Interactions Between the Melanocortin System and Leptin in Control of Sympathetic Nerve Traffic


Abstract—Leptin plays an important role in regulation of body weight through regulation of food intake and sympathetically mediated thermogenesis. The hypothalamic melanocortin system, via activation of the melanocortin-4 receptor (MC4-R), decreases appetite and weight, but its effects on sympathetic nerve activity (SNA) are unknown. In addition, it is not known whether sympathoactivation to leptin is mediated by the melanocortin system. We tested the interactions between these systems in regulation of brown adipose tissue (BAT) and renal and lumbar SNA in anesthetized Sprague-Dawley rats. Intracerebroventricular administration of the MC4-R agonist MT-II (200 to 600 pmol) produced a dose-dependent sympathoexcitation affecting BAT and renal and lumbar beds. This response was completely blocked by the MC4-R antagonist SHU9119 (30 pmol ICV). Administration of leptin (1000 μg/kg IV) slowly increased BAT SNA (baseline, 41±6 spikes/s; 6 hours, 196±28 spikes/s; P =0.001) and renal SNA (baseline, 116±16 spikes/s; 6 hours, 169±26 spikes/s; P =0.014). Intracerebroventricular administration of SHU9119 did not inhibit leptin-induced BAT sympathoexcitation (baseline, 35±7 spikes/s; 6 hours, 158±34 spikes/s; P =0.71 versus leptin alone). However, renal sympathoexcitation to leptin was completely blocked by SHU9119 (baseline, 142±17 spikes/s; 6 hours, 146±25 spikes/s; P =0.007 versus leptin alone). This study demonstrates that the hypothalamic melanocortin system can act to increase sympathetic nerve traffic to thermogenic BAT and other tissues. Our data also suggest that leptin increases renal SNA through activation of hypothalamic melanocortin receptors. In contrast, sympathoactivation to thermogenic BAT by leptin appears to be independent of the melanocortin system.

(Hypertension. 1999;33[part II]:542-547.)

Key Words: autonomic nervous system ▪ renal nerves ▪ sympathetic nervous system ▪ brain ▪ obesity ▪ leptin

Recent studies of monogenic animal models of obesity have implicated several molecular mechanisms responsible for body weight control. These factors include leptin,1 neuropeptide Y (NPY),2,3 corticotrophin-releasing factor,4 and the melanocortin system.5,6 Abnormalities in these systems produce obesity through changes in appetite and food intake. In addition, leptin, neuropeptide Y, and corticotrophin-releasing factor have been shown to influence sympathetic nerve activity (SNA) to brown adipose tissue (BAT), thereby increasing thermogenesis.7–9 In the neural melanocortin system that controls body weight, α-melanocyte-stimulating hormone (α-MSH) derived from proopiomelanocortin (POMC) acts on melanocortin-4 receptors (MC4-Rs) to decrease appetite.5,6 Obesity in humans has recently been linked to the POMC and MC4-R genes.10–12 Melanocortin agonists have been shown to inhibit appetite, but the effects of the melanocortin system on SNA to thermogenic and nonthermogenic tissues are not known. One purpose of this study was to examine the effects of the melanocortin-3 and melanocortin-4 receptor (MC3/4-R) agonist MT-II—Ac-Nle4-c[Asp5,D-Phe7,Lys10]-α-MSH-(4–10)-NH3,13 on SNA to BAT, hind limb, and kidney. We tested the specificity of responses for the melanocortin system by using the MC3/4-R antagonist SHU9119—Ac-Nle4-c[Asp5,D-2’NaF7,Lys10]-α-MSH-(4–10)-NH3.13

Abnormalities in the melanocortin system are known to underlie obesity in the agouti murine obesity syndrome.5,6,14 These mice produce an endogenous antagonist of the hypothalamic MC4-R that blocks the suppression of feeding by α-MSH. Agouti mice are leptin resistant,15 leading to the suggestion that stimulation of the MC4-R by melanocortins is an essential mechanism in the actions of leptin. However, double-mutant leptin-deficient obese mice that also possess the agouti mutation are more obese than mice with leptin deficiency alone, suggesting that the melanocortin system does not play a role in the effects of leptin on body weight.16 The role of MC4-R in the sympathetic effects of leptin has not been reported. We examined the contribution of the hypothalamic melanocortin system in sympathoexcitation to leptin using third cerebral ventricle administration of the MC3/4-R antagonist SHU9119.6,13
Methods

General
All procedures were approved by the University of Iowa and Iowa City Veterans Affairs Animal Research committees. Experiments were performed in free-feeding 3-month-old male Sprague-Dawley rats (300 to 310 g) from Harlan Sprague Dawley Inc (Indianapolis, Ind). Unless otherwise indicated, all procedures were performed as previously reported.9

Procedures
Rats were anesthetized with pentobarbital (Nembutal, 50 mg/kg IP) and secured in a Kopf 900 stereotaxic instrument (David Kopf Instruments) as described previously.17 Briefly, a 23-gauge stainless steel guide cannula (16 mm in length) was lowered 10° from vertical into the third ventricle according to standard stereotaxic procedures. The coordinates with respect to bregma were 1.0 mm anteroposterior, 1.5 mm lateral from the midline, and −9.0 mm dorsalventral from the dura. Placements of the cannula in the third ventricle were considered patent by the acute drinking responses of any remaining nerve activity was used to estimate background noise. 

Design
Studies were performed at least 7 days after placement of the third ventricle catheter. Animals were allowed to stabilize for 45 minutes after placement of nerve electrodes. Baseline measurements of arterial pressure, heart rate, and SNA were made continuously for 30 minutes. After administration of experimental agents, hemodynamic and SNA measurements were made every 5 minutes for 360 minutes. Arterial blood glucose concentrations were measured at baseline and every 30 minutes thereafter. Terminally, rats were euthanized, and any remaining nerve activity was used to estimate background noise. Two protocols were followed.

Role of Melanocortin Receptors in Regulation of Sympathetic Nerve Traffic
Rats instrumented for measurement of BAT and renal SNA received intracerebroventricular injection of vehicle (phosphate-buffered saline; n = 12) or the MC3/4-R agonist MT-II (K, at MC4-R, 6.6 nmol/L) in doses of 200 (n = 11), 400 (n = 12), and 600 (n = 12) pmol. Other rats received the MC3/4-R antagonist SHU9119 (K, at MC4-R, 0.36 nmol/L) either alone (30 pmol; n = 4) or followed 10 minutes later by MT-II (600 pmol; n = 6). Rats instrumented for measurement of lumbar SNA received either intracerebroventricular vehicle (n = 10) or MT-II (600 pmol; n = 16).

Role of Melanocortin Receptors in Sympathoexcitation to Leptin
Rats instrumented for measurement of BAT and renal SNA received intracerebroventricular injection of vehicle (n = 11) or the MC3/4-R antagonist SHU9119 (30 pmol; n = 12). All rats then received murine leptin (1000 μg/kg IV over 3 hours; Amgen Inc) as a 500-μg/kg loading dose over 10 minutes followed by an infusion at 167 μg·kg⁻¹·h⁻¹. This regimen results in a rapid and sustained increase in plasma leptin concentrations to >100 ng/mL.9

Data Analysis
Results are expressed as mean ± SEM. Sympathetic nerve firing rate was corrected for background noise by subtracting postmortem measurements from the measurement obtained at each time point when alive. Values from the 3 separate baseline measurements did not differ significantly for any parameter and were therefore averaged for each animal. Because there is significant interindividual variation in baseline SNA, these data are also expressed as percentage change from baseline. Differences between active treated and
control rats were assessed by use of a repeated-measures ANOVA, with statistical testing by Scheffé’s F test. Statistical analysis was performed by use of StatView software for the Macintosh (version 4, Abacus Concepts Inc). We considered $P < 0.05$ to be statistically significant.

**Results**

**Sympathetic Effects of Melanocortin Receptor Stimulation**

Third cerebral ventricle administration of the MC3/4-R agonist MT-II produced a slow and dose-dependent increase in SNA to BAT, with a 252±53% increase at the highest dose ($P = 0.0001$ versus vehicle, ANOVA; the Table and Figures 1 and 2). MT-II also increased lumbar SNA ($P = 0.001$, ANOVA) and, to a lesser degree, renal SNA ($P = 0.04$, ANOVA; Table and Figure 1). Sympathoexcitation to MT-II was completely blocked by administration of the MC3/4-R antagonist SHU9119 ($P = 0.002$ versus MT-II, ANOVA; Table and Figure 1), which had no effects when given alone. Administration of MT-II or SHU9119 did not significantly alter arterial pressure, heart rate, or blood glucose compared with vehicle (Table).

**Role of Melanocortin Receptor in Sympathoexcitation to Leptin**

Intravenous administration of leptin slowly increased SNA to BAT (455±114%; $P = 0.001$, ANOVA) and kidney (50±21%; $P = 0.014$, ANOVA). The sympathoexcitatory effect of leptin on BAT SNA was not affected by pretreatment with the MC3/4-R antagonist SHU9119 ($P = 0.71$, ANOVA; Figure 3). In contrast, renal sympathoexcitation to leptin was abolished by pretreatment with SHU9119 ($P = 0.007$, ANOVA; Figure 3). Administration of leptin or SHU9119 did not alter arterial pressure, heart rate, or blood glucose compared with vehicle (Table).
The data from these studies demonstrate that stimulation of hypothalamic melanocortin receptors by third cerebral ventricular injection of an MC3/4-R agonist produces progressive sympathoexcitation to BAT, skeletal muscle, and kidney. This is not a nonspecific effect of the agonist because sympathoactivation can be completely blocked by coadministration of the MC3/4-R antagonist SHU9119. Melanocortins do not appear to be involved in leptin-induced thermogenic sympathoactivation because this effect of leptin was not affected by coadministration of the melanocortin receptor antagonist. In contrast, SHU9119 did block renal sympathoactivation to leptin. These results suggest that sympathoactivation caused by leptin has heterogeneous neural mechanisms, which only partly involve the melanocortin system.

The melanocortin system is now understood to comprise a series of peptides derived from POMC, including adrenocorticotrophin, endorphins, and MSH, that bind to at least 5 specific receptors. The agouti yellow obesity syndrome in mice is due to a gene mutation that produces overexpression of agouti protein, an endogenous melanocortin receptor antagonist. Agouti protein produces obesity through antagonism of MC4-R situated in the hypothalamic arcuate and paraventricular nuclei. Pharmacological stimulation of hypothalamic MC4-R suppresses feeding in hyperphagic lean and obese rats, whereas blockade of MC4-R by SHU9119 stimulates feeding. Furthermore, targeted disruption of the MC4-R receptor in mice reproduces the characteristic features of the agouti obesity syndrome.

Leptin decreases weight and adipose tissue mass through inhibition of food intake and stimulation of thermogenic metabolism. The increase in energy expenditure is probably mediated by sympathetically mediated thermogenesis because we and others have shown that leptin increases sympathetic outflow to thermogenic and nonthermogenic tissue. Melanocortin receptor agonists also decrease appetite and body mass and increase body temperature. The mechanisms underlying melanocortin-induced thermogenesis have not been explored before this study. Our finding that activation of the melanocortin system increases sympathetic nerve traffic to BAT, hind limb, and kidney suggests that melanocortins, like leptin, can stimulate sympathetically mediated thermogenesis.

The role of melanocortins in the metabolic and autonomic effects of leptin is controversial. Some lines of evidence suggest an interaction between leptin and melanocortins. First, leptin receptor mRNA is colocalized with POMC mRNA in arcuate-nucleus neurons. Second, leptin-deficient obese mice have reduced hypothalamic POMC mRNA, which can be normalized by leptin replacement. Third, leptin-induced anorexia, expression of BAT uncoupling protein (UCP-1), and weight loss can be prevented by pretreatment with SHU9119. Fourth, obese agouti mice are resistant to the weight-reducing effects of centrally administered leptin. On the other hand, double-mutant leptin-deficient obese mice that also possess the agouti mutation are more obese than mice with leptin deficiency alone. This would suggest that the melanocortin and leptin pathways have independent effects on body weight. No studies have examined the contribution of melanocortins to sympathoactivation caused by leptin, although there is 1 report that stimulation of BAT UCP-1 expression by intracerebroventricular leptin can be blocked by SHU9119. We have shown here that thermogenic sympathoactivation to leptin is not altered by pretreatment with the MC4-R antagonist SHU9119. Our data suggest that the melanocortin system does not play a role in leptin-induced sympathetically mediated thermogenesis. Given that NPY can modulate thermogenic sympathoactivation and that obesity caused by leptin...
Melanocortins and SNS

deficiency can be ameliorated by deletion of the NPY gene. leptin-induced BAT sympathoactivation may be modulated by hypothalamic NPY. In contrast, SHU9119 did block renal sympathoactivation to leptin, supporting a role for melanocortins in the effects of leptin on SNA to kidney. The discrepant effects of SHU9119 on BAT and renal SNA indicate divergent central pathways underlying leptin-induced sympathoactivation to BAT and kidney.

It is of interest that melanocortin receptor stimulation and leptin infusion did not alter arterial pressure or heart rate despite marked increases in renal, hind-limb, and adrenal SNA. The reason may be that the rats were anesthetized or that the degree or duration of sympathoexcitation was insufficient to acutely increase pressure. However, it is possible that the sympathoactivation represents increases mainly in nerve fibers that subserve thermogenesis through activation of uncoupling proteins, without altering vascular tone. Alternatively, the lack of a pressor effect may indicate other actions that oppose sympathetically mediated actions on the heart and blood vessels. These could include activation of vagal tone or neurally mediated vasodilator mechanisms. Given the absence of a change in heart rate and arterial pressure, is the sympathoactivation likely to be of physiological significance? In addition to its important role in short-term cardiovascular regulation, the sympathetic nervous system also stimulates renal tubular sodium excretion and vascular smooth muscle growth. Thus, increases in SNA may be functionally and pathophysiologically relevant in the absence of short-term changes in arterial pressure.

Several potential limitations of this study need to be addressed. First, rats were anesthetized, and it could be argued that different results would have been obtained in conscious rats. However, we have previously demonstrated that the anesthesia regimen used here does not alter efferent renal and lumbar sympathetic responses to baroreflex stimuli or hemorrhage. Second, it is possible that the lack of effect of SHU9119 on leptin-induced BAT sympathoactivation was due to an inadequate dose of the MC3/4-R antagonist. However, this dose was sufficient to block the effect of an MC3/4-R agonist on sympathetic nerve traffic and to block the effect of leptin on renal sympathetic nerve traffic. Third, our study does not delineate the relative contributions of increased thermogenesis and decreased appetite in the weight-reducing effect of melanocortin system. Studies in pair-fed animals are required to do this. Fourth, our study does not address whether the melanocortin system plays a physiological role in regulation of sympathetic nerve traffic. Proof of such a role would require examination of the effects of MC3/4-R blockade on sympathetic responses to stimuli such as hypotension, feeding, or hypothermia. Finally, our conclusions on the role of the melanocortin system in rats cannot be directly extrapolated to humans.

In conclusion, we have demonstrated that stimulation of central nervous system melanocortin receptors increases sympathetic nerve traffic to thermogenic and nonthermogenic tissues. This action may promote weight loss by increasing thermogenic metabolism in addition to the well-known inhibitory effects of melanocortins on appetite. It is also possible that the effects of MC3/4-R stimulation on SNA to nonthermogenic tissues may have deleterious long-term cardiovascular effects. We have also shown that leptin-induced BAT sympathoactivation is not dependent on the melanocortin system. However, the effects of leptin on renal SNA are prevented by MC3/4-R blockade. These data suggest that sympathoactivation caused by leptin has heterogeneous neural mechanisms, which only partly involve the melanocortin system.

Acknowledgments
Research was supported by grants HL-14388, HL-44546 and HL-43514 from the National Heart, Lung, and Blood Institute; by grant DK-25295 from the National Institute of Diabetes, Digestive, and Kidney Diseases; by grant NS-38846 from the National Institute of Neurological Disorders and Stroke; and by funds from the Department of Veterans Affairs and the Juvenile Diabetes Foundation. Dr Haynes is the recipient of a PhRMAF Faculty Development Award.

References
15. Halaz JL, Boozer C, Blair-West J, Fidahusein N, Denton DA, Friedman JM. Physiological response to long-term peripheral and central leptin...


Interactions Between the Melanocortin System and Leptin in Control of Sympathetic Nerve Traffic


Hypertension. 1999;33:542-547
doi: 10.1161/01.HYP.33.1.542

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
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:
http://hyper.ahajournals.org/content/33/1/542

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at: http://hyper.ahajournals.org/subscriptions/