Leptin Signaling in the Nucleus Tractus Solitarii Increases Sympathetic Nerve Activity to the Kidney

Allyn L. Mark, Khrisofor Agassandian, Donald A. Morgan, Xuebo Liu, Martin D. Cassell, Kamal Rahmouni

Abstract—The hypothalamic arcuate nucleus was initially regarded as the principal site of leptin action, but there is increasing evidence for functional leptin receptors in extrahypothalamic sites, including the nucleus tractus solitarii (NTS). We demonstrated previously that arcuate injection of leptin increases sympathetic nerve activity (SNA) to brown adipose tissue and kidney. In this study, we tested the hypothesis that leptin signaling in the NTS affects sympathetic neural outflow. Using a stereotaxic device in anesthetized rats, we microinjected leptin (0.25 to 1.00 μg) or saline into the NTS while recording SNA to kidney and brown adipose tissue. Microinjection of leptin into the commissural and medial subnuclei of the caudal NTS at the level of the area postrema of the Sprague-Dawley rats produced a dose-related increase in renal SNA (+112±15% with leptin 1 μg; n=7; P<0.001) but did not increase SNA to brown adipose tissue (−15±12%; P value not significant). This effect depended on intact functional leptin receptors, because it was not observed in Zucker obese rats that have a missense mutation in the leptin receptor. Rostral NTS injection of leptin failed to increase SNA, indicating that leptin signaling in the NTS is probably confined to the caudal NTS at the level of the area postrema. In summary, this study demonstrates that leptin signaling in the caudal NTS increases SNA to the kidney but not to the brown adipose tissue. The study strengthens the concept of a distributed brain network of leptin action and demonstrates that these distributed brain sites can mediate contrasting sympathetic responses to leptin.

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Key Words: leptin ■ nucleus tractus solitarii ■ sympathetic nerve activity ■ kidney ■ brown adipose tissue

Leptin is an adipocyte-derived hormone that plays a critical role in the regulation of energy homeostasis. The hypothalamic arcuate nucleus was initially regarded as the principal site of leptin action, but there is increasing evidence for a distributed brain network of leptin action.1 Among these distributed brain sites, there is increasing evidence for functional leptin receptors in the nucleus tractus solitarii (NTS), albeit in lower levels than in the arcuate nucleus. Functional leptin receptor mRNA is expressed in the NTS of mice and rats.2–4 Peripheral administration of leptin increases phosphorylated signal transducers and activators of transcription 34,5 and c-fos6,7 immunoreactivities in the NTS. Proopiomelanocortin neurons, which are prominent in mediating leptin action in the arcuate nucleus, also occur in the NTS.8–10 There is controversy centering on whether these NTS proopiomelanocortin neurons do10 or do not11 mediate some of the NTS leptin action, but there is agreement that there are leptin-responsive neurons in the NTS.1–7,9–12 For example, Grill et al3 demonstrated that a low dose of leptin injected into the dorsal vagal complex including the NTS decreased food intake and body weight in rats. In addition, a number of NTS neurons that are activated by gastric distension are also activated by leptin.12,13 and leptin acts in the hindbrain to potentiate the satiety response to gastric distension.12

In addition to effects on appetite and body weight, leptin increases sympathetic nerve activity (SNA).14–17 With systemic,15 cerebroventricular,7,16 or intra-arcuate administration of leptin,17 there are increases in SNA to brown adipose tissue (BAT), which is involved in thermogenic metabolism, and to the kidney, which is involved in cardiovascular regulation. Because the NTS is critically involved in sympathetic regulation and given the emerging evidence for leptin signaling in the NTS, we tested the hypothesis that leptin receptor activation in the NTS regulates sympathetic neural activity.

Methods

Animals

Male Sprague-Dawley rats and Zucker obese and lean rats (Harlan Sprague-Dawley), age 14 to 16 weeks, were studied. Rats were housed in a temperature-controlled room with a 12:12 hour light-dark cycle. Standard laboratory rat chow and tap water were provided ad libitum. The studies and procedures were approved by the University of Iowa Animal Research Committee. All of the

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studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Experimental Preparation**

Rats were anesthetized with IP ketamine (91.0 mg/kg) and xylazine (9.1 mg/kg). After ketamine and xylazine anesthesia and insertion of a venous catheter, α-chloralose 50 mg/kg was administered intravenously in 0.5 mL over 45 minutes followed by 25 mg/kg per hour in 0.5 mL/h. Arterial pressure and heart rate were monitored with a catheter inserted into a caudal artery and a pressure transducer connected to a computer. The trachea was cannulated, and each rat was allowed to breathe oxygen-enriched air spontaneously. Rectal temperature was maintained at 37.5°C using a temperature-controlled surgical table and lamp. The animal was instrumented for sympathetic nerve recordings as described below and was then placed in a stereotaxic frame (David Kopf Instruments) and prepared for stereotaxic microinjections into the NTS as described below.

**Sympathetic Nerve Recordings**

After the induction of anesthesia, we obtained simultaneous multifiber recordings of SNA from a nerve to the kidney and to the BAT. The left kidney was exposed through a retroperitoneal flank incision. Using a dissecting microscope, a renal nerve was carefully dissected free and placed on a bipolar 36-gauge platinum-iridium electrode. When an optimal recording of renal SNA was obtained, the electrode was covered with silicone gel (Kwik-Sil, World Precision Instruments, Inc.). Interscapular BAT was then exposed through a nape incision. A nerve fiber innervating BAT was identified, placed on the bipolar electrode, and secured with silicone gel for recordings of SNA to BAT. Each electrode was attached to a high-impedance probe (HIP-511, Grass Instruments), and the nerve signal was amplified 10^5 times with a Grass P5 AC preamplifier. After amplification, the nerve signal was filtered at a 100- and 1000-Hz cutoff with a nerve traffic analysis system (Model B600c, University of Iowa Bioengineering). The nerve signal was then routed to an oscilloscope (Model 54501A, Hewlett-Packard) for monitoring the quality of the sympathetic nerve recording and to a resetting voltage integrator (Model B600c, University of Iowa Bioengineering).

**Brain Microinjections**

Once the surgery for SNA recordings was complete, the head of the rat was placed in a stereotaxic frame. The head of the rat was flexed ~30° in the stereotaxic device to level the caudal brain stem. For microinjections into the caudal NTS, after surgical exposure, the pipette was placed in the brain stem 0.4 to 0.5 mm rostral to the calamus scriptorius, 0.4 to 0.6 mm lateral to the midline, and 0.5 to 0.7 mm beneath the surface of the brain stem. After injections, the pipette was left in place for 5 minutes and then removed. For microinjection into the rostral NTS, the pipette was placed in the brain stem 0.8 to 1.0 mm rostral to the calamus scriptorius, 0.8 to 1.0 mm lateral to the midline, and 0.5 to 0.7 mm beneath the surface of the brain stem. We mixed fluorescent beads (0.2% FluoSphere, 1 μm, blue fluorescent, Invitrogen) with leptin and vehicle for each injection to assess the accuracy of the injection site. At the end of each experiment, the animal was perfused with 4.0% paraformaldehyde. The brain was sectioned, and brain studies were verified were included.

**Experimental Protocols**

After surgical preparation, rats were allowed to stabilize for 15 to 20 minutes. Baseline recordings of sympathetic nerve activities, arterial pressure, and heart rate were collected twice during a 10-minute control period and averaged for a single value for the control period. In the parent protocol, Sprague-Dawley rats received either vehicle (saline, 50 nL) or recombinant mouse leptin (0.25, 0.50, or 1.00 μg in 50 nL saline; R&D Systems, Inc.) into the left caudal NTS.

**Results**

**Responses to Caudal NTS Injections of Leptin**

Injection of leptin (0.25 to 1.00 μg in 50 nL of saline) into the caudal NTS of Sprague-Dawley rats resulted in a dose-related increase in renal SNA (Figures 1 and 2). Bilateral injection of 0.50 μg of leptin into the caudal NTS produced an increase in renal SNA (+85±13%; n=5) that did not differ (P value not significant) from that seen with injection of 1.00 μg of leptin unilaterally (+112±15%).

In contrast to the effects on renal SNA, unilateral caudal NTS injection of leptin did not increase SNA to BAT (Figures 1 and 2). Bilateral caudal NTS injection of 0.5 μg of leptin also failed to increase SNA to BAT (+2±13%; n=5). Caudal NTS injection of vehicle (50 nL of saline) did not alter either renal or BAT SNA (Figure 2A through 2D).

To ascertain that the lack of increase in SNA to BAT was not caused by a lack of responsiveness of the preparation, we studied responses to cooling at the end of 12 experiments in which NTS leptin injection failed to increase SNA to BAT. Cooling the rats from 37.5° to 30.0°C increased SNA to BAT by +122±41% (P<0.05). Cooling increased SNA to BAT in each of these 12 experiments. These results indicate that the failure of leptin to increase SNA to BAT cannot be explained by a lack of responsiveness of the preparation.
As shown in Figure 3, mean arterial pressure increased significantly (P<0.05 versus vehicle) with the high dose of leptin but not with the low or middle dose when compared with responses to vehicle. Baseline mean arterial pressure was 87±11, 95±4, 86±4, and 94±2 mm Hg in the vehicle and 0.25-, 0.50-, and 1.00-μg leptin groups, respectively. Heart rate increased in both the vehicle and leptin groups, but the increases did not differ between groups (P value not significant). For example, heart rate increased from 229±16 to 299±12 bpm in the vehicle group and from 242±13 to 302±8 bpm in the leptin 0.50-μg group.

The location of the injection sites confirmed to be within the caudal NTS is shown in Figure 4. The injections were distributed in the lateral part of the commissural subnucleus and the dorsomedial part of the medial subnucleus. There was no notable difference in the responses to leptin injection into the lateral part of the commissural subnucleus versus the dorsomedial part of the medial subnucleus.

Responses to NTS Injection of Leptin in Zucker Lean and Obese Rats
To determine whether the SNA responses to caudal NTS leptin are receptor mediated, we compared effects of caudal leptin on renal and BAT SNA in Zucker lean and obese rats.
Dawley and Zucker lean control rats are dependent on an leptin signaling of sympathetic outflow, but not in the Zucker obese rats (Figure 5B). These findings support the conclusion that leptin failed to increase BAT SNA in either Zucker lean or wild-type rats but failed to increase SNA to BAT, kidney or BAT. Injections of pseudorabies virus, a transneuronal tracer, into interscapular BAT result in early infection of the rostral and intermediate NTS, and many neurons so infected are responsive to cold. However, injection of leptin into the caudal NTS increased renal SNA in both Sprague-Dawley and Zucker lean or wild-type rats but failed to increase SNA to BAT.

We considered 2 possible explanations for the failure of caudal NTS to increase SNA to BAT. The first was a nonspecific lack of responsiveness of BAT SNA in our preparation. This was excluded by demonstrating that cooling the rats, which is a stimulus to thermogenic metabolism, increased SNA to BAT. In addition, in our previous report, using the same approach as in the current study, microinjection of leptin into the hypothalamic arcuate nucleus increased both BAT and renal SNA. Second, we considered the possibility that the rostral, as opposed to the caudal, NTS region might regulate SNA to BAT, but the findings suggest that leptin signaling in the NTS is not involved in this process. The major new findings from this study are as follows: (1) leptin receptor signaling in the NTS increases SNA to kidney but not to BAT; (2) sympathoactivation to microinjection of leptin into the NTS was associated with an increase in arterial pressure; and (3) the renal sympathetic nerve responses to NTS leptin appear to emanate from the caudal region of the NTS at the level of the area postrema. Leptin signaling in the NTS produces a strikingly different pattern of sympathetic responses than that emanating from leptin signaling in the hypothalamic arcuate nucleus in our previous study. The current study adds to evidence for functional leptin receptors in the NTS and thereby strengthens the concept of a distributed brain network of leptin action. The results further demonstrate that these distributed brain sites can mediate contrasting responses to leptin.

As discussed in the introduction, our study was prompted by emerging evidence for functionally significant leptin receptors in the NTS. NTS injection of leptin (0.5 μg in 50 nL of saline) into the rostral NTS in Sprague-Dawley rats failed to increase SNA to either BAT (−21±17%) or kidney (+5±25%). A representative photomicrograph of the fluorescent beads in the rostral NTS is shown in Figure S1 (available in the online data supplement at http://hyper.ahajournals.org). The lack of SNA response to rostral NTS leptin is consistent with previous reports that leptin receptor signaling in the NTS is confined to the caudal NTS.1,10,11

Responses to Rostral NTS Injections of Leptin

We also studied responses to leptin injection into the rostral NTS to determine whether this region of the NTS might participate in leptin signaling of sympathetic outflow, because viral tracing has indicated that the rostral and intermediate NTS, but not the caudal NTS, are polysynaptically connected with BAT.19 In the current study, injection of leptin (0.5 μg in 50 nL of saline) into the rostral NTS in Sprague-Dawley rats that have a missense mutation in the leptin receptor18 and lean controls. As shown in Figure 5A, injection of leptin into the caudal NTS increased renal SNA in the Zucker lean but not in the Zucker obese rats. NTS injection of leptin failed to increase BAT SNA in either Zucker lean or obese rats (Figure 5B). These findings support the conclusion that the renal SNA responses to caudal NTS in the Sprague-Dawley and Zucker lean control rats are dependent on an intact leptin receptor.

Figure 4. Schematic diagram of the NTS showing the sites of leptin injection assessed from the location of fluorescent microspheres coinjected with leptin. The injections were localized in the lateral part of the commissural subnucleus and the dorsomedial part of the medial subnucleus of the NTS. The size of each marker reflects the borders of the deposit of microspheres. cNTS indicates commissural subnucleus; iNTS, intermediate subnucleus; mNTS, medial subnucleus; DMX, dorsal vagal motor nucleus; AP, area postrema; sol, solitary tract.

**Discussion**

The major new findings from this study are as follows: (1) leptin receptor signaling in the NTS increases SNA to kidney but not to BAT; (2) sympathoactivation to microinjection of leptin into the NTS was associated with an increase in arterial pressure; and (3) the renal sympathetic nerve responses to NTS leptin appear to emanate from the caudal region of the NTS at the level of the area postrema. Leptin signaling in the NTS produces a strikingly different pattern of sympathetic responses than that emanating from leptin signaling in the hypothalamic arcuate nucleus in our previous study. The current study adds to evidence for functional leptin receptors in the NTS and thereby strengthens the concept of a distributed brain network of leptin action. The results further demonstrate that these distributed brain sites can mediate contrasting responses to leptin.

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Figure 5. Effects of 0.5 μg of leptin injected into the caudal NTS on renal and BAT SNA in leptin receptor mutant Zucker obese (ZO) and control Zucker lean (ZL) rats. A, Comparison of renal SNA response to leptin injection into the NTS between the Zucker obese and lean control rats. B, Comparison of BAT SNA response to leptin injection into the NTS between the Zucker obese and lean control rats. Data are means±SEMs of n=4 to 7 per group. *P<0.05 vs lean controls.
is supported by the findings that increased leptin transgenic expression in the dorsal vagal complex, including the NTS, failed to affect uncoupling protein-1 mRNA expression in the BAT.20

The finding that caudal NTS leptin increased renal SNA, whereas rostral NTS injection of leptin failed to increase SNA, supports previous suggestions3,10,11 that leptin signaling in the NTS occurs primarily in the caudal NTS at the level of the area postrema. Our injections into the caudal NTS were clustered in the lateral part of the commissural subnucleus and the dorsomedial part of the medial subnucleus. There were no notable differences in the renal SNA responses to leptin in these 2 areas. Anatomic studies suggest that the caudal NTS may be indirectly connected with postganglionic sympathetic neurons innervating the kidney,21 and there is evidence that visceral afferents from the kidney ultimately end in the commissural NTS.22

We cannot completely exclude the possibility that the area postrema and/or dorsal motor nucleus of the vagus contributed to the renal SNA responses to the injection of leptin in the caudal NTS. The vast majority of the injection sites into the caudal NTS were centered lateral to the area postrema and dorsal to the dorsal motor nucleus of the vagus (Figure 4), but some leptin may have diffused, albeit at decreasing concentration, into the area postrema or the nucleus of the vagus. Nevertheless, given the distribution of the center of the injection sites in the caudal NTS, the most likely interpretation of our data is that the responses emanated mainly from the commissural and medial subnuclei of the caudal NTS.

From these studies, we cannot identify the NTS neurons activated by leptin to increase SNA. NTS leptin increased renal SNA (which is under baroreceptor control) but not SNA to BAT (which is not under baroreceptor control). Our NTS injection sites include neurons in receipt of baroreceptor afferents.23 These observations suggest that leptin could be acting in the NTS to increase renal SNA by acting on baroreceptor neurons, but our NTS injection sites also include neurons subserving visceral afferents,24 and NTS neurons that are activated by gastric distension are also activated by leptin.25,26

Caudal NTS injection of leptin increased arterial pressure. However, this effect was significant only with the highest dose of leptin. This is consistent with the modest pressor action of leptin, particularly in acute studies.16,17 The pressor action of caudal NTS injection of leptin may be attributable to sympathetically mediated vasocostriction or a consequence of the renal sympathetic antinatriuretic actions of leptin. However, additional studies are needed to test this hypothesis.

Perspectives

A striking and surprising finding was the contrast between the pattern of sympathetic activation with NTS leptin in this study and the pattern of sympathetic responses to arcuate administration of leptin in our previous study.17 NTS injection of leptin increased renal SNA but failed to increase SNA to BAT. In contrast, in our previous study, arcuate injection of leptin (in the same dose injected into the NTS) increased SNA to both BAT and kidney with greater increases in BAT SNA.17 The contrasting pattern of responses to NTS versus arcuate leptin suggests that the responses to NTS leptin are not emanating from the hypothalamic arcuate nucleus. The finding that both NTS and arcuate leptin trigger SNA strengthens the concept of a distributed brain network of leptin action. The contrasting responses to NTS versus arcuate leptin suggest that these distributed brain sites can mediate qualitatively distinct leptin actions. Our study extends the understanding of the brain regions involved in the sympathetic neural actions of leptin and the concept of a distributed brain network of leptin action.

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Disclosures

None.

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LEPTIN SIGNALING IN THE NUCLEUS TRACTUS SOLITARII INCREASES SYMPATHETIC NERVE ACTIVITY TO THE KIDNEY

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A representative photomicrograph of the fluorescent beads in the rostral NTS

Figure S1. Photomicrograph of a representative coronal section showing the site of fluorescent microspheres during a unilateral microinjection into the rostral nucleus tractus solitarii. Abbreviations: 4V, fourth ventricle; NTS, nucleus tractus solitarii; sol, solitary tract.