Leptin and the Control of Food Intake: Neurons in the Nucleus of the Solitary Tract Are Activated by Both Gastric Distension and Leptin

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Leptin reduces food intake by an unspecified mechanism. Studies show that forebrain ventricular leptin delivery increases the inhibitory effects of gastrointestinal (GI) stimulation on intake and amplifies the electrophysiological response to gastric distension in neurons of the medial subnucleus of the nucleus tractus solitarius (mNTS). However, forebrain ventricular delivery leaves unspecified the neuroanatomical site(s) mediating leptin’s effect on intake. Detailed anatomical analysis in rats and mice by phosphorylated signal transducer and activator of transcription 3 immunohistochemistry shows that hindbrain leptin-responsive neurons are located exclusively within the mNTS. Here, we investigate 1) whether leptin and gastric distension affect the same mNTS neurons and 2) whether the intake-inhibitory action of gastric distension is potentiated by hindbrain leptin delivery. Twenty-five minutes after gastric balloon distension or sham distension, rats were injected with leptin or vehicle and killed 35 min later. Double-fluorescent immunohistochemistry for phosphorylated signal transducer and activator of transcription 3 and c-Fos revealed that about 40% of leptin-responsive cells also respond to gastric distension. A paradigm was then developed to examine the relationship between leptin and gastric distension volume on intake inhibition. At subthreshold levels, hindbrain ventricular leptin or distension volume were without effect. When combined, an interaction occurred that significantly reduced food intake. We conclude that 1) leptin-responsive neurons in the hindbrain are primarily located in the mNTS at the level of the area postrema, a key vagal afferent projection zone of the GI system; 2) a significant proportion of leptin-responsive neurons in the mNTS are activated by stomach distension; and 3) leptin delivered to the hindbrain is sufficient to potentiate the intake-suppressive effects of an otherwise ineffective volume of gastric distension. These results are consistent with the hypothesis that leptin acts directly on neurons within the mNTS to reduce food intake through an interaction with GI signal processing. (Endocrinology 148: 2189–2197, 2007)

The contribution of leptin to the control of energy balance is evident by its potent actions on food intake, energy expenditure, body weight, and metabolism both in rodents and in humans (1, 2). Leptin’s effects on feeding are mediated by receptors in the central nervous system (CNS) (1, 3, 4; cf. Ref. 5). The active form of the leptin receptor (ObRb) is expressed in a variety of brain regions, many of potential relevance to feeding control (6–8). Much attention has focused on the ObRb-bearing neurons of the hypothalamus, such as those of the arcuate (ARC) and the ventromedial nuclei (4, 9–11). Indeed, microinjection of leptin into these nuclei can reduce short-term food intake (12). The experiments described here provide anatomical and behavioral data consistent with an alternate model for leptin’s intake-suppressive action, one that localizes leptin action to hindbrain neurons that also process gastrointestinal (GI) signals.

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Ingestion of food gives rise to mechanical and chemical stimulation of the GI tract that activates vagal and spinal afferent fibers and central neurons in the ascending visceral afferent pathway (13). These signals play a prominent role in reducing food intake and, as such, have been referred to as satiation signals (14–18). Exogenous administration of leptin also reduces food intake, with the effect attributed to an action on meal size but not meal frequency control (19, 20). This result suggests that the mechanism underlying leptin’s intake action involves augmenting the strength of GI-triggered satiation signals (11, 21–23). Central leptin treatment increases the number of c-Fos-immunoreactive (IR) neurons observed in response to food ingestion, GI nutrient infusion, or cholecystokinin (CCK) injection (21, 22). Similarly, leptin treatments that are themselves without behavioral effect can increase the suppression of food intake by gastric nutrient loads and CCK injection (21, 22, 24–27). More direct support for the hypothesis that CNS leptin signaling amplifies the processing of GI signals comes from neurophysiological studies of single nucleus tractus solitarius (NTS) neurons that show that forebrain ventricular leptin delivery increased the magnitude of the excitatory response to distension of the stomach (28). Nonetheless, forebrain ventricular administration of leptin provides ligand to ObRb-bearing neurons in many brain regions, and therefore the effective site(s) of leptin action to influence feeding remains undefined.
Leptin receptors are found on neurons of the NTS in the rodent caudal brainstem (8, 29, 30), and low-dose leptin injection directly into the NTS reduces food intake (29). ObRb signaling is not distributed broadly over this large nucleus but is localized to the neurons of one subnucleus, the medial subnucleus (mNTS), at a specific rostrocaudal level of the NTS, the level of the area postrema (AP) (31). Interestingly, neurons in the mNTS are also activated by gastric and intestinal distension and by chemical and nutrient stimulation of the stomach and intestine (32–37). Based on these data, we hypothesized that the same mNTS neurons might be activated both by leptin and by GI afferent input.

Here we show that about 40% of the neurons in the mNTS that are activated by systemic leptin treatment, as measured by phosphorylation of signal transducer and activator of transcription 3 (P-STAT3), are activated by mechanical distension of the stomach, as measured by c-Fos IR. In addition, caudal brainstem injection of leptin as well as a volume of gastric distension that were each without behavioral effect when presented in isolation were effective in suppressing food intake when applied in combination. These results are consistent with the hypothesis that leptin signaling in these caudal brainstem neurons potentiates the effectiveness of GI signals that mediates the observed reduction in food intake.

Materials and Methods

Common materials

Recombinant mouse leptin was purchased from Dr. E. Parlow (National Institute of Diabetes and Digestive and Kidney Diseases, Torrance, CA). Supplies for immunohistochemistry (IHC) were purchased from Sigma Chemical Co. (St. Louis, MO), and the ABC Vectastain Elite kit was from Vector Laboratories (Burlingame, CA). Diaminobenzidine (DAB) staining was done using the ABC Vectastain Elite kit (Vector). Fluorescent donkey antigoat and donkey antirabbit Ig conjugates were from Molecular Probes (Eugene, OR), and donkey serum was from Invitrogen Life Technologies, Inc. (Carlsbad, CA).

Anatomical experiment 1: detailed mapping of leptin-responsive neurons in the caudal brainstem

Animals. Male Sprague Dawley rats (80–100 g; Charles River Laboratories, Wilmington, MA) were individually housed in plastic bins under a 12-h light, 12-h dark cycle. Chow diet and water were available ad libitum, except that animals were fasted (but water was available) for 24 h before injections. These experiments were carried out according to the guidelines of the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee.

Leptin injections and P-STAT3 IHC. Rats were injected ip with leptin (4.0 mg/kg body weight) or vehicle (PBS) and 40–45 min later deeply anesthetized with ketamine (90 mg/kg) and xylazine (2.7 mg/kg), transcardially perfused with isotonic saline followed by 10% buffered formalin. Brains were removed, postfixed for 12–15 h in formalin solution, and cryoprotected in a 20% sucrose solution. Brains were then sectioned in 25-μm-thick coronal sections on a sliding microtome, collected in five series, and stored in 0.02% sodium azide containing PBS at 4 °C until further use. Two adjacent series were subjected either to P-STAT3 DAB IHC or Nissl staining as we have described earlier (8, 31). Images were captured under a bright-field light source with a digital camera (AxioCam; Carl Zeiss, Thornwood, NY) mounted on a Zeiss microscope (Axioskop2; Carl Zeiss).

Anatomical experiment 2: anatomical analysis of caudal brainstem cells activated by distension and leptin

Animals. Male Sprague Dawley rats (80–100 g; Charles River) were individually housed in plastic bins under a 12-h light, 12-h dark cycle. Pelleted chow (5011 diet; Ralston Purina, St. Louis, MO) and water were available ad libitum, except the night before surgery and for a 15- to 17-h period before experiments when food was withheld but water was available. These experiments were carried out according to the Institutional Animal Care and Use Committee guidelines of the University of Pennsylvania.

Distension and leptin stimulation experiments. Rats were anesthetized with ketamine (90 mg/kg), xylazine (2.7 mg/kg), and acepromazine (0.64 mg/kg) delivered im and were implanted with a gastric cannula. The cannula (5 mm internal diameter) was fixed to the ventral forestomach according to the method of Weingarten and Powley (38). Briefly, the stomach was exteriorized after a midline laparotomy, and a small puncture wound was made in the fundus. A stainless steel cannula was inserted into the stomach and secured with purse-string sutures. The cannula was exteriorized through an abdominal puncture. Rats recovered 4 d before the start of the experiments. The rats were divided into four subgroups according to the experimental condition they were to receive. Four rats received leptin (4 mg/kg, ip) after balloon distension (4 ml), four rats received leptin after sham distension (balloon insertion but no distension), four rats received PBS after distension, and four rats received PBS after sham distension. On the day of the experiment, the cannula screw was opened and the gastric lumen visualized. Stomach contents, if any, were gently removed by a saline lavage and with a cotton-swab. The empty balloon (adhered to a threaded plastic sleeve with a pressure shut-off valve) was screwed into the gastric cannula and the empty balloon positioned in the proximal stomach. For the distension conditions, the animals rested for 1–3 min after balloon positioning, and then the balloon was inflated by infusing 4 ml warm saline (dyed with food coloring for postmortem leak identification) at a rate of 1–1.5 ml/min. For the leptin/sham distension condition, the balloon was inserted but not inflated. Twenty-five minutes after the start of distension, animals were given an ip injection of leptin or PBS in a volume of 1 ml/kg; distension was maintained throughout the period after drug injection (total distension time = 60 min). Thirty minutes after the leptin/PBS injection, rats received an ip overdose of a ketamine (90 mg/kg), xylazine (2.7 mg/kg), and acepromazine (0.64 mg/kg) mixture and were transcardially perfused as described in experiment 1. After the perfusion, gastric inflation was confirmed and the location of the inflated balloon within the stomach was determined by examination of the stomach and by estimation of the percentage of the total volume of the balloon in the fundus and corpus, with the limiting ridge delineating the border between fundus and corpus. The balloon contents (saline) were withdrawn and measured and the abdomen inspected for the presence of dye to verify that the intended volume was delivered. Data are presented from rats whose balloon distension was confirmed after postmortem observation.

Double-fluorescent P-STAT3 and c-Fos IHC. Brain sections were generated as described in experiment 1. Double-fluorescent P-STAT3 and c-Fos IHC was performed as described below. In brief, the sections were first pretreated with 1% NaOH and 1% H2O2 in H2O for 20 min, 0.3% glycine for 10 min, and 0.03% SDS for 10 min. After that, sections were blocked for 1 h with 3% donkey serum in PBS with 0.25% Triton X-100 and 0.2% sodium azide. Sections were then incubated with the rabbit anti-P-STAT3 antibody (1:200) together with the goat anti-c-Fos antibody (1:100) at room temperature overnight. On the next day, sections were incubated with a fluorescent-labeled secondary donkey antirabbit (1:200) and donkey antigoat (1:200) antibody (at room temperature overnight, generating green and red fluorescence, respectively). On the color fluorolight day, the cells were visualized using fluorescence light sources and captured with a Zeiss digital camera (AxioCam) mounted on a Zeiss microscope (Axioskop2). Adobe Photoshop software (Adobe, San Jose, CA) was used to merge fluorescence photographs via red green blue (RGB) channels to visualize double-labeled cells (31).

Cell counting. One of every five brain series from each rat was subjected to single or double IHC as described above. Sections were organized in a rostral-to-caudal manner according to the rat brain atlas (39). All
sections in each series that contained P-STAT3-positive cells were counted bilaterally to obtained numbers of single- or double-labeled cells. Numbers and percentages are provided as averages ± SEM.

Behavioral experiment 3: effects of gastric distension and/or fourth ventricular leptin on short-term food intake

Animals and gastric and fourth ventricular cannula surgery. Eleven male Sprague Dawley rats (same breeder) weighing 275–325 g at surgery were individually housed in hanging wire cages under a 12-h light, 12-h dark cycle (lights on at 0800 h). Pelleted chow and water were available ad libitum, except the night before surgery and for a 15- to 17-h period before experiments where food was withheld but water was available. Rats were injected im with ketamine-xylazine-acepromazine anesthesia (as above). Rats received a fourth intracerebroventricular (icv) guide cannula (Plastics One, Inc., Roanoke, VA; 22-gauge) with its tip positioned 2.0 mm above the fourth cerebral ventricle (coordinates were on the midline, 2.5 mm anterior to the occipital suture, and 5.2 mm ventral to the skull surface). Cannulas were cemented in place with dental acrylic and jeweler’s screws attached to the skull and closed with an obturator. Rats were implanted with a gastric cannula (see methods above) during the same surgical session.

Ventricle cannula placement verification. Approximately 7 d after surgery, icv cannula placement was confirmed by measurement of the sympathetic-adrenal-mediated glycemic response to hindbrain parenchymal cytologicuscopy induced by injection of 5-thio-d-glucose at a dose of 210 μg in 2 μl of artificial cerebral spinal fluid (Harvard Apparatus Inc., Torrance, CA). The criterion for subject inclusion was an increase in preinjection baseline plasma glucose level of no less than 100% measured 45 min after injection.

Habituation. Rats were deprived of food for 15–17 before their habituation to experimental procedures and for each experimental condition. They then received several manipulations involving the opening of the screw in the gastric cannula and the insertion of an empty balloon to adapt them to these procedures. Rats were habituated to the empty and to 5-ml balloon inflation. The balloon device (the distal end, 40 mm, of the small finger of a nitrile glove glued to a threaded plastic sleeve connected to a pressure shut-off valve) was inserted into the stomach and screwed into the threads of the lumen of the gastric cannula.

The effect of balloon distension volume on food intake. Rats were tested in the morning after a 15- to 17-h food deprivation period. The cannula screw was opened and the gastric lumen visualized. Stomach contents, if any, were gently removed with a saline lavage and a cotton swab. The empty balloon apparatus was screwed into the gastric cannula and the empty balloon positioned in the proximal stomach. Rats were tested under distended and sham-distended conditions using a counterbalanced design with conditions separated by 1 d. For the distension conditions, the balloon was inflated with three different volumes (2, 4, and 5 ml) of room temperature saline (dyed with food coloring for leak identification) at a rate of 1–1.5 ml/min. For the nondistension or sham condition, the balloon was inserted but not inflated. Fifteen minutes after the start of distension, animals were returned to their home cages and given access to a premeasured amount of pelleted chow. Food intake was remeasured 30, 60, and 90 min after access to chow, taking into account spillage. Distended balloons were deflated immediately after the 90-min food intake measurement.

The combined effects of a 4-ml gastric distension and fourth ventricular leptin delivery. Results of the distension dose-response study (see below) showed that the 4-ml volume did not significantly reduce food intake measured at any time point. Similarly, pilot experiments showed that a 5-μg dose of leptin (recombinant mouse leptin dissolved in sodium bicarbonate and delivered icv in a 2-μl volume) was without effect on short-term intake in rats maintained on this paradigm. Approximately 1 wk after the three-volume distension experiment, the same rats underwent a four-condition leptin-distension experiment with each condition separated by 1 d. Rats received an icv injection of either leptin or vehicle, counterbalanced in combination with either a 4-ml distension or a nondistension condition. Seventy-five minutes after the leptin or vehicle injection, the balloon device was inserted and either inflated or remained deflated. Fifteen minutes after balloon insertion, the rats were given access to pelleted chow. Cumulative food intake readings were made 30, 60, and 90 min after food access. Intake was also assessed at 24 h after injection to examine any longer-term effects of the conditions. After the 90-min intake measurement, the balloon apparatus was withdrawn, and in the distension case, its contents (saline) were first retrieved and measured to verify full recovery of the initial volume.

Data analysis. Results of each behavioral experiment were analyzed statistically using PC-SAS software (version 8.02; SAS Institute, Cary, NC) and expressed as mean ± SEM. For the volume-dose response experiment, cumulative intakes at 30 and 60 min were analyzed separately by one-way ANOVA, with distension volume as the main variable. For the leptin-distension experiment, cumulative chow intakes at 30, 60, and 90 min were analyzed separately by two-way ANOVA, with drug and distension conditions as the main variables. For both behavioral experiments, significant differences among treatment means were analyzed by Tukey honestly significant difference test for planned comparisons, with P < 0.05 considered significantly different.

Results

Anatomical experiments

Because a detailed anatomical description of leptin-responsive neurons in the caudal brainstem of the rat has not previously been reported, we first performed an experiment to investigate this question using P-STAT3 IHC. This method is useful to identify individual neurons that respond directly to leptin (8, 31). Figure 1 displays representative microphoto-
graphs of P-STAT3 IHC in the NTS of leptin-treated rats (Fig. 1, top row) and PBS-treated rats (Fig. 1, middle row). Sections were ordered in a rostral-to-caudal manner. Within the NTS, leptin-dependent P-STAT3-IR cells were found only in the mNTS at the level of the AP. Very few P-STAT3-IR cells were found rostral or caudal to the AP (Fig. 1). Likewise, very few P-STAT3-positive cells were detected in the NTS of PBS-treated rats (Fig. 1, middle row). We also noted P-STAT3-IR cells in the AP of the leptin-treated rats. By performing Nissl staining in the adjacent sections of series from the leptin-treated rats, with only a 25-μm difference between each P-STAT3 and Nissel section (Fig. 1, bottom row), the landmarks in the Nissl-stained sections can be applied to the P-STAT3 IHC sections. Based on this anatomical data (see Fig. 2), we conclude that within the NTS, P-STAT3-positive cells are detected only in the mNTS, just dorsal to the dorsal motor nucleus of the vagus. In five rats, we counted an average of 261 ± 26 cells in each 1.5 series, which yields an estimated 1303 ± 129 P-STAT3-positive cells in the entire NTS of the rat. Within the AP, we counted 146 ± 26 P-STAT3 cells per 1.5 series.

As shown in Fig. 3, and consistent with previous studies (33, 34, 36, 37), gastric distension robustly induced c-Fos IR in NTS neurons, primarily within the mNTS (Fig. 3A). In rats with sham distension, very few c-Fos-IR cells were detected in the NTS (Fig. 3B). Therefore, we tested the hypothesis that distension of the stomach in rats induces c-Fos in neurons that also respond directly to leptin. Figures 4 and 5 show that c-Fos IR was present in a significant number of the leptin-responsive cells (P-STAT3 IR; 40.6 ± 2.0%) by applying double-fluorescent IHC. In the AP, only 4.8 ± 1.5% of P-STAT3 cells showed double IR for c-Fos (n = 3). This result demonstrates that a significant proportion of the leptin-responsive cells in the NTS are activated by mechanical stimulation of the stomach. For rats with sham distension given PBS, very few c-Fos-IR or P-STAT3-IR cell bodies were detected in the NTS. In addition, in sham-distension rats, leptin, over this short time course, did not significantly stimulate c-Fos IR in the NTS (Fig. 4B). Finally, leptin did not affect the number of c-Fos-IR cells in rats subject to distension under these testing conditions. It has been reported that leptin amplified the numbers of distension-induced c-Fos cells in NTS (21). We reason that we did not see this amplification in our studies due to the shorter time (35 vs. 135 min) of leptin treatment.

Behavioral experiments

The effect of balloon distension volume on food intake. One-way ANOVA showed a significant main effect of gastric distension volume on 60-min cumulative food intake (F1,40 = 3.097; P < 0.04). As shown in Fig. 6, the intake-inhibitory effects of gastric distension were volume related. Specifically, 2- and 4-ml gastric balloon distension yielded a percent intake suppression of 1.4 and 16.6%, respectively, compared with sham distension. In neither case, however, was the suppression of intake significant. The 5-ml distension, however, produced a significant suppression of food intake (33.3%, P < 0.025). For measurements taken at 30 min, there were no significant effects of any volume of distension on intake.

The combined effects of a 4-ml gastric distension and fourth ventricular leptin delivery. Two-way ANOVA showed a significant main effect of gastric distension on cumulative intake at 60 min (F1,40 = 10.73; P < 0.01) and 90 min (F1,40 = 5.97; P < 0.04). There was no significant effect of drug (leptin, vehicle) treatment on intake under these conditions. However, there was a significant interaction between gastric distension and leptin treatment on intake assessed at 60 min (F1,40 = 5.34; P < 0.05) and 90 min (F1,40 = 14.14; P < 0.005). Figure 7 illustrates that neither 4-ml gastric distension nor icv leptin treatment (5 μg/2 μl) alone yielded a significant effect on intake at 30, 60, or 90 min. When combined; however, gastric distension and leptin significantly suppressed intakes at 60 and 90 min by 36.3 and 33.6%, respectively (P < 0.015 and P < 0.007, respectively). Although not quite reaching statistical significance, the interaction between gastric distension and icv leptin produced a tendency toward an inhibitory effect on intake at 30 min (F1,40 = 3.44; P = 0.097). To assess the longer-term anorectic effects of icv leptin and distension, 24-h food intake was measured. Two-way ANOVA showed a significant intake-inhibitory effect of drug (leptin) treatment on 24-h food intake (F1,40 = 8.73; P < 0.005). There was no effect of gastric distension on 24-h food intake.

Discussion

Leptin reduces food intake, but the central site of its action and the mechanism mediating this behavioral effect remain unspecified. The literature emphasizes leptin signaling in ARC neurons in triggering the effect of leptin on feeding

Fig. 2. Leptin-responsive neurons are exclusively located in the medial-subnucleus of the NTS at the AP level. Shown are two representative images of P-STAT3 IHC (A) and Nissl staining (B). Identification of NTS subnuclei is based on the Nissl staining: c, Central subnucleus of the NTS; cc, central canal; cm, commissural subnucleus of the NTS; dm, dorsal-medial sub-nucleus of the NTS; DMX, dorsal motor nucleus; m, mNTS; ts, solitary tract; vl, ventrolateral subnucleus of the NTS. Scale bars, 200 μm.
effect (9–11). This hypothalamic event is presumably linked to the amplification of the hindbrain-based processing of GI signals via known direct descending projections from the paraventricular nucleus and lateral hypothalamus (LH) to NTS neurons. Here, we investigated an alternate site and mechanism for the inhibitory effect of leptin on intake. Anatomical experiments showed that within the NTS, leptin-responsive neurons are limited to the mNTS and demonstrated that about 40% of these neurons are also activated by distension of the stomach. Behavioral experiments identified a dose of hindbrain injected leptin and a volume of stomach distension that were without effect on food intake when presented alone. When the two were presented in combination, however, short-term food intake was significantly reduced by approximately 35%. These results suggest a role for hindbrain neurons in triggering the inhibitory effect of leptin on intake. The same hindbrain neurons were shown to be activated by leptin and gastric distension, suggesting that the site of interaction between leptin and gastric distension (NTS) is also the site of leptin signaling and the site of processing of this interaction.

Our data show that leptin rapidly stimulates cellular STAT3 phosphorylation in cells located in the mNTS at the level of the AP. We hypothesized that those mNTS leptin-responsive neurons were also activated by gastric distension. We combined gastric distension with peripheral leptin treatment, using c-Fos IR as the marker for neuronal activation induced by distension and P-STAT3 IR as the marker for direct leptin action. To avoid the effects of anesthesia on c-Fos IR, we performed the gastric distension in awake, behaving Sprague Dawley rats. Because studies have shown that leptin itself can induce c-Fos in the NTS with its peak at 90 min (40, 41), we limited the time between leptin treatment and killing to 35 min. We showed that leptin by itself did not elevate c-Fos in the NTS at this time. In contrast, a modest level of gastric balloon distension robustly induced Fos IR in the NTS, primarily within the medial subnucleus. The finding that a significant proportion of leptin-responsive cells also respond to gastric distension confirms our hypothesis.

Leptin signaling has been shown to interact with GI satiety signals controlling for food intake (21, 22, 24–27). A variety of these studies suggest that this interaction is one of leptin potentiating the intake-inhibitory effects of the GI signals. At supraphysiological levels, either leptin or meal-cued

![Fig. 3. Gastric distension increases the number of c-Fos-IR cells in the mNTS. Gastric balloon distension (4 ml) was performed in rats that had recovered for 1 wk after surgical placement of gastric fistulas. Sixty minutes after completion of the distension, rats were killed. The NTS sections were subjected to c-Fos fluorescence IHC. Shown are two representative microphotographs from distension (A) and sham distension (B).](https://academic.oup.com/endo/article-abstract/148/5/2189/2501936)

![Fig. 4. Gastric distension increases c-Fos IR in leptin-responsive neurons in the mNTS. Gastric balloon distension (4 ml saline) was performed in rats that had recovered for 1 wk after surgical placement of gastric fistulas. Twenty-five minutes after completion of the distension, rats were injected with leptin (4 mg/kg, ip) or vehicle and killed 35 min later. Sections from the NTS were subjected to double-fluorescence IHC for P-STAT3 (green fluorescence) and c-Fos (red fluorescence). Shown are representative merged microphotographs of double IHC (P-STAT3 and c-Fos).](https://academic.oup.com/endo/article-abstract/148/5/2189/2501936)
signals can reduce food intake (29, 42). Likewise, physiological levels of GI satiation signals are sufficient to reduce intake (43–45). Recent findings of Zhang et al. (46) suggest that endogenous leptin signaling is also sufficient to control for food intake, because chronic infusion of a competitive leptin receptor antagonist increased daily food intake. Thus, although previous reports suggest that leptin is a potentiator of GI satiation signaling, our findings in conjunction with those of Zhang et al. (46) leave open the possibility of a synergistic interaction between leptin and gastric distention. Leptin delivered to the fourth cerebral ventricle enhanced the intake-inhibitory action of gastric distension. This result is consistent with the results of other studies that suggest that the mechanism underlying the intake-suppressive action of central leptin involves a process, most likely localized to the hindbrain, whereby leptin potentiates or amplifies the intake-inhibitory effects of GI stimuli that control meal size (21, 22, 27, 47). Prior studies delivered leptin to the forebrain ventricles, making the ligand available to leptin-responsive neurons in periventricular areas throughout the brain. We delivered leptin to a more restricted region, the caudal brainstem ventricle, and yet observed comparable potentiation by leptin of the intake-inhibitory effects of GI stimulation. Leptin delivered to the fourth ventricle supplies the ligand to receptor-bearing neurons in the parenchyma of the dorsal medulla. Data presented here and elsewhere (31) show those ObRb-bearing neurons are located in the mNTS at the level of the AP in the rat and mouse. The icv administration or microinjection of leptin directly into the NTS (at the level of the AP) is sufficient to reduce short-term food intake and body weight gain (29). This suggests a role for leptin signaling at the level of the dorsal vagal complex (includes NTS, AP, and dorsal motor nucleus of the vagus nerve) in food intake control. Given the proximity of the dorsal vagal complex to the hindbrain ventricle, it seems reasonable to suggest that stimulation of neurons expressing ObRb in this region contributes to the effects of icv leptin delivery observed in our experiment. Although we cannot entirely rule out a forebrain action, available information is inconsistent with the notion that icv injections also provide leptin to the hypothalamic parenchyma. First, ink or dye injected into the fourth ventricle (in volumes similar to those used here) was observed at and caudal to the injection site, but not rostral to it (48, 49). Second, the behavioral effects of a number of peptide ligands (e.g. oxytocin, cocaine-amphetamine-related transcript, and angiotensin II) are determined by whether they are injected into the fourth or into the forebrain ventricles (49–53). A case in point is the robust water intake

FIG. 5. A significant proportion of leptin-responsive cells respond to gastric distension. A representative merged microphotograph of double IHC (P-STAT3 and c-Fos) from gastric distension combined with leptin-treated rats is shown on the left. On the right are shown high magnifications (top, P-STAT3 green fluorescence IHC; middle, c-Fos red fluorescence IHC; bottom, merged microphotograph from the double IHC) of the area marked on the left. Examples of double-labeled cells are shown in yellow. cc, Central canal. Scale bars, 200 μm.

FIG. 6. The volume of gastric distention determines the magnitude of intake. Within-meal gastric balloon distension (2, 4, and 5 ml) resulted in a volume-dependent reduction of 60-min cumulative chow intake compared with sham distension intakes. Food intake was not significantly suppressed by either 2- or 4-ml distension. However, 5-ml gastric distension significantly suppressed food intake compared with within-subject sham-distension intakes. *, P < 0.05.

FIG. 7. Leptin delivered to the hindbrain is sufficient to potentiate the intake-suppressive effects of an otherwise ineffective volume of gastric distension. Cumulative chow intake was not significantly affected by either 4-ml gastric distension or icv leptin treatment (5 μg/2 μl) alone at 30, 60, or 90 min. However, when combined, gastric distension and leptin significantly suppressed cumulative intakes at 60 and 90 min compared with vehicle/sham-distension intakes. *, P < 0.05.
response observed after forebrain ventricular delivery of angiotensin II (51, 52), with no drinking observed, however, even when high doses of this ligand were delivered to the fourth ventricle (51). This indicates, at least for this treatment, that hindbrain ventricular delivery does not supply ligand that reaches the medial basal hypothalamus to trigger a behavioral response and suggests that our findings are a result of leptin action within the caudal brainstem, which includes the NTS.

The results reported here are consistent with the hypothesis that leptin’s intake-inhibitory action is mediated, at least in part, by direct signaling in mNTS neurons (29–31). We cannot rule out a role for the AP, although we find far fewer double-labeled cells there. Under some circumstances, leptin signaling in ARC neurons also appears to contribute to the intake-inhibitory effects. For example, microinjection of leptin directly into the ARC of the hypothalamus, and into the ventromedial nucleus or LH, can reduce short-term food intake (12). In addition, viral-mediated expression of leptin receptors in the arcuate region of Koletsky rats, which lack functional receptors throughout the CNS, is sufficient to attenuate the chronic hyperphagia associated with this genetic deficiency (10). Moreover, ARC neurons may contribute to the intake-inhibitory effects of CCK as reported by Morton and colleagues (11). They showed that the behavioral sensitivity to CCK is diminished in the Koletsky rats lacking ObRb but that restoration of a subset of these receptors with injection of adenoviral vectors into the ARC enhanced sensitivity to the intake-inhibitory effects (and increased expression of Fos IR in the NTS) observed with peripherally injected doses of CCK. These leptin-dependent actions in the hypothalamus may be explained by descending pathways from paraventricular nucleus and LH, because these nuclei are both innervated by leptin-responsive ARC neurons and project to the hindbrain where they may modulate GI signal processing in the NTS (54, 55). Additional work will be needed to reconcile and explain the data indicating that several forebrain and hindbrain regions are independently capable of reducing short-term intake in response to leptin. Alternatively, it is possible that each region serves distinct, yet unknown, functions under different physiological conditions that require leptin signaling to reduce short-term food intake.

Leptin, injected into the forebrain ventricle, has been shown to increase the excitatory neurophysiological response of NTS neurons to gastric distension (28). Similarly, an excitatory action of leptin on NTS neurons was inferred by the increased Fos IR observed in NTS neurons after peripheral or central leptin treatment (7, 40). The c-fos gene is an early immediate gene, which is associated with increased cellular excitability based on its presence in regions showing increases in intracellular calcium or cAMP (56). In both examples, leptin was administered 1–2 h before the observed responses. Recently, Williams and Smith (57) reported that 58% of NTS neurons in caudal brainstem slices were rapidly hyperpolarized by the addition of leptin to the holding chamber. Only a small proportion of these NTS neurons (~13%) responded with a depolarization. It is unclear whether the neurons that display the short-latency inhibitory response and those neurons that display a longer-latency excitatory neurophysiological or Fos-IR response are the same neurons or whether they represent two distinct phases of leptin activity in the caudal brainstem neurons (57). Leptin receptors are also found on vagal afferent neurons that terminate on NTS neurons. The Williams and Smith study (57) also reported that leptin decreased excitatory input to NTS neurons evoked by electrical stimulation of the tractus solitarius, suggesting a presynaptic effect on vagal terminals. On the other hand, other data show that leptin application depolarized nodose ganglion cells examined in culture (58) and that leptin administration to the gastric lumen of an ex vivo stomach/vagus/hindbrain preparation resulted in a vagally mediated and acute increase in the neuronal activity of NTS neurons (59). It appears that there are also different populations of leptin-responsive neurons outside the NTS with potentially opposite responsiveness to leptin. There is a diversity of effects of leptin on neuronal excitability within a given nucleus and across different levels of the neuraxis and the periphery. It is as yet unclear how this diversity in leptin’s effects on cellular excitability contributes to defining mechanisms of intake control.

Our data are consistent with the hypothesis that leptin acts on specific NTS neurons to augment the neurophysiological response of gastric distension and thereby to increase the intake-inhibitory potency of satiation signals arising in the GI tract. The neurochemical identity of these leptin- and gastric-distension-responsive hindbrain neurons is not as yet defined. Proglucagon- and proopiomelanocortin (POMC)-expressing neurons are found in this region of the hindbrain (60). Functional data suggest that each may play a role in behavioral responses to GI stimulation (61, 62); however, the leptin responsiveness of neurons with these phenotypes is less clear. We have previously shown that peripheral leptin treatment does not result in the expression of P-STAT3 IHC in POMC neurons of mice (31). Those data strongly suggest that POMC is not the phenotype of the ObRb-bearing, gastric-distension-driven neurons identified here (cf. Ref. 63). Nonetheless, ObRb mRNA has been reported to colocalize with proglucagon-producing neurons in the NTS of mice (64). Although Elias et al. (40) have shown that leptin induces c-Fos in proglucagon-producing neurons in the NTS of rats, we have recently reported that rat proglucagon neurons, in stark contrast to mice, do not respond directly to leptin (65). First, this suggests that c-Fos activation in proglucagon neurons by leptin in the rat is mediated via indirect pathways. Second, because gastric distension does induce c-Fos in proglucagon neurons in the rat (61), we conclude that at least two distinct populations of distension-responsive neurons exist in the rat: one set that responds to leptin and does not express proglucagon and another set that expresses proglucagon but does not respond to leptin.

Based on the results of these studies we conclude that 1) leptin-responsive neurons in the hindbrain are primarily located in the mNTS at the level of the AP, a key vagal afferent projection zone of the GI tract (66); 2) a significant proportion of the leptin-responsive cells in the NTS are activated by stomach distension; and 3) leptin delivered to the hindbrain is sufficient to potentiate the intake-suppressive effects of an otherwise ineffective volume of gastric distension. These results are consistent with the hypothesis that leptin acts, at
least in part, in the mNTS to reduce food intake through an interaction with the processing of GI signals. These conclusions provide a complementary perspective to the view that leptin’s effects on feeding are mainly triggered by an action in ARC neurons, one of multiple sites expressing functional leptin receptors. The conclusions are consistent with data from chronic decerebrate rats that show that the central brainstem circuits mediate the intake-inhibitory action of GI signals and with the perspective that the neural control of energy balance is distributed rather than localized to one region of the brain (67). Additional studies are needed to determine 1) the cellular and circuit properties of hindbrain neurons that mediate leptin’s potentiation of GI signals that in turn control meal size and 2) the neurochemical phenotype of the mNTS neurons that exhibit coresponsiveness to leptin and GI signals.

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