Role of capsaicin-sensitive peripheral sensory neurons in anorexic responses to intravenous infusions of cholecystokinin, peptide YY-(3–36), and glucagon-like peptide-1 in rats

Roger Reidelberger,1,2 Alvin Haver,1,2 Krista Anders,2 and Bettye Apenteng2

1Veterans Affairs Research Service, Veterans Affairs Nebraska Western Iowa Health Care System, Omaha, Nebraska; and 2Department of Biomedical Sciences, Creighton University, Omaha, Nebraska

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Reidelberger R, Haver A, Anders K, Apenteng B. Role of capsaicin-sensitive peripheral sensory neurons in anorexic responses to intravenous infusions of cholecystokinin, peptide YY-(3–36), and glucagon-like peptide-1 in rats. Am J Physiol Endocrinol Metab 307: E619–E629, 2014. First published August 12, 2014; doi:10.1152/ajpendo.00024.2014.—Cholecystokinin (CCK)-induced suppression of feeding is mediated by vagal sensory neurons that are destroyed by the neurotoxin capsaicin (CAP). Here we determined whether CAP-sensitive neurons mediate anorexic responses to intravenous infusions of gut hormones peptide YY-(3–36) [PYY-(3–36)] and glucagon-like peptide-1 (GLP-1). Rats received three intraperitoneal injections of CAP or vehicle (VEH) in 24 h. After recovery, non-food-deprived rats received at dark onset a 3-h intravenous infusion of CCK-8 (5, 17 pmol·kg−1·min−1), PYY-(3–36) (5, 17, 50 pmol·kg−1·min−1), or GLP-1 (17, 50 pmol·kg−1·min−1). CCK-8 was much less effective in reducing food intake in CAP vs. VEH rats. CCK-8 at 5 and 17 pmol·kg−1·min−1 reduced food intake during the 3-h infusion period by 15, 33, and 70% in VEH rats and 13, 30, and 33% in CAP rats. In contrast, PYY-(3–36) and GLP-1 were similarly effective in reducing food intake in VEH and CAP rats. PYY-(3–36) at 5, 17, and 50 pmol·kg−1·min−1 reduced food intake during the 3-h infusion period by 15, 33, and 70% in VEH rats and 13, 30, and 33% in CAP rats. These results suggest that anorexic responses to PYY-(3–36) and GLP-1 are not primarily mediated by the CAP-sensitive peripheral sensory neurons (presumably vagal) that mediate CCK-8-induced anorexia.

CHOLECYSTOKININ (CCK), glucagon-like peptide-1 (GLP-1), and peptide YY-(3–36) [PYY-(3–36)] are postulated to act as hormonal signals from gut to brain to inhibit food intake. Studies showing an increase in food intake in response to systemic administration of receptor antagonists for CCK, GLP-1, and PYY-(3–36) suggest that these gut peptides play essential roles in meal-induced satiety (8, 16, 39, 46, 49, 62). There is now strong evidence that CCK, secreted from epithelial cells in the mucosa of the small intestine in response to a meal, acts through paracrine stimulation of intestinal vagal sensory neurons to inhibit food intake. Studies have identified CCK enteroendocrine cells in the innermost epithelial layer lining the lumen of the small intestine (10), vagal sensory nerve terminals in adjacent lamina propria of the mucosal layer (10), and CCK1 receptors within vagal afferent nerves (40).

Functional studies have shown that exogenous and endogenous CCK stimulate intestinal vagal afferent neurons (19, 35) and that CCK1 receptor antagonists (49, 63–65) and vagal denervation (50) both attenuate anorexic responses to exogenous CCK (49, 50) and nutrient delivery to the small intestine (50, 63–65). However, a recent study by Zhang and Ritter (66) suggests that CCK may also act by a nonvagal endocrine mechanism (via the systemic circulation) to reduce food intake.

Evidence supporting a similar paracrine mechanism of action for GLP-1 and PYY-(3–36) to reduce food intake is less clear. Studies have identified GLP-1- and PYY-(3–36)-secreting cells in the luminal epithelial lining of the small and large intestines (3, 20), as well as receptors for these peptides in vagal sensory neurons (30, 41). However, vagal denervation has been reported to both attenuate (1, 27, 29, 30, 34, 44, 52, 57, 66) and have no effect (25, 52, 57, 66) on anorexic responses to systemic administration of GLP-1 (1, 27, 29, 34, 44, 52, 57, 66) and PYY-(3–36) (1, 25, 30, 57) receptor agonists. Possible reasons for these discrepancies include methodological differences in 1) vagal denervation (subtotal pharyngeal vagotomy, selective vagal deafferentation, systemic capsaicin administration); 2) administration of peptides, including site (intraperitoneal, portal vein, systemic venous), duration (injection, infusion), and timing (light vs. dark cycle) of administration; and 3) feeding paradigm (fasted or non-fasted, light vs. dark phase eating, liquid vs. solid food, single meal vs. intake over longer periods).

Several studies have reported that poorly acclimatized animals receiving intraperitoneal injections of PYY-(3–36) and well-acclimatized, fasted, vagotomized animals receiving intraperitoneal injections of PYY-(3–36) consume less food presumably because of stress, which masks the anorexigenic effects of PYY-(3–36) (2, 25, 26). In all other studies (1, 5, 27, 29, 30, 34, 44, 52, 57) except one (57) reporting attenuated anorexic responses to exogenous GLP-1 receptor agonists or PYY-(3–36) in vagal- or capsaicin-denervated subjects, the denervated subjects also appeared to exhibit lower baseline food intake. Thus, studies reporting attenuated anorexic responses to exogenous PYY-(3–36) and GLP-1 following vagal denervation may reflect stress-induced artifacts caused by manual injection of peptide and/or the vagal denervation procedure itself, rather than neuronal mediation of peptide-induced anorexia.

Despite the potential for producing stress, intraperitoneal injection continues to be the method used by most investigators to study mechanisms of action of putative satiety peptides in rodents because it is the easiest and least costly means to administer the substances. Several investigators have also ar-
gued that intraperitoneal administration of gut peptides like CCK, PYY-(3–36), and GLP-1 better mimics physiological paracrine actions of the peptides in the lamina propria of the small intestine (22, 23, 27, 37, 52, 66). Greenberg et al. (22, 23) and Lo et al. (37) showed that acute administration of CCK-8 intraorally or intravenously, respectively, had little if any effect on food intake when administered at doses that inhibit food intake when administered intraperitoneally. Thus, Greenberg et al. (22) concluded that their results support the hypothesis that endogenous CCK primarily acts through an intestinal paracrine mechanism. Lo et al. (37) provided further support for this hypothesis by showing that intraperitoneal CCK-8 injection, compared with intravenous injection, produces a larger and more prolonged increase in CCK-8 levels in mesenteric lymph, which includes lymph formed in lacteals within the lamina propria. However, the pharmacokinetics of intravenously and intraperitoneally injected peptide are likely to be quite different if duration of administration is relatively short and the peptide has a relatively short half-life, such as those for CCK-8, GLP-1, and PYY-(3–36). For example, plasma CCK-8 levels rapidly rise and fall within 5 min of intravenous injection (28), whereas intraperitoneal injection of CCK-8 appears to produce a blunter, more prolonged rise in plasma CCK-8 (36) that may produce a greater suppression of feeding. Indeed, for two other gut peptides, GLP-1 and PYY-(3–36), we have shown that prolonging the delivery of the same total intravenous dose from 15 min to 3 h produces a significantly greater reduction in food intake (12, 13). Thus, to directly assess whether an intraperitoneally administered peptide reduces feeding via the systemic circulation, one would need to measure changes in systemic plasma levels of the peptide following intraperitoneal administration and then determine whether reproducing these changes by intravenous infusion reduces food intake. This has not been done for any of the intraperitoneally administered peptides postulated to preferentially stimulate a vagal sensory nerve ending (10) and a dense capillary network (11–14, 45, 46). Rats are free to move, eat, and drink within their home cages, and their indwelling catheters remain functional for many months. Measurement of food bowl weight, recorded by computer every 20 s, permits daily assessment of the instantaneous effects of dose and pattern of administration of substances on food intake and meal patterns. We used this experimental model to help resolve a significant controversy on whether PYY-(3–36) reduces food intake and body weight (6, 11, 13, 24, 43, 59). We demonstrated that 3-h intravenous infusion of PYY-(3–36), intended to simulate postprandial secretion of the gut hormone, dose-dependently reduces short-term food intake in rats and that daily intermittent PYY-(3–36) infusion reduces body weight in lean and diet-induced obese rats (11, 13, 15). We concluded that, because of its short half-life, bolus systemic injection of PYY-(3–36) to rodents, the method routinely used by most investigators to study mechanisms of action of putative satiety hormones, does not reliably reduce food intake and body weight. Others have also argued that bolus intraperitoneal injections can produce acute stress and that stress can mask the inhibitory effect of anorexigenic substances on food intake (2, 26). This is not a factor with our experimental model.

Here we used peripheral administration of the neurotoxin capsaicin to assess whether capsaicin-sensitive neurons (presumably vagal) mediate anorexigenic responses to 3-h intravenous infusions of CCK-8, PYY-(3–36), and GLP-1 at dark onset in non-food-deprived rats consuming rat chow. Three-hour intravenous infusions were employed both to simulate duration of postprandial secretion of the gut peptides and to activate potential endocrine and paracrine mechanisms of gut peptide action. The mucosal lamina propria adjacent to the peptide-secreting cells in the villi of the small intestine contains both vagal sensory nerve endings (10) and a dense capillary network, which allows circulating peptides ready access to these neurons.

**METHODS**

Subjects. Male rats (~400 g at start of study; Sasco Sprague-Dawley; Charles River Laboratories, Kingston, NY) 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). Animals were provided rat chow (Labdiet, 5001 Rodent diet; PMI Nutrition International, Brentwood, MO) and water ad libitum. The Animal Studies Subcommittee of the Omaha Veterans Affairs Medical Center approved the experimental protocol.

CCK-8, PYY-(3–36), and GLP-1. CCK-8 was purchased from Tocris Bioscience (R&D Systems, Minneapolis, MN). Rat PYY-(3–36) and GLP-1 were synthesized by 9-fluorenylmethoxycarbonyl solid-phase methodology (4) and purified by reverse-phase high performance liquid chromatography. Proof of structure was provided by electrospray mass spectrometry.

Capsaicin treatment. Intraperitoneal injection of capsaicin was used to destroy small unmyelinated primary afferent neurons as described previously (66). Capsaicin (Sigma, St. Louis, MO) was dissolved in a vehicle consisting of Tween 80 (10%), ethanol (10%), and 0.9% NaCl (80%) to produce a capsaicin concentration of 50 mg/ml. Each rat (n = 25) received three intraperitoneal injections of capsaicin (25, 50, and 50 mg/kg) over a 24-h period. At each injection, rats were anesthetized with isoflurane, and mechanical ventilation was used as needed to aid respiration. Control rats (n = 26) received intraperitoneal injections of vehicle under the same conditions. Rats were allowed at least 2 wk to recover before implantation of a jugular vein catheter. A corneal chemosensory test was used to assess efficacy of capsaicin treatment in destroying small unmyelinated afferent neurons (66). When a drop of 1% NH₄OH was applied to the eye, all capsaicin-treated rats responded by rapidly wiping the eye. None of the capsaicin-treated rats exhibited this behavior either before or after the series of peptide administration experiments was completed.

Jugular vein catheterization. Procedures for implanting a jugular vein catheter for administration of CCK-8, PYY-(3–36), and GLP-1 were as described.
were described previously (63). Jugular vein catheters were filled with heparinized saline (40 U/ml), plugged with stainless steel wire, and flushed every other day to maintain patency. Catheters were connected to 40-cm lengths of tubing passed through a protective spring coil connected between a lightweight saddle worn by the rat and a single-channel infusion swivel (Instech Laboratories, Plymouth Meeting, PA).

Effects of capsaicin treatment on anorexigenic responses to intravenous infusions of CCK-8, PYY-(3–36), and GLP-1. Five experiments were performed in chronological order. The first experiment, performed ~1 mo after capsaicin or vehicle treatments, determined the effects of 3-h intravenous infusion of CCK-8 (0, 5, and 17 pmol·kg⁻¹·min⁻¹) at dark onset on food intake and meal patterns in control and capsaicin-treated rats. The next four experiments of similar design determined the effects of intravenous infusions of PYY-(3–36) (0, 5, and 17 pmol·kg⁻¹·min⁻¹), PYY-(3–36) (0 and 50 pmol·kg⁻¹·min⁻¹), GLP-1 (0, 17, and 50 pmol·kg⁻¹·min⁻¹), and CCK-8 (0 and 17 pmol·kg⁻¹·min⁻¹) on feeding in the control and capsaicin-treated rats. Three-hour intravenous infusions were employed both to simulate duration of postprandial secretion of the gut peptides and to activate potential endocrine and paracrine mechanisms of gut peptide action. Doses were based on our previous findings using the same experimental model that showed that 3-h intravenous infusions of CCK-8, PYY-(3–36), and GLP-1 dose dependently reduce 3-h food intake with mean effective doses of 14, 15, and 23 pmol·kg⁻¹·min⁻¹, respectively (12, 13, 47). Previous studies showed that systemic treatment with capsaicin significantly attenuates CCK-8-induced anorexia (51, 55, 66). Here we bracketed the PYY-(3–36) and GLP-1 experiments with CCK-8 experiments to assess the extent to which capsaicin-induced denervation persisted across the series of experiments, which were completed in ~1 mo (~ 2 mo after capsaicin/ vehicle treatments).

Animals were permitted at least 1 wk to recover from implantation of catheters. 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 h before onset of the dark period and peptide infusions. We have used this non-food-deprived approach in numerous studies examining the effects of putative anorexigenic substances and their receptor antagonists on food intake in order to achieve more normal baseline meal sizes and pattern during the first 3 h of the dark period when treatments are administered. However, first meal parameters after onset of peptide infusion can be affected not only by the peptide itself but also by food eaten immediately before infusion onset, and we know that rats will awaken to eat some food after fresh food is provided. Thus, here we have chosen not to report the effects of infused peptide on first meal parameters and 1-h food intake because the data are quite variable [e.g., when vehicle was infused, coefficient of variation in 1-h intake is 2–3 times larger than those for 3- and 6-h intakes (60, 30, and 20%, respectively)].

In the first experiment, non-food-deprived control and capsaicin-treated rats (n = 16 each) received a 3-h intravenous infusion of CCK-8 (0, 5, or 17 pmol·kg⁻¹·min⁻¹; 2 ml/h) or vehicle (0.15 M NaCl, 0.1% BSA) that began 15 min before dark onset because catheter dead-space volume was about 0.5 ml. Cumulative food intake at 3, 6, and 12 h after dark onset was determined, as described previously, from continuous computer recording of changes in food bowl weight (63). Meal parameters (mean meal size and number of meals) during these periods were determined using a minimum meal size criterion of 50 mg, and a minimum intermeal interval criterion of 15 min, as recommended by Zorrilla et al. (67). Infusions were administered using a syringe infusion pump (Harvard Apparatus, South Natick, MA); pumps were turned on and off by a computer program. Within an experiment, each rat received each treatment in random order at intervals of at least 48 h. At the end of the 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 in the catheter. In the next four experiments of similar design, control and capsaicin-treated rats (n = 16 each) received intravenous infusions of PYY-(3–36) (0, 5, and 17 pmol·kg⁻¹·min⁻¹), PYY-(3–36) (0 and 50 pmol·kg⁻¹·min⁻¹), GLP-1 (0, 17, and 50 pmol·kg⁻¹·min⁻¹), and CCK-8 (0 and 17 pmol·kg⁻¹·min⁻¹).

Statistical analyses. Values are presented as group means ± SE. Effects of capsaicin treatment on changes in cumulative food intake, mean meal size, and number of meals in response to 3-h intravenous infusions of CCK-8, PYY-(3–36), and GLP-1 were evaluated at 3, 6, and 12 h after infusion onset using a two-factor, split-plot ANOVA design, with the between-group factor reflecting vehicle or capsaicin treatment and the within-group factor reflecting peptide dose. Separate ANOVAs were used for each time period because cumulative food intakes, mean meal sizes, and number of meals at the different time points are not independent measures. Differences were considered significant if P < 0.05.

RESULTS

Effects of capsaicin treatment on body weight, food intake, and meal patterns. Body weights in control and capsaicin-treated rats did not differ significantly across the various phases of the study (Fig. 1). Cumulative food intakes and mean meal sizes at 3, 6, and 12 h were also similar in control and capsaicin-treated rats receiving vehicle infusions in the various peptide infusion experiments (Fig. 2, A–C). On the other hand, capsaicin treatment slightly increased the number of meals consumed during the 12-h dark period in these experiments (Fig. 2C). ANOVA showed a significant main effect of capsaicin on number of meals during the 12-h dark period [F(1,137) = 7.3, P < 0.01], yet no main effect of experiment [F(4,137) = 0.4, P > 0.05], or interaction of capsaicin and experiment [F(4,137) = 0.4, P > 0.05]. Main effects and interactions were not statistically significant for cumulative number of meals at 3 and 6 h or for cumulative food intake or mean meal size at 3, 6, and 12 h.

Effects of capsaicin treatment on anorexigenic responses to intravenous infusion of CCK-8. CCK-8 infusion for 3 h at dark onset was much less effective in reducing food intake in capsaicin-treated vs. control rats (Fig. 3A). CCK-8 at 5 and 17
pmol·kg$^{-1}$·min$^{-1}$ reduced mean 3-h food intake in control rats by 39 and 71%, respectively, and in capsaicin-treated rats by 7% and 18%, respectively. At 3 and 6 h after infusion onset, ANOVA demonstrated significant main effects of CCK-8 $[F(2,56) = 33.8, P < 0.001 \text{ and } F(2,56) = 19.1, P < 0.001,$ respectively] and capsaicin $[F(1,28) = 28.7, P < 0.001 \text{ and } F(1,28) = 9.5, P < 0.01,$ respectively] on cumulative food intake, as well as significant interactions between CCK-8 and capsaicin $[F(2,56) = 12.3, P < 0.001 \text{ and } F(2,56) = 15.5, P < 0.001,$ respectively]. CCK-8 reduced cumulative food intake in

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**Fig. 2.** Baseline cumulative food intake (A), mean meal size (B), and no. of meals (C) in control and capsaicin-treated rats receiving vehicle infusions in the 5 peptide infusion experiments. Non-food-deprived rats ($n = 13–16$) received a 3-h iv infusion of vehicle beginning 15 min before dark onset. Values are means ± SE. *Main effect of capsaicin and no interaction between capsaicin and experiment. CCK-8, cholecystokinin-8; GLP-1, glucagon-like peptide-1; PYY, peptide YY.

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**Fig. 3.** Effects of CCK-8 infusion on cumulative food intake (A), mean meal size (B), and no. of meals (C) in control and capsaicin-treated rats. Non-food-deprived rats ($n = 14–16$) received a 3-h iv infusion of CCK-8 (5 or 17 pmol·kg$^{-1}$·min$^{-1}$) or vehicle beginning 15 min before dark onset. Values are means ± SE. *Interaction between CCK-8 and capsaicin.
control rats by decreasing the number of meals (Fig. 3, B and C). In contrast, CCK-8 produced little if any effect on meal parameters in capsaicin-treated rats. At 3 h, ANOVA demonstrated significant main effects of CCK-8 \([F(2,56) = 4.6, P < 0.01]\) and capsaicin \([F(1,28) = 10.3, P < 0.01]\) on number of meals, as well as a significant interaction between CCK-8 and capsaicin \([F(2,56) = 3.3, P < 0.05]\). At 6 h, ANOVA demonstrated a significant main effect of capsaicin \([F(1,28) = 5.5, P < 0.05]\) on number of meals, as well as a significant interaction between CCK-8 and capsaicin \([F(2,56) = 5.6, P < 0.01]\). Main effects of CCK-8 and capsaicin and their interactions were not statistically significant for mean meal size at 3, 6, or 12 h.

Effects of capsaicin treatment on anorexic responses to intravenous infusion of PYY-(3–36). PYY-(3–36) infusion for 3 h at dark onset reduced food intake similarly in capsaicin-treated and control rats (Figs. 4A and 5A). PYY-(3–36) at 5, 17, and 50 pmol·kg\(^{-1}\)·min\(^{-1}\) reduced mean 3-h food intake by 15, 33, and 70% in control rats and by 13, 30, and 33% in capsaicin-treated rats. In the experiment examining the effects of low doses of PYY-(3–36) (5 and 17 pmol·kg\(^{-1}\)·min\(^{-1}\)), ANOVA demonstrated significant main effects of PYY-(3–36) on cumulative food intake at 3, 6, and 12 h \([F(2,56) = 19.1, P < 0.001]\) and no significant main effects of capsaicin \([F(1,28) = 1.9, P > 0.05]\), yet no significant main effects of capsaicin \([F(1,28) = 2.4, P > 0.05]\), or interactions between PYY-(3–36) and capsaicin \([F(2,56) = 0.003, P > 0.05]\), yet no significant main effects of capsaicin \([F(1,28) = 1.7, P > 0.05]\), or interactions between PYY-(3–36) and capsaicin \([F(2,56) = 0.7, P > 0.05]\) at these time points. In the experiment examining the effects of the high dose of PYY-(3–36) (50 pmol·kg\(^{-1}\)·min\(^{-1}\)), ANOVA demonstrated significant main effects of PYY-(3–36) on cumulative food intake at 3, 6, and 12 h \([F(1,29) = 7.8, P < 0.01]\) and no significant main effects of capsaicin \([F(1,29) = 11.3, P < 0.01]\), yet no significant main effects of capsaicin \([F(1,29) = 0.2, P > 0.05]\), or interactions between PYY-(3–36) and capsaicin \([F(2,56) = 0.7, P > 0.05]\) at these time points.

PYY-(3–36) reduced food intake in control and capsaicin-treated rats by similarly decreasing mean meal size (Figs. 4, A–C, and 5, A–C). In the experiment examining the effects of low doses of PYY-(3–36) (5 and 17 pmol·kg\(^{-1}\)·min\(^{-1}\)), ANOVA demonstrated significant main effects of PYY-(3–36) on mean meal size at 3, 6, and 12 h \([F(2,56) = 10.3, P < 0.001]\) and no significant main effects of capsaicin \([F(1,28) = 0.1, P > 0.05]\), or interactions between PYY-(3–36) and capsaicin \([F(2,56) = 0.3, P > 0.05]\) at these time points. In the experiment examining the effects of the high dose of PYY-(3–36) (50 pmol·kg\(^{-1}\)·min\(^{-1}\)), ANOVA demonstrated significant main effects of PYY-(3–36) on mean meal size at 3 and 6 h but not 12 h \([F(1,30) = 4.6, P < 0.05]\) and \([F(1,29) = 11.3, P < 0.01]\), yet no significant main effects of capsaicin \([F(1,28) = 0.8, P > 0.05]\), yet no significant main effects of capsaicin \([F(1,28) = 0.3, P > 0.05]\) at these time points.

Effects of PYY-(3–36) on food intake at 3, 6, and 12 h. Values are means ± SE. aMain effect of PYY-(3–36) and no interaction between PYY-(3–36) and capsaicin. 

Values are means ± SE. aMain effect of PYY-(3–36) and no interaction between PYY-(3–36) and capsaicin.
Effects of capsaicin treatment on anorexic responses to intravenous infusion of GLP-1. GLP-1 infusion for 3 h at dark onset reduced 3-h food intake similarly in capsaicin-treated and control rats (Fig. 6A). GLP-1 at 17 and 50 pmol·kg\(^{-1}\)·min\(^{-1}\) reduced mean 3-h food intake by 48 and 60% in control rats and by 30 and 52% in capsaicin-treated rats. GLP-1-induced anorexia, however, was of shorter duration in capsaicin-treated vs. control rats. After 6 h, GLP-1 reduced mean cumulative food intake by 40 and 66% in control rats and by 17 and 34%
in capsaicin-treated rats. After 12 h, GLP-1 reduced mean cumulative food intake by 15 and 28% in control rats and by 2 and 9% in capsaicin-treated rats. At 3 h, ANOVA demonstrated a significant main effect of GLP-1 on cumulative food intake [$F(2,52) = 70.1$, $P < 0.001$], yet no significant main effect of capsaicin [$F(1,26) = 0.02$, $P > 0.05$], or interaction between GLP-1 and capsaicin [$F(2,52) = 2.9$, $P > 0.05$]. At 6 and 12 h, ANOVA demonstrated significant main effects of GLP-1 on cumulative food intake [$F(2,52) = 92.7$, $P < 0.001$ and $F(2,52) = 19.5$, $P < 0.001$, respectively] and no significant main effects of capsaicin [$F(1,26) = 3.0$, $P > 0.05$ and $F(1,26) = 2.7$, $P > 0.05$, respectively], yet it now demonstrated significant interactions between GLP-1 and capsaicin [$F(2,52) = 13.4$, $P < 0.001$ and $F(2,52) = 5.6$, $P < 0.01$, respectively].

GLP-1 reduced food in control rats by decreasing both meal size and number of meals, and capsaicin treatment prevented the decrease in number of meals but not meal size (Fig. 6, B and C). ANOVA demonstrated significant main effects of GLP-1 on mean meal size at 3, 6, and 12 h [$F(2,52) = 21.1$, $P < 0.001$; $F(2,52) = 23$, $P < 0.001$; $F(2,52) = 7.7$, $P < 0.01$, respectively], yet no significant main effects of capsaicin [$F(1,26) = 0.03$, $P > 0.05$; $F(1,26) = 0.004$, $P > 0.05$; $F(1,26) = 0.6$, $P > 0.05$, respectively], or significant interactions between GLP-1 and capsaicin [$F(2,52) = 0.3$, $P > 0.05$; $F(2,52) = 0.1$, $P > 0.05$; $F(2,52) = 0.2$, $P > 0.05$, respectively] at these time points. Main effects and interactions of GLP-1 and capsaicin on cumulative number of meals were not significant at 3 or 12 h. In contrast, at 6 h there was a significant main effect of GLP-1 [$F(2,52) = 7.2$, $P < 0.01$], no significant main effect of capsaicin [$F(1,26) = 2.6$, $P > 0.05$], yet a significant interaction between GLP-1 and capsaicin [$F(2,52) = 4.6$, $P < 0.05$]. Thus, the shorter duration of GLP-1-induced anorexia observed in capsaicin-treated rats (Fig. 6A) may have been due in part to an effect of capsaicin treatment alone to increase the number of meals consumed during the dark period in freely feeding rats (Fig. 2C).

Effects of capsaicin treatment on anorexic responses to intravenous infusion of CCK-8. This final experiment, which was performed about 2 mo after capsaicin treatment and 1 mo after the first CCK-8 experiment, again examined the effects of CCK-8 infusion at 17 pmol·kg$^{-1}$·min$^{-1}$ on food intake in control and capsaicin-treated rats. In the initial experiment, CCK-8 (17 pmol·kg$^{-1}$·min$^{-1}$) reduced mean cumulative food intake at 3 and 6 h by 71 and 37%, respectively, in control rats, and by 18 and 1% in capsaicin-treated rats (Fig. 7A). In the second CCK-8 experiment 1 mo later, this CCK-8 dose was again much less effective in reducing food intake in capsaicin-treated vs. control rats (Fig. 7, A and B). CCK-8 reduced mean cumulative food intake at 3 and 6 h by 56 and 45% in control rats and by 12 and 9% in capsaicin-treated rats. At 3 h, ANOVA demonstrated a significant main effect of CCK-8 [$F(1,25) = 23.4$, $P < 0.001$] on cumulative food intake, no significant main effect of capsaicin [$F(1,25) = 3.8$, $P > 0.05$], and a significant interaction between CCK-8 and capsaicin [$F(1,25) = 10.6$, $P < 0.01$]. At 6 h, ANOVA demonstrated a significant main effect of CCK-8 [$F(1,25) = 30.1$, $P < 0.001$] on cumulative food intake, a significant main effect of capsaicin [$F(1,25) = 4.5$, $P < 0.05$], and a significant interaction between CCK-8 and capsaicin [$F(1,25) = 14.0$, $P < 0.001$]. Thus, capsaicin treatment significantly attenuated the anorexic response to CCK-8 infusion in a similar manner in the two experiments.

DISCUSSION

Here we used peripheral administration of the neurotoxin capsaicin to assess whether intestinal vagal afferent neurons mediate anorexic responses to 3-h intravenous infusions of CCK-8, PYY-(3–36), and GLP-1 at dark onset in non-food-deprived rats consuming rat chow. Berthoud (9) describes the current methodology used to manipulate vagal afferents as “rudimentary at best, as they do not allow selective ablation or stimulation of functionally specific neurons.” Previous studies examining the role of intestinal vagal afferents in mediating PYY-(3–36) and GLP-1-induced anorexia used subdiaphragmatic vagotomy (1, 5, 30, 44, 66), selective vagal deafferentation (27, 29, 34, 52), and systemic capsaicin (57, 66) to ablate intestinal vagal afferents. Each of these methods has advantages and disadvantages. Subdiaphragmatic vagotomy involves
bilateral transection of left and right esophageal vagal nerves. This procedure not only ablates all intestinal vagal afferents but also all vagal afferents from stomach, pancreas, and liver, and all vagal efferents to stomach, intestines, pancreas, and liver, which produces significant disturbances in gastrointestinal motility and secretion, and reductions in baseline meal size, food intake, and body weight (32). Selective vagal deafferentation involves transecting the left dorsal vagal rootlets as they enter the brain as well as the left dorsal esophageal vagal nerve (42). This procedure not only ablates all intestinal vagal afferents but also all vagal afferents from stomach, pancreas, and liver, one-half of vagal afferents from tissues anterior to the diaphragm, including the esophagus, and one-half of vagal efferents to stomach, intestines, pancreas, and liver. Selective vagal deafferentation appears to reduce eating rate, meal size, and food intake (27, 29, 34, 52). Systemic capsaicin causes degeneration of vagal and nonvagal small-diameter unmyelinated sensory neurons (C type) (21). Thus, it does not ablate all vagal afferents, and it is not selective for vagal sensory nerves (60). Here we show that systemic capsaicin did not reduce baseline food intake, meal size, or body weight. Talsania et al. (57) also reported no effects of systemic capsaicin on baseline food intake. It remains to be determined whether the reduced eating in rats with selective vagal deafferentation is a nonspecific effect of the procedure or reflects a more complete vagal afferent lesion than that produced by capsaicin administration.

Previous studies show that the anorexic response to intraperitoneal injection of CCK-8 is primarily mediated by capsaicin-sensitive vagal sensory neurons (55, 66). Here we show that intraperitoneal administration of the neurotoxin capsaicin blocked anorexic responses to 3-h intravenous infusions of CCK-8 at dark onset in non-food-deprived rats consuming rat chow. In contrast, capsaicin treatment had little if any effect on anorexic responses to 3-h intravenous infusions of PYY-(3–36) and GLP-1 during the 3-h infusion period, although duration of GLP-1-induced anorexia beyond the infusion period was reduced in capsaicin-treated rats. These results suggest that the anorexic response to intravenous infusion of CCK-8 is mediated by capsaicin-sensitive sensory (presumably vagal) neurons and that anorexic responses to intravenous infusions of PYY-(3–36) and GLP-1 are not primarily mediated by these neurons.

Here we showed that intravenous infusion of GLP-1 reduced food intake by decreasing meal size and number of meals, consistent with our earlier findings (12), and that capsaicin treatment prevented the peptide-induced decreases in meal number but not meal size. However, we also observed that capsaicin treatment alone increased the number of meals consumed during the dark period in our freely feeding rats. Thus, it remains to be determined whether the shorter duration of GLP-1-induced anorexia observed in capsaicin-treated rats beyond the 3-h infusion period was due in part to an independent effect of capsaicin to increase the number of meals.

Our results confirm and extend the findings of Zhang and Ritter (66), which showed that neither capsaicin treatment nor subdiaphragmatic vagotomy attenuates anorectic responses to 60-min intravenous infusions of GLP-1 during the light period in 4-h fasted rats consuming 15% sucrose solution. However, in contrast to our results, Zhang and Ritter (66) reported that anorectic responses to intravenous infusion of CCK-8 are only partially attenuated by vagotomy and not affected by capsaicin treatment. The reason for this discrepancy may be the higher CCK-8 doses employed by Zhang et al. (~60 and 120 pmol·kg \(^{-1}\)·min \(^{-1}\) vs. 5 and 17 pmol·kg \(^{-1}\)·min \(^{-1}\) in our study), which may have stimulated redundant nonvagal and vagal mechanisms. Delivery of a larger proportion of infused CCK-8 to the vagal sensory nerve terminals in the intestinal lamina propria may also have occurred in our rats because chow consumption, compared with ingestion of sucrose solution, likely produces a greater postprandial hyperemia (33). Together, these results suggest that the anorectic response to circulating CCK-8 is mediated by capsaicin-sensitive sensory (presumably vagal) neurons and that anorectic responses to circulating PYY-(3–36) and GLP-1 are not primarily mediated by these neurons.

Our results are also consistent with those of Rüttimann et al. (52) demonstrating that the anorectic response to acute hepatic portal vein infusion of GLP-1 does not require vagal afferent signaling. In contrast, they reported that selective vagal deafferentation blocked the anorectic response to a remote acute infusion of GLP-1 in the intraperitoneal cavity. Thus, they argued that intraperitoneal GLP-1 might better mimic a paracrine vagal-mediated mechanism of GLP-1 action in the lamina propria of the small intestine, whereas intravenous GLP-1 may act directly in the brain.

As discussed in the introduction, there is no direct evidence that intraperitoneal administration of any intestinal hormone, including GLP-1, preferentially stimulates a paracrine mechanism in the lamina propria of the small intestine. Furthermore, it is unlikely that intraperitoneally administered peptide diffuses through several thick layers of gut wall to reach the lamina propria (38). Rather, it is more likely that the peptide first enters the hepatic portal vein and then, like intravenously administered peptide, reaches the lamina propria via the arterial system and capillary networks within villi of the small intestine.

Assuming then that anorectic responses to intravenously and intraperitoneally administered GLP-1 are mediated by the same mechanism(s), why did selective vagal deafferentation appear to block the effects of intraperitoneal but not intravenous GLP-1 in the study by Rüttimann et al. (52)? This may have been because of a lower level of baseline intake in the denervated rats that masked the inhibitory response to intraperitoneally administered peptide. Several studies have now demonstrated that lower baseline food intakes in vagotomized mice (25), and in unacclimatized mice and rats receiving intraperitoneal injections of PYY-(3–36) (2, 26) can mask the anorexigenic effects of PYY-(3–36). In all other studies (1, 5, 27, 29, 30, 34, 44, 52, 57) except one (57) reporting attenuated anorectic responses to exogenous GLP-1 receptor agonists or PYY-(3–36) in vagal- or capsaicin-denervated subjects, the denervated subjects also appeared to exhibit lower baseline food intake. Thus, attenuation of peptide-induced anorexia in these subjects may have been a consequence of lower baseline food intake rather than blockade of a vagally mediated mechanism of peptide action. The one study reporting similar baseline food intakes in denervated and control animals showed that, in mice, systemic capsaicin treatment attenuated the anorexic response to a single intraperitoneal dose of long-acting GLP-1 receptor agonist exendin-4 but not to a single intraperitoneal dose of PYY-(3–36) (57). In the present study, capsaicin treatment altered neither baseline food intakes nor
anorexie responses to 3-h intravenous infusions of GLP-1 or PYY-(3–36) during the 3-h infusion period.

Rüttimann et al. (52) and Labouesse et al. (34) provide evidence that selective vagal deafferentation attenuates anorexie responses to remote intraperitoneal infusion of GLP-1 agonists (=2.5 min in duration) only within the first meal or first hour after infusion onset in the dark period. Together, these results suggest that vagal sensory neurons play an essential role in mediating only the early satiating effects of GLP-1. Here we administered GLP-1 and PYY-(3–36) during the first 3 h of the dark period in control and capsaicin-treated rats and reported food intake data only at 3, 6, and 12 h after infusion onset. The reason why first meal and first hour intake data were not reported is offered in METHODS under Effects of capsaicin treatment on anorexie responses to intravenous infusion of CCK-8, PYY-(3–36), and GLP-1. Thus, it could be argued that our failure to observe a capsaicin-induced attenuation of anorexie responses to GLP-1 and PYY-(3–36) was because we did not measure the early satiating effects of these treatments. However, using the same experimental design, we showed that CCK-8-induced reductions in cumulative food intake at 3 and 6 h were blocked by capsaicin pretreatment. Together, these results suggest that anorexie responses to PYY-(3–36) and GLP-1 are not primarily mediated by the capsaicin-sensitive peripheral sensory neurons (presumably vagal) that mediate CCK-8-induced anorexia.

Studies showing an increase in food intake in response to systemic administration of receptor antagonists of GLP-1 (62) and PYY-(3–36) (46) suggest that these gut peptides play essential roles in meal-induced satiety. Our results, as well as those of Zhang and Ritter (66) and Rüttimann et al. (52), suggest that anorexie responses to intravenous infusions of GLP-1 and PYY-(3–36) are not primarily mediated by capsaicin-sensitive vagal sensory neurons. These results do not preclude an important role for these neurons in mediating anorexie responses to GLP-1 and PYY-(3–36) if the peptides stimulate redundant vagal and nonvagal mechanisms. If GLP-1 and PYY-(3–36) act through paracrine stimulation of intestinal vagal sensory neurons to inhibit food intake, it would be important to determine whether local gut administration of their receptor antagonists [exendin-(9–39) and BIIE-0246, respectively] stimulates food intake like that shown for the CCK antagonist devazepide (16) and whether this effect is mediated by intestinal vagal afferents.

If GLP-1 and PYY-(3–36) act as blood-borne signals from gut to brain to decrease food intake, then it would be important to determine whether food intake is inhibited by intravenous doses of GLP-1 and PYY-(3–36) that reproduce meal-induced increases in plasma GLP-1 and PYY-(3–36). If inhibition were to occur, this would suggest that postprandial plasma levels are sufficient to inhibit food intake. If little or no effect were observed, then either the peptides do not inhibit food intake by an endocrine mechanism or other factors interact with circulating GLP-1 and PYY-(3–36) in an additive or potentiating manner to produce satiety.

Several human studies suggest that postprandial increases in plasma GLP-1 are sufficient to decrease food intake (61). In rats, intravenous infusion of GLP-1 at 10 pmol·kg\(^{-1} \cdot \text{min}^{-1}\) was reported to increase plasma GLP-1 to a degree comparable to that produced by food intake (58), and we have shown that this dose is sufficient to decrease food intake (13). With respect to PYY-(3–36), postprandial plasma levels have been reported to be both sufficient (6) and insufficient (7, 56) to inhibit food intake. However, a wide variability in basal and stimulated plasma GLP-1 and PYY-(3–36) values has been reported, which likely reflects differences in antisera and standards used in GLP-1 and PYY-(3–36) assays and whether plasma samples were extracted to remove interfering factors before being assayed (6, 7, 17, 18, 31, 53, 54). Furthermore, most of these studies did not report concentrations of specific molecular forms of GLP-1 and PYY-(3–36) in plasma or the ability of specific forms to decrease food intake (48). Thus, it remains to be established that meal-induced changes in plasma levels of GLP-1 and PYY-(3–36) are sufficient or necessary to inhibit food intake.

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DISCLOSURES

Contents of this publication do not represent the views of the Department of Veterans Affairs or the United States Government.

AUTHOR CONTRIBUTIONS


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