Leptin-induced satiation mediated by abdominal vagal afferents

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Leptin-induced satiation mediated by abdominal vagal afferents. Am J Physiol Regul Integr Comp Physiol 288: R879–R884, 2005. First published December 9, 2004; doi:10.1152/ajpregu.00716.2004.—Leptin is a hormone secreted into the systemic blood primarily by white adipose tissue. However, leptin also is synthesized and stored by cells in the gastric mucosa. Because gastric mucosal leptin is secreted in response to ingestion of a meal, we hypothesized that it might contribute to satiation (meal termination) by acting on gastrointestinal vagal afferent neurons. To test whether leptin is capable of acutely reducing short-term food intake, we measured consumption of a liquid meal (15% sucrose) following low-dose leptin administration via the celiac artery, which perfuses the upper gastrointestinal tract. Leptin (1, 3, 10 μg) was infused via a chronically implanted, nonocclusive celiac arterial catheter or via a jugular vein catheter with its tip in the right cardiac atrium. Fifteen percent sucrose intake was then measured for 30 min. We found that leptin dose-dependent inhibited sucrose intake when infused through the celiac catheter but not when infused into the general circulation via a jugular catheter. Plasma leptin concentrations in the general circulation following celiac arterial or jugular leptin infusions were not significantly different. Celiac arterial leptin infusion did not reduce meal size in vagotomized or capsaicin-treated rats. Finally, we also found that reduction of meal size by celiac leptin infusion was markedly enhanced when coinfused with cholecystokinin, a gastrointestinal satiety peptide whose action depends on vagal afferent neurons. Our results support the hypothesis that leptin contributes to satiation by a mechanism dependent on gastrointestinal vagal afferent innervation of the upper gastrointestinal tract.

leptin; vagal afferents; cholecystokinin; satiation

The hormone leptin provides a negative-feedback signal that controls adipose tissue mass through actions on the central nervous system (CNS). For example, plasma leptin concentration varies directly with white adipose mass (6, 16), and systemic or intracerebral administration of recombinant leptin triggers the reduction of food intake and loss of white adipose tissue (WAT) (8, 16). A variety of experimental results indicate that leptin’s effects are mediated via leptin receptors in the brain, especially in the arcuate nucleus of the hypothalamus (15, 23). However, expression of leptin receptor (Ob-R) mRNA has been detected in rat vagal afferent neurons (4, 5), including some that innervate the gastric fundus (11). Vagal afferent neurons carry sensory information from the abdominal viscera to the hindbrain. This sensory information includes signals that contribute to the process of satiation, which controls meal size. The presence of Ob-R mRNA in vagal afferent neurons suggests that leptin might directly activate vagal afferent neurons. In fact, extracellular recordings from vagal afferent fibers have detected increased firing following leptin injection (21). Furthermore, we recently reported that leptin acutely activates vagal afferent neurons in vitro (12).

The ability of leptin to rapidly activate vagal afferent neurons seems surprising for a hormone whose systemic blood levels do not change rapidly. However, it now appears that, in addition to white adipose cells, chief cells and some mucosal endocrine cells in the gastric mucosa synthesize leptin (1). In contrast to adipocyte-derived leptin, gastric leptin is stored within the secretory cells and is rapidly secreted in response to stimuli such as food intake and CCK, a gut peptide secreted in response to dietary nutrients in the upper gastrointestinal (GI) tract (1). Thus it is possible that gastric leptin may be released in paracrine fashion to act as a satiation signal via vagal afferents innervating the GI tract (1, 21).

To evaluate this hypothesis we infused low doses of leptin into the celiac artery, which perfuses the stomach, upper small intestine, and other upper abdominal organs, via chronically implanted catheters. Leptin delivered via the celiac artery accesses the upper GI innervation before being diluted in the systemic circulation. We found that 5-min intraceliac infusions of leptin (1, 3, 10 μg) significantly reduced consumption of a liquid meal (15% sucrose) compared with identical infusions made into the right atrium via jugular vein catheters. Blood concentrations of leptin resulting from these infusions remained within physiological levels. Additionally, we observed that subdiaphragmatic vagotomy and systemic capsaicin pretreatment abolished the leptin-induced suppression and that coapplication of leptin and CCK resulted in synergistic suppression of sucrose intake compared with exposure to either ligand individually.

METHODS

Animals. Adult male Sprague-Dawley rats (320–360 g), purchased from Simonsen Laboratories, were subjects in all experiments. The animals were housed under a 12:12-h light-dark cycle and provided with ad libitum pelleted chow, except for the vagotomy study where recovering animals received wetted powdered chow. Animals were housed in an American Association for Accreditation of Laboratory Animal Care-accredited quarters. All procedures performed were approved by the Washington State University Institutional Animal Care and Use Committee. For all surgical procedures, the rats were deeply anesthetized (ketamine 25 mg/100 g, xylazine 2.5 mg/100 g), and surgeries were performed under aseptic conditions.

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Celiac artery catheterization. A 0.33-mm outer-diameter polyurethane catheter (Microrenathane, Braintree Scientific, Braintree, MA), with a tip tapered to 0.15 mm, pulled in vegetable oil (145°C), was inserted into the left carotid artery, passed via the descending aorta into the abdominal aorta. The catheter tip could then be visualized through the wall of the aorta and guided manually into the celiac artery, such that it rested, nonocclusively, ~5 mm from the aortic celiac orifice. The catheter was then secured in place within the neck, and the end was fed subcutaneously to the back, where it was secured subcutaneously using an implanted polypropylene mesh patch (Davol, Cranston, RI) with the end of the catheter exposed externally along the midline between the scapulas. Following surgery the animals were allowed to recover for 7–10 days. In all cases, operated rats maintained their body weight and were normophagic before experimentation began. The patency of the catheters was maintained with daily flushing (50 U of heparin/0.1 ml saline) and filling the catheter with a fresh blocking solution consisting of 50% glycerol with 5% gentamicin and 300 U/ml heparin. After completion of all experimental manipulations, each rat was deeply anesthetized, and its catheter placement in the celiac artery was confirmed by infusion of a dye (Evans Blue 4.5 mg/ml, -1.30 s-1, Harvey Laboratories, Philadelphia, PA) and direct visual confirmation. To be accepted as properly placed, infusion of Evans blue into the catheter had to result in transient blue staining of the stomach within 2 s or less from the start of infusion. In addition, the tip of a celiac catheter had to be visually located within the celiac artery.

Jugular vein catheterization. A 0.33-mm diameter polyurethane catheter without a tapered tip was introduced into the right jugular vein and secured into place with the tip resting celiac without a tapered tip was introduced into the right jugular retrograde transport. Animals exhibiting this profile were considered indicating a successful intraperitoneal injection, but interruption of the DMN was absent, while there was still light labeling of the AP, accumulated the tracer via retrograde transport by intact vagal motor labeling of the dorsal motor nucleus of the vagus (DMN), which circulating fluorogold, and there was intense bilateral fluorogold rescence filters. In intact animals, light fluorogold fluorescence label- ing media (Vectashield, Vector Laboratories, Burlingame, CA), cov- postrema. The sections were mounted on slides in fluorescent mount-

decussation of the pyramidal tracts and the rostral border of the area frozen sections were prepared of the hindbrain between the

stored in a 20% sucrose solution at 4°C until processing to detect postfixed for 4 hr in the 4% PF solution at room temperature, then stained for 20% sucrose solution at 4°C until processing to detect retrogradely transported fluorogold in the hindbrain. Forty-micrometer thin sections were prepared of the hindbrain between the

decussation of the pyramidal tracts and the rostral border of the area postrema. The sections were mounted on slides in fluorescent mount- ing media (Vectashield, Vector Laboratories, Burlingame, CA), cov- erslipped, and microscopically examined using appropriate epifluo rescence filters. In intact animals, light fluorogold fluorescence label- ing was present in the area postrema (AP), which accumulates circulating fluorogold, and there was intense bilateral fluorogold labeling of the dorsal motor nucleus of the vagus (DMN), which accumulated the tracer via retrograde transport by intact vagal motor neurons. In the hindbrain of successfully vagotomized rats labeling of the DMN was absent, while there was still light labeling of the AP, indicating a successful intraperitoneal injection, but interruption of retrograde transport. Animals exhibiting this profile were considered to have complete subdiaphragmatic vagotomies.

Capsaicin treatment. Animals receiving systemic capsaicin treatment were fasted overnight and pretreated with 0.1 ml of atropine (Phoenix Pharmaceuticals, St. Joseph, MO). All capsaicin injections were performed under isoflurane anesthesia (5% induction/2–3% maintenance) and positive-pressure ventilation. Each animal received three separate injections (ip) of capsaicin, the first at 25 mg/kg, on day 1 of the treatment schedule, and the subsequent two at 50 mg/kg in the morning and afternoon of day 2 of the treatment schedule. The procedures for systemic capsaicin treatment have previously been described (14). Systemic capsaicin treatment destroys unmyelinated primary sensory neurons, including most vagal afferent neurons (9). Efficacy of the capsaicin treatment was confirmed using the corneal chemosensitivity test (18). Briefly, a drop of 0.1% NH4OH is placed in the eye. Intact rats wipe the eye one to five times within 5 s of placement of the stimulus, whereas capsaicin treated rats do not. In addition we evaluated the effect of capsaicin treatment on vagal sensory function by testing the ability of an intraperitoneal CCK (2 μg ip) injection to reduce 30 min sucrose intake. Capsaicin treatment has previously been shown to attenuate reduction of food intake by CCK, due to destruction of vagal afferent fibers (13).

Intraceliac artery and intrajugular vein infusions. Infusions of either vehicle (0.9% saline) or peptides (leptin and/or CCK) were made using syringe infusion pumps (Razel Scientific Instruments, Stamford, CT) at a rate of 12 μl/min. For the celiac-catheterized animals, infusion time was 5 min, which began 2 min before sucrose presentation. The infusion time for the jugular controls was modified to compensate for the difference in residual volume between the jugular and celiac catheters. This modification ensured that the both the celiac and jugular groups received equivalent amounts of hormone before sucrose presentation as well as equivalent hormone infused throughout the experiment.

Sucrose intake. Intake of sucrose (15%) was measured from burets every 5 min for a total of 30 min. To measure the sucrose intake, animals were removed from their home cages and placed in opaque plastic cylinders. These cylinders allowed infusions to be performed simultaneously with access to sucrose. Before surgery, rats were acclimated to the experimental protocol, and surgery was performed only after a stable baseline of sucrose intake was established. Following recovery from surgery (7 days), animals were tested on consecutive days until the completion of the experiment. Data are expressed as a percent reduction from the saline control. Control infusions were run the day before and following each test infusion, with the intake from these 2 days averaged and expressed as a baseline from which the intakes on test days were compared. Since >90% of the total intake occurred within the first 15 min, percent reduction data are reported using total consumption up to this time point. At least two control infusions were interspersed between peptide infusions. In the dose-response study the order of different peptide doses was random- ized. In the leptin CCK-interaction study, the order of leptin or CCK alone was random, but the combined exposure always occurred last. All testing started 1 h after lights on and continued over the next 4–5 h. However, each individual rat was consistently tested within the same time block during the testing period.

Contrast angiography. To visualize the distribution of the celiac infuses in a living rat, one animal with a celiac catheter was anesthetized and positioned for radiographic imaging. The iodine-rich dye iohexaline (300 mg/ml) was infused via a celiac catheter and imaged using a radiographic imaging system (MD3, Phillips). An image was taken ~5 s after the start of the infusion with data collected digitally at 1,024 × 1,024 pixels in a 64-cm2 field.

Plasma leptin measurements. Plasma leptin concentrations in systemic plasma were measured in separate groups of rats, not used for behavioral experiments, using a radioimmunoassay (RIA) (LINCO Research, St. Charles, MO). For these measurements, rats were anesthetized and implanted with catheters in the femoral vein and with either a celiac artery or jugular catheter, as used in behavioral experiments. Leptin infusions were made via the celiac artery or
jugular catheter, as described above, with 6 (0.6 ml) serial blood samples collected from each animal through the femoral catheter. The total blood volume taken from each animal was 3.6 ml. Plasma replacement was not necessary with the low volumes of blood collected. Blood sampling began before the start of leptin infusion and continued simultaneously with and following completion of leptin infusion. The blood samples were mixed with 20 µl aprotinin, to inhibit protease activity, and 1.5 mg EDTA in 15% NaCl to prevent coagulation. After collection the samples were centrifuged (6,000 g) for 10 min at 4°C. The plasma was then removed and stored at −80°C until utilized in the RIA procedure.

Statistical analyses. Experiments involving infusions were a within-subjects design where every animal received each drug treatment. Effects resulting from leptin doses infused via the celiac or jugular catheters were analyzed with a repeated-measures ANOVA followed with a Dunnett’s post hoc analysis. Control baselines were established by averaging the sucrose intake following infusions of saline on the day prior and subsequent to the test day. Peak concentrations of circulating leptin were analyzed using t-tests. Total leptin exposure was determined as the integrated area under the curve and analyzed using t-tests. In the experiment administering 10 µg leptin via a celiac catheter in intact, vagotomized, or capsaicin-treated animals, the data were analyzed using paired t-tests against saline controls. To determine if coinfusion of CCK and leptin resulted in a greater than additive suppression of sucrose intake, we compared the response to coapplication of CCK and leptin with the summed responses of both compounds when infused individually. In all analyses the confidence limit for significance was P < 0.05.

RESULTS

Figure 1 is a contrast radiographic image that illustrates the distribution of an infusion made through one of our celiac arterial catheter preparations. The image was collected ~5 s after the start of the infusion and demonstrates that infusions made by this route perfuse the celiac circulation, including the hepatic, fundic, left gastric, and splenic arterial supplies.

When leptin (3, 10 µg) was infused into the celiac artery it significantly reduced short-term sucrose intake (Fig. 2). This effect was most pronounced at the 15-min time point but remained significant throughout the 30-min experiment. Similar infusions through the jugular vein produced no significant reduction in sucrose intake (Fig. 2).

Infusion of leptin (1, 3, 10 µg) through either the celiac artery or jugular vein elevated plasma levels of leptin in the systemic general circulation compared with baseline (Fig. 3). The peak response for each leptin dose occurred within 5–7 min following the start of the infusion. Although the 1 µg dose of leptin infused into the jugular vein produced a significantly greater peak response compared with the same dose administered into the celiac artery, there was no significant difference between celiac and jugular routes for peak leptin levels after the 3 or 10 µg leptin infusions (Fig. 4A). The total amount of leptin exposure the animals received as represented by the integrated area under the curve did not differ significantly between the jugular and celiac infusions at any of the leptin doses tested (Fig. 4B). A total of 3.6 ml of blood was removed from each rat. However, it seems clear that this procedure did not significantly contribute to elevated leptin concentrations because examination of leptin levels in the 1-µg infusion experiment reveals the leptin levels returned to preinfusion baseline within 20 min, at the time when blood volume deficit would be maximal. This volume of blood removal did not significantly influence leptin concentrations in our studies, and this same procedure was used to determine leptin concentrations following 3- and 10-µg infusions of leptin.

In vagotomized rats, celiac arterial leptin infusion (10 µg) did not reduce 15% sucrose intake (Fig. 5). Vagotomized animals maintained a significantly greater level of basal sucrose intake compared with intact and capsaicin-treated animals [baseline sucrose intake (in ml): intact, 7.03 ± 0.14, n = 6; capsaicin treated, 9.3 ± 0.13, n = 3, vagotomized, 14.3 ± 1.3*, n = 6, *P < 0.05 by ANOVA]. All surgical vagotomies were confirmed as complete by the bilateral absence of retrogradely transported fluorogold in the dorsal motor nucleus (DMN) 76 h after intraperitoneal injection of fluorogold. Cap-
saicin pretreatment, which destroys unmyelinated primary afferent neurons, including the majority of vagal afferents, also abolished the reduction of sucrose intake by celiac arterial leptin administration (Fig. 5).

Finally, when leptin (1 μg) or CCK (90 ng) was infused through the celiac catheter alone, they produced no significant effect on 15% sucrose intake (Fig. 6). However, when these same amounts were coinfused via the celiac artery, they produced a significantly greater reduction of 15% sucrose intake than the sum of the effects of CCK or leptin administered alone (Fig. 6).

**DISCUSSION**

Intraceliac arterial infusions of leptin significantly inhibited consumption of a liquid meal, whereas infusion of the same leptin dose via a jugular vein catheter did not decrease intake. From these results we conclude that leptin reduces meal size by acting at a site(s) perfused by branches of the celiac artery. It is well established that systemic administration of milligram doses of leptin (8) or injection of microgram amounts of leptin into the forebrain (15, 22) or hindbrain (7) ventricles reduces 24-h food intake and decreases meal size. However, the acute reduction of meal size we observed in response to intraceliac

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**Fig. 3.** Time course of changes in systemic arterial leptin concentration following leptin infusions via celiac arterial or jugular vein catheters. The subject rats were infused with either 1 μg (A), 3 μg (B), or 10 μg (C) of leptin over 5 min, in a total volume of 60 μl. The solid bar over each trace represents the duration of infusion. Blood was collected from the femoral artery at the times indicated. Plasma leptin immunoreactivity was determined by RIA analysis. Leptin concentrations are expressed as ng/ml of plasma (n = 6).

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**Fig. 4.** A: peak plasma leptin concentrations following celiac arterial or jugular vein leptin infusions. Leptin concentrations were significantly higher in rats infused via the jugular vein catheters at the 1 μg dose than they were for rats infused with the same dose via a celiac catheter (n = 6, *P < 0.05), but there were no significant differences in peak leptin concentrations between celiac artery and jugular vein infusions at any of the other leptin doses. B: total leptin exposure as estimated by integrating the areas under the curves in Fig. 3. None of the values differ significantly between celiac arterial and jugular vein infused rats at all of the leptin doses tested (n = 6).

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**Fig. 5.** Fifteen-minute intake of 15% sucrose following celiac arterial infusion of leptin in intact rats, vagotomized (VagoX) rats, or rats treated systemically with capsaicin (Cap). Bilateral subdiaphragmatic vagotomy (n = 6) or systemic capsaicin treatment (n = 3) abolished the ability of leptin (10 μg) infused via a celiac arterial catheter to reduce sucrose consumption. Control animals with intact vagus nerves significantly reduced their sucrose intake in response to leptin infused by the celiac arterial route (n = 13, *P < 0.05).
arterial leptin cannot be due to an action of leptin on the brain because infusions of leptin into the systemic circulation via the jugular vein did not decrease meal size, even though plasma leptin concentrations, as well as the pattern of change in leptin concentration, following celiac arterial and jugular leptin infusions were not different. Therefore, the brain was exposed to comparable amounts of leptin by both the effective celiac infusions and the ineffective jugular infusions.

Reduction of meal size by celiac arterial leptin infusion was abolished by systemic capsaicin treatments and vagotomy. Capsaicin, at the doses we employed, destroys unmyelinated primary afferent neurons in the vagus and the spinal nerves. Thus reduction of food intake after celiac arterial leptin might be mediated by either spinal or vagal primary afferent fibers. However, since subdiaphragmatic vagotomy also abolished the response to celiac arterial leptin, it appears the capsaicin-sensitive abdominal vagal afferents mediate the response to leptin that we observed. The fact that leptin receptor mRNA is present in the neurons of the nodose ganglia (4, 5), and that leptin activates vagal afferents in vivo (21) and in vitro (12), are consistent with our conclusion. It is conceivable that systemic capsaicin or vagotomy resulted in an altered blood flow to this area, resulting in a decrease in drug delivery and a false negative effect; however, during confirmation of catheter placement the tissue appeared to be healthy. Further, we observed no noticeable restriction in the perfusion of the stomach and intestine during postexperiment infusion of Evan’s blue dye, in vagotomized or capsaicin-treated rats, indicating that the perfusion of putative target tissues in these animals was comparable to control animals. Thus the lack of effect is most likely attributable to the removal of leptin-sensitive nerve endings.

We cannot specify the exact abdominal site(s) where celiac arterial leptin acts to reduce food intake because the celiac artery perfuses multiple abdominal organs (stomach, proximal small intestine, liver, pancreas, and spleen). The spleen is devoid of vagal sensory innervation (3), and therefore is unlikely to be a site action mediating leptin’s effect on meal size. The liver, pancreas, small intestine, and stomach all receive vagal afferent innervation. Recently, Bado and colleagues (1) demonstrated that leptin is produced and stored by cells in the gastric mucosa. Furthermore, gastric mucosal leptin is depleted postprandially (1), suggesting that it is secreted during meals. The total amount of leptin secreted by the stomach is probably small relative to that released by white adipose tissue; thus gastric leptin may not contribute significantly to the levels of endocrine leptin in the systemic circulation. However, leptin secreted by the stomach might act close to its site of release in a paracrine fashion. Consequently, vagal afferent fibers in the lamina propria of the stomach could encounter high concentrations of leptin, which would not reach the brain and other vagal afferent neurons. The demonstration that a number of vagal afferents express leptin receptors, some of which innervate the stomach, is consistent with this hypothesis. Further, Yuan and colleagues (24) have demonstrated that in an ex vivo stomach/vagus/hindbrain preparation, leptin administration to the lumen of the stomach resulted in an acute increase of neuronal activity in the nucleus of the solitary tract, an effect dependent on the gastric branch of the vagus. Taken together these observations suggest a paracrine mode of action for gastric leptin on vagal afferents. Our behavioral results add additional support to this hypothesis, and they further suggest at least one physiological parameter for which this action is important.

CCK, a gut-peptide secreted in response to fat and protein entering the small intestine (20), reduces food intake by acting on abdominal vagal afferent neurons (19). Further, several investigators have reported that leptin enhances CCK-induced reduction of food intake (2) and that CCK enhances leptin-induced reduction of body weight (10). In contrast to our findings, however, these CCK/leptin interactions apparently required that several hours elapse between the time of leptin and CCK administration (2, 10). Therefore, these previously reported interactions likely require leptin-induced neuronal transcriptional changes to occur to produce enhanced reduction of food intake or body weight. In contrast to these previous findings, our results indicate that leptin and CCK infused via the celiac artery produce a reduction of meal size that occurs within minutes of infusion. Specifically, coadministration of behaviorally subthreshold amounts of leptin (1 μg) and CCK (90 ng) via the celiac artery resulted in a greater than additive reduction of sucrose intake compared with reductions seen with either substance infused alone. Other investigators have reported short latency interactions between leptin and CCK during extracellular recording from vagal afferent fibers (21). We also have found that many CCK-sensitive vagal afferent neurons in primary culture respond to leptin with very short latencies (12). These observations demonstrate that the interaction of leptin and CCK at the level vagal afferents seems to involve membrane mechanisms, rather than transcriptional responses that may underlie CCK/leptin interactions at other levels of the neuroaxis.

In summary we have demonstrated that leptin, acting locally within the upper GI organs, reduces liquid food intake (15% sucrose). Leptin-induced reduction of food intake occurred rapidly and was mediated by capsaicin-sensitive vagal afferent neurons. Additionally, coadministration of subthreshold doses of leptin and CCK infused via the celiac artery lead to significant reduction of food intake. Since endogenous leptin is synthesized and secreted by the stomach, we propose that leptin acting in a paracrine fashion on abdominal vagal affer-
ents could cooperate with CCK and other visceral feedback signals in the process of satiation for food. If this interpretation of our results is correct, it suggests that leptin has multiple actions to influence food intake and body weight: a rapid and acute response perhaps mediated by gastric leptin acting on vagal afferent endings, and more sustained effects mediated by adipose release of leptin acting on hypothalamic and/or hindbrain sites. Whether these two events are completely independent of one another, or whether they form branches of a unified regulatory system, remains to be determined.

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GRANTS

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