Hypothalamic Arcuate Nucleus Mediates the Sympathetic and Arterial Pressure Responses to Leptin

Kamal Rahmouni, Donald A. Morgan

Abstract—Leptin is an adipocyte-derived hormone that plays an important role in the regulation of energy homeostasis through its action in the central nervous system. Leptin decreases body weight by promoting satiety and increasing thermogenesis via increasing sympathetic nerve activity (SNA) to brown adipose tissue. Leptin also increases renal SNA and arterial pressure. The arcuate nucleus is considered as a major nucleus for leptin action on energy homeostasis. We tested whether leptin action in the arcuate nucleus simultaneously activates SNA to brown adipose tissue and the kidney. The sympathetic and cardiovascular responses to intra-arcuate injection of leptin were compared with those evoked by intracerebroventricular administration of leptin in rat. Intracerebroventricular administration of leptin (10 μg) caused a significant increase in SNA to brown adipose tissue and the kidney. Intracerebroventricular leptin also increased mean arterial pressure. Direct injection of leptin (500 ng) into the arcuate nucleus increased both brown adipose tissue (254 ± 49%; P < 0.001 versus vehicle) and renal SNA (111 ± 31%; P < 0.001 versus vehicle). Microinjection of leptin into the arcuate nucleus also produced a substantial increase in mean arterial pressure (from 82 ± 3 to 100 ± 7 mm Hg; P = 0.02). These data demonstrate that leptin action in the arcuate nucleus of the hypothalamus is important for the control of sympathetic outflow to both brown adipose tissue and the kidney. These results also suggest that the cardiovascular effects of leptin might be evoked by the action of this hormone in the arcuate nucleus of the hypothalamus. (Hypertension. 2007; 49[part 2]:647-652.)

Key Words: leptin ■ obesity ■ sympathetic nervous system ■ hypothalamus ■ thermogenesis ■ blood pressure

The adipocyte-derived hormone leptin plays a critical role in the regulation of energy homeostasis.1 Leptin action in the central nervous system decreases body weight by suppressing appetite and increasing energy expenditure. Consistent with its effects on energy expenditure, leptin increases sympathetic nerve activity (SNA) to the thermogenic brown adipose tissue (BAT),2,3 but it also causes sympathoactivation to the kidney, adrenal gland, and hindlimb.4,5 Activation of the autonomic function by leptin has implications for the cardiovascular system. Indeed, systemic, as well as central, administration of leptin increased arterial pressure in the rat.5,6 Transgenic overexpression of leptin in mice also results in higher baseline arterial pressure.7 In contrast, absence of leptin, as in the ob/ob mice, is associated with lower arterial pressure.8

Adrenergic blockade reversed the increase in arterial pressure induced by leptin demonstrating the importance of the sympathetic nervous system in the arterial pressure response to leptin.9 Renal sympathoactivation to leptin seems to play a pivotal role in the increase in arterial pressure induced by this hormone. Indeed, preservation of leptin action on renal SNA, such as in the diet-induced obese mice, is associated with hypertension and a preserved arterial pressure response to leptin.10 In contrast, loss of leptin’s ability to increase renal SNA, such as in the melanocortin-4 receptor knockout mice, is associated with normal baseline arterial pressure and lack of arterial pressure increase after leptin treatment.11–13

The hypothalamus is recognized as an important site of leptin action in the central nervous system.14 The long form of the leptin receptor, which appears to mediate most of the biological effects of leptin, is expressed in several hypothalamic nuclei with higher expression in the arcuate nucleus (ARC).15 Within the ARC, the long form of the leptin receptor is expressed in ≥2 classes of neurons that are critical for the regulation of energy homeostasis: the anorexigenic neurons that express proopiomelanocortin peptide and the orexigenic neurons expressing neuropeptide Y and agouti-related peptide.14,15

The observation that ARC-specific gene transfer of the long form of the leptin receptor in rats16 or mice17 lacking functional leptin receptors results in an amelioration of the obesity phenotype demonstrates the importance of leptin signaling in this nucleus for energy homeostasis. Moreover, local injection of leptin into the ARC decreases food intake and body weight in a dose-dependent manner.18 Conversely, lesions of the ARC blunt the feeding- and weight-reducing actions of leptin.19,20 The thermogenic SNA response to leptin was also blocked after the ARC was destroyed.21 Further-
more, direct administration of leptin into the ARC increased lumbar SNA in rats. Together, these data suggest that the sympathetic effects of leptin may emanate from this nucleus.

However, to our knowledge, the role of ARC in the renal SNA response to leptin has not been investigated. Therefore, in the present study, we assessed in rats the effects of injecting leptin directly into the ARC on SNA that subserve thermogenic BAT and cardiovascular (kidney) tissues. The changes in arterial pressure and heart rate were recorded simultaneously with SNA. The sympathetic and hemodynamic changes induced by intra-ARC leptin were compared with those evoked by intracerebroventricular (ICV) administration of this hormone.

Methods

Animals
Male Sprague–Dawley rats (Harlan Sprague–Dawley) were used. All of the rats (300 to 350 g) 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 University of Iowa Animal Research Committee approved all of the procedures. All of the studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Cannulation
Rats were anesthetized by intraperitoneal injection of ketamine (91 mg/kg) and xylazine (9.1 mg/kg) and placed in a stereotactic frame (David Kopf Instruments). The skull was exposed by an incision and leveled between lambda and bregma. ICV cannulation of the third ventricle was performed as described previously. The ARC was cannulated unilaterally using a 25-gauge cannula, which was implanted with a 10° angle with the following coordinates relative to bregma: −3.6 mm anteroposterior, +2 mm lateral from the midline, and −8.6 mm dorsoventral from cortex. Each cannula was fixed in place using dental cement, and rats were kept in individual cages postoperatively. Rats were allowed ≥1 week of recovery before experimentation. The position of the cannulae was verified at the end of each experiment as described below.

Sympathetic Nerve Recordings
Simultaneous multifiber recording of SNA was obtained, in anesthetized (ketamine/xylazine mixture, IP) rats, from 2 nerves subserving different beds: BAT and kidney. The left kidney was exposed retroperitoneally through a flank incision. Using a dissecting microscope, a renal nerve was carefully dissected free and placed on a table and a lamp. The nerve signal was then routed to an oscilloscope (model 54501A, Hewlett-Packard) for monitoring the quality of the sympathetic nerve activity and arterial pressure. As shown in Figure 1A, there was a significant increase in sympathetic nerve activity in hour 4 compared with hour 6 after leptin treatment (P < 0.0001). Renal sympathetic activation to ICV leptin was less marked compared with the BAT SNA response (Figure 1B). Renal SNA increased from a baseline of 46 ± 7 to 87 ± 19 spikes per second (P = 0.02) in hour 6 after leptin treatment.

Experimental Protocol
Rats were anesthetized using intraperitoneal ketamine (91 mg/kg) and xylazine (9.1 mg/kg) and instrumented for SNA recordings as described above. Hemodynamic parameters (arterial pressure and heart rate) were monitored through a pressure transducer connected to the catheter inserted in the femoral artery using a pressure transducer connected to a computer. Anesthesia was maintained with α-chloralose (50 mg/kg per hour) via a catheter inserted in the femoral vein. The trachea was cannulated, and each rat was allowed to breathe spontaneously oxygen-enriched air. Rectal temperature was maintained at 37.5°C using a temperature-controlled surgical table and a lamp.

After surgery, rats were allowed to stabilize for 15 to 20 minutes. Baseline SNA for each bed, arterial pressure, and heart rate were then collected on 2 occasions during a 10-minute control period and averaged to obtain a single value for this control period. Each rat then received 1 injection of leptin ICV (10 μg in 4 μL) or unilaterally into the ARC (500 ng in 200 nL) or vehicle (saline, 4 μL ICV or 200 nL intra-ARC); there were 7 rats in each group. After treatment, arterial pressure, heart rate, and SNA measurements were made every 15 minutes for 6 hours.

At the end of the study, the injector was retracted and filled with Evans blue solution and the dye injected ICV (4 μL) or into the ARC (200 nL) to verify the injection site. The animals were euthanized with a lethal dose of ketamine/xylazine and the brain removed. The ICV cannulation was considered successful when the dye was present in the third ventricle. To verify the ARC cannulation, coronal sections (50 μm) were cut with a freezing Microm cryostat. The slides were mounted on slides and the location of the dye verified under microscope. The atlas of Paxinos and Watson was used to map the labeled microinjection site. Only animals that showed ARC-specific location of the injections (dye) were included in the present study. Seven rats, including 2 treated with leptin, were excluded from the studies for improper ARC cannulation.

Data Analysis
To ensure that electrical noise was excluded in the assessment of sympathetic outflow, each SNA recording was corrected for postmortem background activity. Because of the animal-to-animal variability in baseline SNA, the data for SNA are expressed as a percentage change from baseline. Results are presented as mean ± SEM. Data were analyzed using unpaired t test or repeated-measures ANOVA followed by Newman–Keuls posthoc test. A value of P < 0.05 was considered significant.

Results

Effects of ICV Administration of Leptin
ICV administration of leptin caused a significant increase in sympathetic nerve activities and arterial pressure. As shown in Figure 1A, there was a thermogenic BAT SNA response to leptin was robust (increasing from a baseline of 25 ± 3 to 84 ± 9 spikes per second in hour 6 after leptin treatment; P = 0.0001). Renal sympathetic activation to ICV leptin was less marked compared with the BAT SNA response (Figure 1B). Renal SNA increased from a baseline of 46 ± 7 to 87 ± 19 spikes per second (P = 0.02) in hour 6 after leptin treatment.

ICV administration of leptin raised mean arterial pressure from a baseline of 85 ± 4 to 103 ± 8 mm Hg (P = 0.03). The effect of ICV leptin on mean arterial pressure was significantly greater than the effect of ICV vehicle (Figure 2A). This elevation in mean arterial pressure after ICV leptin resulted from an increase in systolic (22 ± 6 mm Hg; P = 0.02 versus vehicle) and diastolic (11 ± 5 mm Hg; P = 0.04 versus vehicle) arterial pressure.

Although heart rate tended to increase after leptin treatment, this was not different from the effect of vehicle treatment (Figure 2B). ICV administration of vehicle caused no significant change in SNA or arterial pressure.
Sympathetic Nerve Responses to Intra-ARC Administration of Leptin

Administration of leptin directly into the ARC increased regional SNA (Figures 3 and 4). Intra-ARC leptin caused a robust BAT sympathoactivation (Figure 4A). The increase in BAT SNA after leptin treatment was slow and reaches a maximum of $254\%$ in hour 6 of treatment, increasing from $18$ to $65$ spikes per second ($P=0.0006$). As shown in Figure 4B, renal SNA was also responsive to microinjection of leptin into the ARC. As with the ICV injection, renal sympathoactivation to intra-ARC leptin was less pronounced, compared with the BAT SNA response, with a maximum increase of $111\%$ after 6 hours of treatment, increasing from $57\%$ to $115\%$ spikes per second ($P=0.001$). Intra-ARC administration of vehicle did not alter BAT or renal SNA (Figure 4).

Cardiovascular Responses to Intra-ARC Administration of Leptin

Arterial pressure was also responsive to the microinjection of leptin into the ARC (Figures 3 and 5A). Mean arterial pressure increased from a baseline of $82\pm3$ to $100\pm7$ mm Hg in hour 6 after leptin treatment ($P=0.02$). The rise in mean arterial pressure caused by intra-ARC leptin was much greater than the effect of vehicle treatment (Figure 5A). The increased mean arterial pressure after microinjection of leptin into the ARC reflected an elevation of both systolic ($18\pm6$ mm Hg; $P=0.03$ versus vehicle) and diastolic ($11\pm5$ mm Hg; $P=0.04$ versus vehicle) arterial pressure.

As with the ICV injection, intra-ARC treatments increased heart rate. However, this effect was not significantly different between the leptin and control groups (Figure 5B).

Discussion

In the present study, we investigated the sympathetic and cardiovascular effects of leptin signaling in the ARC of the hypothalamus. The main finding is that leptin action in the ARC increases sympathetic nerve outflow to thermogenic BAT, as well as the kidney. Furthermore, intra-ARC administration of leptin increased arterial pressure. The sympathetic and cardiovascular responses to intra-ARC leptin mimic those induced by ICV administration of this hormone. These data suggest that the ARC is an important site for the sympathetic and cardiovascular effects of leptin.

Early studies have demonstrated the importance of the ARC in transducing the leptin signaling into physiological responses. Indeed, local injection of leptin into the ARC caused a dose-dependent reduction in food intake and body weight. Conversely, lesioning the ARC blocks the appetite- and weight-reducing actions of leptin. Our finding of BAT sympathoactivation to intra-ARC leptin is in agreement with the previous finding showing that lesioning the ARC blunted the BAT sympathoactivation to systemic administration of leptin. Together, these data demonstrate that the ARC is a major site for the regulation of thermogenic sympathetic outflow by leptin.

The increase in SNA to tissues other than the BAT after microinjection of leptin into the ARC suggests that leptin signaling in this nucleus is also important for the control of nonthermogenic sympathetic tone. Indeed, microinjection of leptin into the ARC has been shown to increase lumbar SNA in rats. Furthermore, our present data demonstrate that renal SNA is also activated after local injection of leptin into this nucleus. Of importance, we found that intra-ARC leptin increased arterial pressure, which was comparable to the effect induced by ICV administration of this hormone. These
results suggest that the cardiovascular effects of leptin might be evoked by the action of leptin in the ARC.

The anatomic aspects of the neural connections between the ARC and BAT, as well as the kidneys, have been characterized previously using viral retrograde tracing techniques. Indeed, after injection of the pseudorabies virus into the BAT25,26 or kidneys,22,27 infected cells were found in several brain regions, including the ARC. Furthermore, Oldfield et al28 have shown that the infected neurons in the ARC, after the viral inoculation of BAT, express proopiomelanocortin and leptin receptors. Together these studies provide a neuroanatomical basis for the control of BAT and renal SNA by leptin action in the ARC. The difference in the magnitude of BAT versus renal sympathoactivation in response to leptin is consistent with the concept that sympathetic outflow to thermogenic and cardiovascular effectors is differentially regulated.29

Although in the present study the effect of microinjecting leptin into the ARC was compared with the effect of ICV administration of this hormone, the sympathetic and cardiovascular effects of activating the leptin receptor in other hypothalamic nuclei were not investigated. Previous studies have reported differential sympathetic and cardiovascular responses after leptin administration into the hypothalamic nuclei other than the ARC in rats. For instance, Marsh et al30 have reported that injection of leptin into the ventromedial hypothalamic nucleus increased renal SNA and arterial pressure, whereas its injection into the dorsomedial part of the hypothalamus increased only arterial pressure. Reports on the cardiovascular effects of leptin action in the paraventricular nucleus are controversial. Indeed, microinjection of leptin into this nucleus was found to cause no change in arterial pressure or renal SNA,10 to increase lumbar SNA but not
arterial pressure,

or to increase arterial pressure and sympathetic vasomotor tone. Several factors may account for such discrepancies in the reported sympathetic and cardiovascular responses induced by microinjection of leptin into the paraventricular nucleus including whether the injections were unilateral or bilateral, the amount of leptin injected, and differences in the exact location within the nucleus of the site of the injection.

In contrast to our findings, Satoh et al have shown that microinjection of leptin into the ARC caused no significant change in plasma catecholamines in rats. A leptin-induced increase in catecholamine secretion was found to be caused by the action of leptin in the ventromedial hypothalamus. It should be stressed, however, that plasma levels of catecholamine depend on several processes, including secretion, tissue clearance, and reuptake. Therefore, the sensitivity of plasma catecholamine in detecting changes in sympathetic activity is limited.

A major strength of the present study consists in the combination of site-specific injection of leptin into the ARC with simultaneous recording of hemodynamic parameters and SNA to 2 different beds. There are also limitations that need to be addressed. First, we used a dye to ascertain the specificity of the microinjection to the ARC. The reliability of the dye distribution as an indicator of leptin diffusion is not established. Nonetheless, this approach is commonly used to ensure the specificity of the microinjections in the brain. Second, the experiments were performed in the presence of anesthesia, which may have influenced the cardiovascular and sympathetic responses. For instance, a significant increase in heart rate was observed after ICV and intra-ARC treatments. The fact that bradycardia was evoked by both leptin and vehicle treatments suggest that it may be because of anesthesia. The increase in heart rate did not seem, however, to interfere with the arterial pressure and sympathetic nerve responses to leptin, because the bradycardia occurring in the vehicle-treated rats did not alter the baseline arterial pressure and sympathetic nerve activities. Furthermore, in a previous study we found that presence of anesthesia does not affect the sympathetic responses to different stimuli, including baroreflex activation and hemorrhage.

Third, we have assessed the short-term sympathetic and cardiovascular responses to leptin. Whether similar responses will be obtained with chronic administration of leptin remains to be determined. Fourth, the downstream mechanisms mediating the SNA and arterial pressure responses to leptin action in the ARC were not examined. We have shown previously the pivotal role of phosphoinositol 3-kinase in the renal SNA response to leptin. Microinjection of phosphoinositol 3-kinase inhibitors into the ARC before leptin should reveal whether this signaling pathway in the ARC mediates the sympathetic and cardiovascular responses to leptin.

**Perspectives**

Obesity is known to be associated with leptin resistance. Studies of 2 animal models of obesity (agouti and diet-induced obese mice) indicate that leptin resistance might not be uniform. In these models, leptin resistance appears to be selectively restricted to the metabolic (feeding- and weight-reducing) actions of leptin, whereas the cardiovascular sympathoexcitatory effects of leptin are preserved. Interestingly, the preservation of the regional SNA responses to leptin are not homogeneous but are instead specific to the kidney. Indeed, although renal sympathoactivation to leptin was preserved, the BAT and lumbar SNA responses to leptin were significantly attenuated in obese mice. This selectivity in leptin resistance in parallel with the hyperleptinemia that is commonly present in obesity might represent the link between the increase in fat mass and hypertension. However, the processes underlying the selectivity in leptin resistance remain unknown. Future studies are required to understand the mechanisms, within the ARC, that account for the differential resistance to leptin of the regional SNA that is associated with obesity.

**Acknowledgments**

We thank Drs Allyn L. Mark and Curt D. Sigmund for the critical reading of the article and helpful comments. We also thank Paul Casella for English-language editing and Vickie L. Akers for secretarial assistance.

**Source of Funding**

K.R. is supported by a Scientist Development Grant from the American Heart Association National Center (grant 0530274N).

**Disclosures**

None.

**References**


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Hypertension. 2007;49:647-652; originally published online December 26, 2006; doi: 10.1161/01.HYP.0000254827.59792.b2
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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