Enterostatin suppresses food intake in rats after near-celeic and intracarotid arterial injection

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Enterostatin suppresses food intake in rats after near-celeic and intracarotid arterial injection. Am J Physiol Regulatory Integrative Comp Physiol 278: R1346–R1351, 2000.—Enterostatin (Ent) selectively suppresses the intake of dietary fat after peripheral and central administration. To further investigate the site of action of Ent, we compared the feeding responses to Ent injected intra-arterially near the celiac artery, into the carotid artery, or intravenously in rats adapted to a high-fat diet. After near-celeic arterial injection there was an immediate dose-dependent (0.05–13.5 nmol) inhibition of food intake occurring within 5 min in overnight-fasted rats that lasted up to 20 min. Carotid arterial Ent had a similar, immediate dose-related response, and the inhibitory effect was long lasting. The response to intravenous Ent was only evident at the highest dose (13.5 nmol) and was delayed for at least 120 min. Pretreatment with capsaicin, which causes degeneration of vagal sensory neurons, abolished the inhibitory responses to near-celeic Ent but not to intravenous or intracarotid Ent. These results provide further evidence for both a gastrointestinal site of action for peripheral Ent and a central site of action for intracarotid Ent and suggest that the delayed response to intravenous Ent may reflect either binding or slow uptake of this peptide into the central nervous system.

Enterostatin (Ent) is the amino-terminal pentapeptide released from pancreatic procolipase by tryptic hydrolysis during fat digestion (4). Colipase serves as an essential cofactor to pancreatic lipase for fat digestion (26). The amino acid sequence of Ent is highly conserved across a range of animal species. A series of studies over the past decade has suggested that Ent selectively inhibits the intake of dietary fat and may act as a feedback regulator or endogenous signal to determine fat intake and selection (6, 9–11, 14, 18, 27). Using different feeding paradigms, we and others have shown that the peptide inhibits the food intake in rats fed on a single-choice, high-fat diet, but not low-fat diet (9, 18), and selectively reduces intake of the high-fat diet when rats select from a high-fat and low-fat two-choice selection regimen without any compensatory increase of the low-fat diet (8, 19). Furthermore, Ent selectively decreases fat intake when rats are fed a three-choice macronutrient diet (18). The anorectic effects of Ent are evident using various routes of administration: intraperitoneal, intravenous, oral, intracerebroventricular, and after injection onto specific regions of the brain (amygdala and paraventricular nucleus) (11). The response to peripheral Ent is thought to involve an afferent vagal signaling pathway, because both the feeding response and the brain c-fos expression in response to intraperitoneal Ent were abolished either by transection of the hepatic vagus or by capsaicin (Cap)-induced vagal deafferentation (24, 27).

In addition to the pancreas, procolipase mRNA and protein have been demonstrated in the antral stomach and duodenal mucosa of rats (17). Immunoreactive Ent has also been identified in the intestinal content of rats and humans after a satiating meal (2, 13). Immunohistochemical studies have shown the presence of Ent in enterochromaffin cells of the gastrointestinal tract, in the antral part of the stomach, and in the duodenum (23). These observations suggest that Ent may be released in the gastrointestinal tract to act either locally by a paracrine mechanism to activate the vagal system or distantly by an endocrine mechanism into the circulation to reach the brain. To obtain further insight into the sites of action of Ent, we have used an intra-arterial infusión technique to investigate the role of gastrointestinal and central nervous system (CNS) sites of action and compared this to the response to Ent given intravenously. Near-arterial injections of Ent would be expected to inhibit food intake much faster and at a lower dose than intravenous Ent if the Ent is given into the local arterial supply to its site of action.

MATERIAL AND METHODS

Animals and diet. For these studies, a total of 71 male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) with a beginning weight of 250 ± 2 g was used. Rats were housed in hanging stainless steel cages in a temperature-controlled room (22–23°C) with a 12:12-h light-dark cycle (lights on at 0700) and with free access to an automatic watering system. They were adapted to either a high-fat (HF) diet (56% of energy as fat, 4.78 kcal/g) or a three-choice macronutrient diet (18). The anorectic response to intraperitoneal Ent was abolished either by transection of the hepatic vagus or by capsaicin (Cap)-induced vagal deafferentation (24, 27).

Food cups were secured in the cages with a stainless steel spring, and fresh diet was provided daily. When food choices were provided, the position of the food cups was reversed daily. All

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experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee.

Surgery. Animals were anesthetized with pentobarbital sodium (Nembutal; 0.1 ml/100 g body wt ip). For near-celiac artery cannulations, the left common carotid artery was exposed after a midline incision over the neck and dissection of the overlying fascia and muscles. Polyurethane tubing (ID 0.40 mm, OD 0.76 mm, PhysioCath, Data Science International, St. Paul, MN) filled with heparinized saline (30 U/ml) was inserted through the left carotid artery caudally. It was slowly passed into the aorta until its tip resided 10–15 mm above the celiac-arterial junction. This required an ~70-mm length of tubing inside the artery. The distal end of the catheter was connected to a 20-mm length of 25-gauge stainless steel tubing and tightly covered with a cap made from PE tubing. It was exteriorized through the dorsal skin of the neck and secured to the skull with acrylic dental cement anchored to three stainless steel screws.

In a separate group of the rats, a catheter was implanted into the left common carotid artery in a rostral direction. The tip of this intracarotid catheter was placed close to the junction of internal and external branches. All catheters were filled with a solution of 50% polyvinylpyrrolidone (molecular weight 40,000, Sigma Chemical, St. Louis, MO) in saline containing 1,000 U heparin per/ml. This solution prevented occlusion of the cannulas. Intravenous injections were made through the tail vein, which was warmed under an infrared lamp.

Cap treatment. Rats received either Cap (Sigma Chemical) or vehicle (10% ethanol, 10% Tween 80, 80% 0.9% wt/vol saline) injections intraperitoneally. A series of three Cap injections was given at doses of 25, 25, and 50 mg/kg body wt with 18- and 6-h intervals between the injections. The effectiveness of Cap treatment was confirmed by the rat’s failure to exhibit a corneal reflex in response to 1% NH4OH.

Peptide and injections. Ent was synthesized by the Core Laboratory at Louisiana State University Medical School (New Orleans, LA). The purity of Ent was at least 95% as established by HPLC and mass spectrometry. The peptide or vehicle (0.9% saline) was administered as a bolus in a volume of 0.1 ml over a 30-s period, and cannulas were flushed with 0.1 ml of heparinized saline (30 U/ml). The dose range of Ent used was from 0.05 to 13 nmol.

Procedures. Catheterized rats were given at least 10 days to recover before the experimental test procedures. The rats were food deprived overnight but allowed free access to water. After injections, rats were replaced in the test cages and presented with food in preweighed cups. Diet consumption was measured at either 5, 10, 20, 30, or 60 min for the arterial injections or up to 6 h after intravenous injections. All food intake data were corrected for spillage.

At the end of the experiments, the patency of catheters for near-celiac arterial delivery was checked by infusion of 0.1 ml green dye (McCormick, Adams Extract, Austin, TX) into anesthetized rats surgically opened to expose the abdominal organs. Green coloring of the gastric vasculature within 2 min was taken as indicative of a near-celiac arterial location for the cannula.

Data analysis. Cumulative food intake (g or kcal) or food intake expressed as a percentage of saline controls are presented as means ± SE. The data were analyzed by ANOVA, and post hoc comparisons were made by Duncan’s test.

RESULTS

Time course of intra-arterial and intravenous Ent on feeding. The effects of Ent administered through different routes on food intake in overnight food-deprived rats are shown in Fig. 1. Near-celiac Ent at a dose of 2 nmol suppressed feeding within 5 min (saline 1.37 ± 0.03 vs. Ent 0.40 ± 0.23 g; P < 0.05), and this inhibition lasted for 20 min, after which the difference between experimental groups was no longer statistically significant. Intracarotid Ent (2 nmol) also produced a rapid inhibition of feeding (saline 1.97 ± 0.07 vs. Ent 0.00 ± 0.00 g; P < 0.05) (Fig. 1B). In contrast, there was no immediate suppression of food intake after intravenous injection of Ent but a delayed reduction (beginning 3 h later) was observed in response to a much higher dose (13 nmol) of Ent (saline 6.35 ± 0.51 vs. Ent 3.80 ± 0.50 g; P < 0.05) (Fig. 1C).

Dose-response curve for intra-arterial and intravenous Ent. Dose-response curves were performed for each route of injection. Rats received saline vehicle and either four (near celiac), three (intracarotid), or two (intravenous) doses of Ent in a blocked design. Data are expressed as a percentage of the intake after control vehicle injection at the time point corresponding to the maximum inhibitory effect: 5 min for both arterial routes and 3 h for the intravenous injection (Fig. 2). It is evident that Ent was effective at much lower doses after near-celiac arterial and carotid injections than after intravenous administration. Near-celiac Ent resulted in ~60 and 70% inhibition at 0.5- and 2-nmol

![Fig. 1. Cumulative intake of a high-fat diet in response to enterostatin (Ent) administered through different routes. All values are means ± SE for groups of 4–6. ANOVA indicated effects of Ent treatment for near-celiac [A; F(1,6) = 6.86, P < 0.05], intracarotid [B; F(1,6) = 16.83, P < 0.01], and intravenous [C; F(1,10) = 9.24, P < 0.05] administration. Significant differences were defined by *P < 0.05, **P < 0.01 compared with respective saline group at same time point.](http://ajpregu.physiology.org/Downloadedfrom/10.1152/ajpregu.00129.2016)
Fig. 2. Comparison of suppressive effects of Ent on food intake after near-celiac artery, intracarotid, and intravenous injections. Data are expressed as percentage of intake of respective vehicle control rats at time of maximum inhibition (5 min for arterial injections, 3 h for intravenous injection). ANOVA indicated significant effects of Ent treatment of near-celiac [F(4,24) = 3.26, P < 0.05], intracarotid [F(3,16) = 61.4, P < 0.01], and intravenous [F(2,20) = 15.87, P < 0.01] administration. * P < 0.05, **P < 0.01 compared with respective vehicle control group.

doses, respectively. Carotid Ent caused 60 and 100% reduction of food intake at doses of 0.5 and 2 nmol, respectively. After intravenous administration, a low dose of Ent (2 nmol) had no effect on feeding compared with saline control but, at a higher dose (13 nmol), Ent produced a 30% inhibition of food intake.

Effect of Cap treatment on the response to Ent. The food intake in response to Ent or vehicle was compared in the rats before and after Cap treatment. Optimal doses of Ent identified in the previous experiment (2, 0.5, and 13 nmol) were selected for near-celiac arterial, intracarotid, and intravenous routes, respectively. Only data from a single time point are shown in Fig. 3 for simplification, although the data were collected at the same time points as in the previous experiment (see MATERIAL AND METHODS). ANOVA of the complete data set indicated that Cap treatment abolished the response to near-celiac arterial injection of Ent over 1 h [Cap × Ent interaction: F(1,11) = 9.29, P = 0.012; simple main effects of Ent pre-Cap treatment: F(1,5) = 8.39, P = 0.03 and post-Cap treatment: F(1,5) = 1.15, P = 0.33]. Figure 3A shows the significant inhibition of food intake in Cap-treated rats 5 min after near-celiac Ent treatment (pre-Cap saline 1.37 ± 0.03 vs. Ent 0.40 ± 0.23 g, P < 0.05; post-Cap saline 1.17 ± 0.07 vs. Ent 1.43 ± 0.15 g). However, neither the responses to intracarotid [Cap × Ent interaction: F(1,11) = 0.0007, P = 0.98; simple main effects of Ent pre-Cap: F(1,4) = 21.69, P = 0.009 and post-Cap: F(1,7) = 7.83, P = 0.027] nor intravenous Ent [Cap × Ent interaction: F(1,19) = 0.1, P = 0.93; simple main effects of Ent pre-Cap: F(1,14) = 4.34, P = 0.05, post-Cap: F(1,5) = 7.53, P = 0.04] were affected by Cap treatment.

Effect of Ent on two-choice HF/LF diet selection. Because peripheral and central Ent selectively inhibit intake of fat in rats allowed to select their diets, we tested the response to near-celiac Ent in rats adapted to a two-choice HF/LF diet selection (Fig. 4) using an optimal dose for this route (2 nmol). Ent decreased the intake of the HF diet at the earliest time point measured (5 min, saline 4.46 ± 0.79 vs. Ent 0.72 ± 0.32 kcal; P < 0.05), but this inhibitory effect had disappeared by 30 min. In contrast, intake of the LF diet was not affected by Ent during the first 20 min, but an increase in intake was observed thereafter (60 min: saline 12.08 ± 2.58 vs. Ent 18.11 ± 1.64 kcal), although this was not significantly different from the saline control level (P > 0.05). ANOVA indicated that total caloric intake over the 1-h period was not affected by Ent treatment [F(1,10) = 0.47, P = 0.51; data not shown].

DISCUSSION

The present study provides several new observations relevant to our understanding of the site and mechanism of action of Ent. Near-celiac arterial injection of Ent produced an immediate dose-dependent suppression of food intake, and intracarotid Ent had a similar rapid effect on feeding. In contrast, intravenous injection of Ent had a delayed response and required a higher dose. Vagal deafferentation by Cap treatment abolished the suppression of food intake caused by near-celiac Ent but not the responses to either intravenous or intracarotid Ent administration. Furthermore, near-celiac arterial Ent had a selective effect to reduce intake of HF diet in rats allowed a two-choice diet selection. These data provide strong evidence to sug-
Gest that the upper gastrointestinal tract is one peripheral site of action of Ent and that this response is mediated through a vagal-afferent pathway. The slow response to intravenous Ent may reflect the slow uptake of the peptide into the central nervous system, because injection of Ent as a bolus dose into the carotid artery induced an immediate suppression of food intake that was independent of the vagal afferent neurons.

Near-arterial sites of injection have been used previously to investigate the site of action of other peptides, e.g., CCK and bombesin (3, 7). When injected into the arterial supply to the target tissue, it is expected that the peptide will be effective at a much lower dose than is required for intravenous or parenteral routes and also have a shorter lag time for the response. This was evident for near-celiac arterial administration of Ent. Through this route, Ent was effective at a dose of 0.5 nmol with no lag time. In contrast, 2 nmol of Ent given intravenously were without effect, although a delayed (>2 h) and smaller inhibition (30% for intravenous vs. 65% for near-celiac artery) was observed at the much higher dose of 13 nmol. The delay in response to intravenous Ent and the effective dose observed in our studies were similar to those reported previously (14). The effective dose of Ent for intraperitoneal injection was 60 times higher (30 nmol) than that for near-celiac injections (0.5 nmol), and again the response was delayed (18).

Ent had a selective inhibitory effect toward HF diet when the two-choice HF/LF feeding regimen was used. This macronutrient being selective toward fat is consistent with previous data (8, 19) from studies using different routes of administration. Because the celiac artery supplies both the stomach and proximal regions of the small intestine, these data strongly suggest that the peripheral site of action of Ent is in this region. Previous observations that Ent would inhibit food intake after both oral (J. Chen and D. A. York, unpublished observation) and duodenal administration (15) also support a gastrointestinal site of action, particularly because the uptake of Ent from the gastrointestinal lumen is limited and slow and mainly through the thoracic duct (25). Other organs perfused through the near-celiac artery are the liver and the pancreas. Previous studies have shown that Ent inhibits glucose-induced insulin secretion from isolated rat islets (5, 20). The longer time course of this response is not consistent with the rapid, short duration of the feeding response to near-celiac arterial Ent, although it is possible that Ent could modulate its effect through some other pancreatic factor. Although the liver is also a potential site of action for near-celiac arterial Ent, this seems unlikely. Both intragastric and intraduodenal Ent have rapid effects to reduce food intake, whereas uptake of Ent via the hepatic portal vein is very slow and limited (25).

Afferent-vagal signaling is thought to mediate the satiety response to a number of anorectic or orexigenic agents that act locally at gastrointestinal or hepatic levels, including CCK, glucose, and β-mercaptoacetate (16, 21, 28, 29). The responses to such agents are blocked or attenuated by selective branch vagotomy or Cap treatment (21, 28, 29). The inhibition of food intake induced by intraperitoneal Ent was blocked by both Cap treatment and by selective hepatic vagotomy (24, 27). Similarly, local tetracaine anesthesia blocked the response to intraduodenal Ent (15). Systemic treatment with Cap destroys fine diameter unmyelinated primary sensory neurons, including most of visceral sensory neurons without damaging motor neurons. In the current series of experiments we demonstrated that the response to near-celiac arterial Ent was also prevented by prior treatment with Cap, confirming the role of the afferent vagal system in this response.

We currently believe that the afferent vagus serves to signal to the CNS the presence of Ent and that these signals directly modulate the activity of specific neuronal sites that affect feeding behavior and macronutrient selection. This interpretation is supported by observations on c-Fos protein induction (24). However, alternative explanations are possible. Because the inhibitory effect of Ent is dependent on the presence of a signal related to dietary fat, it is possible that the afferent vagus transmits a “fat signal” rather than an “Ent signal.” This seems unlikely, because the response to neither intracerebroventricular Ent (27) nor intracarotid or intravenous Ent was blocked by vagal deafferentation. Second, it is possible that Ent initiates a vagal-vagal reflex to inhibit stomach emptying. Although intracerebroventricular Ent does inhibit gastric emptying, this was not related to its anorectic effect across several strains of rats (12), and it is unclear how such a mechanism could be selective toward dietary fat.

Intracarotid arterial injection of Ent inhibited food intake at low doses (0.5–1.5 nmol) with virtually no delay in this response, whereas intravenous Ent was only effective at a high dose (13 nmol) and the response was delayed for >2 h. The rapid and sensitive response to intracarotid Ent is consistent with a central site of action of Ent. This is supported by previous observations that Ent injections into brain ventricles or specific...
that are known to affect feeding behavior. However, the role of Ent in the regulation of feeding behavior is complex and involves both the gastrointestinal tract and the CNS. As such, it is possible that the high concentration (0.5–1.5 nmol in 0.1 ml vehicle) attained by local arterial injection provides a concentration gradient that would allow rapid transfer into the brain. This concentration gradient would not be achieved after dilution of the intravenous dose (13 nmol) in the circulation. Second, it is possible that Ent is bound in the circulation and that this limits uptake into the brain. The extremely long half-life of 125I-labeled Ent in the circulation (Lin and York, unpublished observations) would support this hypothesis as would the observations that antisera to Ent cross-react with high molecular weight proteins in the plasma. Alternatively, it is possible that rapid hydrolysis of Ent to smaller peptide fragments in the circulation (1) prevents uptake into the brain. Finally, it is possible that circulating procolipase rather than Ent is the major route through which Ent enters the brain. To date, it has not been possible to show expression of the precursor protein procolipase gene in the CNS (17). Ent is one of the most potent peptides affecting food intake after central administration, suggesting either that Ent is a hormone that is transported from the circulation into the brain or that there is some other natural ligand for its receptor. However, recent data (21) have shown that the procolipase precursor protein is transported by a carrier-mediated process into the CNS. Whichever mechanism is involved, it is clear that the peripheral and central systems that are responsive to Ent are temporally very different: the gastrointestinal system responding rapidly to an individual meal, whereas the central system appears to have a much longer response time that may alter appetite for dietary fat.

Our current data are consistent with both a central and peripheral site of action for Ent. Both procolipase mRNA- and Ent-like immunoreactivity have been identified in the gastric mucosa, and procolipase protein and Ent are present in gastric juice (2, 13, 18, 23). Furthermore, Rippe et al. (21) recently showed that there is a rapid uptake of procolipase from the plasma into the stomach. As both oral and intraduodenal Ent inhibit feeding behavior, the evidence suggests that Ent secreted into the gastrointestinal lumen may initiate the feeding response. This suggests that there must either be a process for the local uptake of Ent and its transport across the mucosal layers to activate the vagal nerve terminals or that Ent may interact with receptors on the apical membranes of mucosal epithelial cells to trigger the release of other paracrine effectors that themselves activate afferent vagal activity.

Perspectives

The current study, together with the previous reports, suggests that Ent may have sites of action in both the gastrointestinal tract and the CNS. As such, it is similar to other gastrointestinal peptides, e.g., CCK, that are known to affect feeding behavior. However, the selectivity of Ent toward dietary fat implies interaction between the feeding pathways affected by Ent and the taste and/or olfactory systems that allow the rat to recognize dietary fat. The site of this interaction is unclear, but the nucleus of the solitary tract would be a prime candidate because both sensory information and the neural pathway affected by Ent are transmitted through this region.

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