative intake [$F(1,28) = 7.3$, $P < 0.05$; $F(1,24) = 7.2$, $P < 0.01$; and $F(1,24) = 2.1$, $P > 0.05$, respectively]. In the sham-vagotomized rats, devazepide produced a significant, sustained increase in cumulative food intake from 2 to 17 h after dark onset, with a peak stimulation of 92% at 2 h ($P < 0.05$), decreasing to 12% stimulation by 17 h ($P < 0.05$). Vagotomy alone significantly reduced cumulative food intake with a peak inhibition of 57% at 4 h ($P < 0.001$), decreasing to 30% inhibition by 17 h ($P < 0.001$). Devazepide did not stimulate food intake in the vagotomized rats receiving the intragastric peptone infusion. Cumulative intakes at all time points in vagotomized animals receiving devazepide were not different from those observed at the same time points in the same animals receiving vehicle. Figure 3B shows the effects of intravenous injection of devazepide (2.5 μmol/kg) in vagotomized rats not receiving the peptone infusion. Devazepide produced a significant, sustained increase in cumulative food intake from 3 to 9 h after dark onset, with a peak stimulation of 59% at 3 h ($P < 0.05$), decreasing to 19% stimulation by 9 h ($P < 0.05$).

Figure 4 shows the effects of a 3.5-h intravenous infusion of A-70104 (3 μmol·kg$^{-1}$·h$^{-1}$) or vehicle (0.15 M NaCl, 0.1% BSA, 1% DMSO) at dark onset on food intake in sham-vagotomized rats receiving the 2-h peptone infusion and in vagotomized rats not receiving the peptone infusion. In the sham-vagotomized rats, A-70104 produced a significant, sustained increase in cumulative food intake from 3 to 17 h after dark onset, with a peak stimulation of 54% at 3 h ($P < 0.01$), decreasing to 10% stimulation by 17 h ($P < 0.01$). A-70104 did not stimulate food intake in the vagotomized rats. Cumulative intakes at all time points in vagotomized animals receiv-

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**Fig. 2. Effects of intravenous infusion of CCK-8 (0.3 nmol·kg$^{-1}$·h$^{-1}$, A; 1 nmol·kg$^{-1}$·h$^{-1}$, B; and 10 nmol·kg$^{-1}$·h$^{-1}$, C) on food intake in control rats and vagotomized rats.** Non-food-deprived rats ($n = 13–15$/dose) received a 3.25-h intravenous infusion of CCK-8 or vehicle beginning 15 min before dark onset. Food intake was during the first 17 h after dark onset. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ compared with response at the same time to vehicle administration in the same rats.
CCK1 receptor antagonists differing in blood-brain barrier permeability (devazepide penetrates, A-70104 does not) were used to test the hypothesis that satiety is mediated in part by CCK action at CCK1 receptors on vagal sensory fibers innervating the small intestine. In rats with intact vagal nerves both devazepide and A-70104 stimulated food intake and attenuated the anorexic response to intragastric infusion of peptone. In contrast, only devazepide was effective in stimulating food intake in rats with total abdominal vagotomy. Thus endogenous CCK appears to act both at CCK1 receptors beyond the blood-brain barrier and by a CCK1 receptor-mediated mechanism involving abdominal vagal nerves to inhibit food intake.

Results of the present study are similar to those of other studies showing that vagal neural lesions block the anorexic response to intraperitoneal injection of low doses of CCK-8 (22, 29). This method of CCK administration, however, does not reproduce the prolonged secretion of intestinal CCK that likely occurs with ingestion of a meal. The present study extends these earlier findings by demonstrating that subdiaphragmatic vagotomy also blocks the anorexic response to a prolonged intravenous infusion of low, but not high, doses of CCK-8.

Mechanisms mediating the anorexic response to high systemic doses of CCK in vagotomized rats remain to be defined. Subdiaphragmatic vagotomy does not destroy the cell bodies of vagal afferent neurons sectioned by the vagotomy (25). Perhaps high doses of CCK activated the cell bodies of these remnants, which reside in the nodose ganglia, contain CCK1 receptors, and respond to CCK administration (48). CCK1 receptors have also been identified in enteric neurons, pyloric circular muscle, pancreas, and in discrete regions throughout the brain, including the area postrema, which lacks a blood-brain barrier (27). CCK does not readily penetrate the blood-brain barrier (34), so it is unlikely that systemically administered CCK acts at CCK1 receptors beyond the blood-brain barrier to inhibit food intake. The anorexic response to high doses of CCK in the vagotomized rats may have been mediated in part by one or more non-vagally mediated mechanisms involving CCK1 receptors in the area postrema, pyloric muscle, and enteric neurons.

Results of the present study confirm our previous results demonstrating that devazepide and A-70104 both increase food intake in rats (37, 38) and that devazepide increases food intake similarly whether or not rats are vagotomized (36) or pretreated with capsaicin to lesion visceral sensory nerves (42). The present study extends these findings by showing that A-70104
fails to increase food intake in vagotomized rats under the same conditions in which devazepide increases food intake in these animals. No prior study has demonstrated that vagal neural lesions prevent the orexigenic response to selective blockade of peripheral CCK1 receptors. These results, together with those demonstrating the existence of CCK-secreting endocrine cells in the epithelium of the upper small intestine (5, 47), CCK1 receptors within vagal afferent nerves (30, 53), activation of intestinal vagal afferent neurons by exogenous and endogenous CCK (14, 18, 24), and similar attenuation by CCK1 receptor antagonists and vagal neural lesions of anorexic responses to nutrient administration (41), provide strong evidence that satiety is mediated in part by CCK action at CCK1 receptors on vagal sensory fibers innervating the small intestine. Simasko and Ritter (45) recently provided evidence suggesting that CCK may activate both A- and C-type vagal afferent neurons to inhibit food intake.

In rats with intact vagal nerves the CCK1 receptor antagonist devazepide stimulated food intake when given alone and attenuated the anorexic response to intragastric infusion of peptone. Devazepide also stimulated food intake in vagotomized rats but not when they received the intragastric infusion of peptone. It is not clear why the peptone infusion prevented the orexigenic response to devazepide in the vagotomized animals. Our previous work suggests that in normal rats devazepide is more effective in reversing the anorexia produced by duodenal delivery of glucose, oleic acid, and peptone when the nutrients are delivered at doses that are on the lower end of their dose-response curves (50–52). This would be consistent with the idea that CCK plays a partial, indispensable role in mediating the satiety response to low rates of delivery of nutrients to the small intestine and that larger rates of nutrient delivery produce a greater stimulation of redundant CCK-independent satiety mechanisms. Subdiaphragmatic vagotomy stimulates gastric emptying of liquids (49). Perhaps peptone infusion into the stomach of the vagotomized rats caused a relatively large rate of delivery of peptone to the small intestine, which prevented the orexigenic response to devazepide.

There are several lines of evidence suggesting that CCK may also act as a neurotransmitter or neuromodulator within two different brain regions to produce satiety, one region that includes the nucleus tractus solitarius in the hindbrain and another more distributed region within the medial-basal hypothalamus. This conclusion is supported by studies showing that food intake releases hypothalamic CCK (15, 43), the hypothalamus and caudal brain stem contain CCK1 receptors (32), site-specific injections of CCK into the medial-basal hypothalamus and the caudal brain stem inhibit food intake (7), and brain injections of CCK antisera (16) and CCK receptor antagonists (12, 17, 19, 44) stimulate food intake. We previously speculated about the possible origin of endogenous CCK acting on central CCK1 receptors to inhibit food intake (7), so that discussion will not be repeated here.

Results of the present study provide further evidence that endogenous CCK acts at CCK1 receptors beyond the blood-brain barrier to inhibit food intake. Devazepide stimulated food intake in vagotomized rats under the same conditions in which A-70104 failed to increase intake. Because the only apparent difference between these CCK1 receptor antagonists is that devazepide can penetrate the blood-brain barrier and A-70104 cannot, then this finding suggests that in the vagotomized rats, devazepide blocked an endogenous CCK action at CCK1 receptors beyond the blood-brain barrier that normally reduces food intake. Whether such a central CCK mechanism also functions in normal rats to inhibit food intake was not specifically addressed by our study. Subdiaphragmatic vagotomy and the associated weight loss produced by this procedure may have altered the central neural circuitry controlling food intake or the physiology of CCK secretion and action in periphery and brain, in a manner that permitted the expression of a central CCK1 receptor-mediated inhibition of food intake. Subdiaphragmatic vagotomy has been reported to decrease the number of neurons expressing CCK1 receptors in nodose ganglia (10), decrease the density of CCK1 receptor fibers in gastric mucosa (46), and have no effect on CCK1 receptor binding in the area postrema, nucleus of the solitary tract, and pyloric muscle (30, 31). Food deprivation has been reported to decrease CCK1 receptor binding in the hypothalamic supraoptic and paraventricular nuclei (33) yet have no effect on the number of neurons expressing CCK1 receptors in nodose ganglia (10) or CCK1 receptor binding in the area postrema and nucleus of the solitary tract (33). These limited findings do not support the idea that vagotomy and weight loss induce the unique expression of a central CCK1 receptor-mediated mechanism to inhibit food intake.

Studies of the effects of brain injections of CCK receptor antagonists on food intake in rats have provided contradictory evidence. Earlier studies demonstrated an orexigenic response to intracerebroventricular and paraventricular nucleus injection of proglumide, a relatively weak, nonspecific antagonist of CCK1 and CCK2 receptors (12, 44). Intracerebroventricular administration of more potent and selective CCK1 and CCK2 receptor antagonists has been reported both to stimulate (17, 19) and to have no effect (8, 12) on food intake. The study by Ebenezer (19), which demonstrated an orexigenic response to devazepide, used much lower doses of devazepide (2.4–240 pmol) than the 0.5- to 5-nmol doses used in the study by Corp et al. (12) and the 2.4- to 720-nmol doses used in the study by Brenner and Ritter (8), both of which showed no effect of devazepide on food intake. Devazepide is poorly soluble in water. Perhaps significant precipitation of devazepide occurred within the aqueous, slow flow environment of the ventricles after intracerebroventricular injection of the higher doses of devazepide. This may explain why devazepide produced an inverted “U-shaped” dose-response curve in Ebenezer’s study (no effect of the 240-pmol dose) and why orexigenic intraperitoneal doses of devazepide were ineffective when injected intracerebroventricularly in the study by Brenner and Ritter. None of these studies assessed the bioavailability of antagonist doses administered intracerebroventricularly by determining whether they could block the anorexigenic response to brain injection of CCK.

Dorré and Smith (17) reported that intracerebroventricular injection of 1.2- to 620-nmol doses of the potent, selective CCK2 receptor antagonist PD-135158 stimulated food intake in rats. In rodents, PD-135158 is ~400 times more selective for the CCK2 than the CCK1 receptor (21). Perhaps PD-135158, at the high doses administered, stimulated food intake by blocking CCK1 rather than CCK2 receptors. Low doses of a CCK1 receptor antagonist were not administered for comparison in the same study. In an earlier study from the same laboratory, intracerebroventricular injection of 0.1- to 5-nmol doses of the
potent, selective CCK2 receptor antagonist L-365260 had no effect on food intake under the same conditions in which the relatively weak, nonspecific CCK antagonist proglumide stimulated food intake (12).

Bi et al. (6) recently provided evidence suggesting that CCK may act at CCK1 receptors in the dorsomedial hypothalamus (DMH) to inhibit food intake. The DMH normally contains a dense population of CCK1 receptors (32) and is a site where we previously demonstrated that CCK potently inhibits food intake (7). The adult Otsuka Long-Evans Tokushima Fatty (OLETF) rat, which lacks CCK1 receptors, is hyperphagic and obese. Young, preobese OLETF rats exhibit a fivefold elevation in DMH neuropeptide Y (NPY) expression compared with that observed in lean, Long-Evans Tokushima Otsuka (LETO) control rats. Thus Bi et al. (6) postulated that hyperphagia in the OLETF rat is due in part to the absence of a DMH CCK1 receptor-mediated mechanism that normally inhibits a DMH NPY-mediated mechanism that stimulates food intake. It remains to be determined whether systemic devazepide administration increases DMH NPY expression in normal rats. It would also be useful to determine whether OLETF rats exhibit alterations in caudal brain stem regions that normally contain CCK1 receptors and are linked to the control of food intake.

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REFERENCES