# Receptor-mediated Regional Sympathetic Nerve Activation by Leptin

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## Abstract

**Leptin is a peptide hormone produced by adipose tissue which acts centrally to decrease appetite and increase energy expenditure. Although leptin increases norepinephrine turnover in thermogenic tissues, the effects of leptin on directly measured sympathetic nerve activity to thermogenic and other tissues are not known. We examined the effects of intravenous leptin and vehicle on sympathetic nerve activity to brown adipose tissue, kidney, hindlimb, and adrenal gland in anesthetized Sprague-Dawley rats. Intravenous infusion of mouse leptin over 3 h (total dose 10–1,000 μg/kg) increased plasma concentrations of immunoreactive murine leptin up to 50-fold. Leptin slowly increased sympathetic nerve activity to brown adipose tissue (+286±64% at 1,000 μg/kg; $P = 0.002$). Surprisingly, leptin infusion also produced gradual increases in renal sympathetic nerve activity (+228±63% at 1,000 μg/kg; $P = 0.0008$).The effect of leptin on sympathetic nerve activity was dose dependent, with a threshold dose of 100 μg/kg. Leptin also increased sympathetic nerve activity to the hindlimb (+287±60%) and adrenal gland (388±171%). Despite the increase in overall sympathetic nerve activity, leptin did not increase arterial pressure or heart rate. Leptin did not change plasma glucose and insulin concentrations. Infusion of vehicle did not alter sympathetic nerve activity. Obese Zucker rats, known to possess a mutation in the gene for the leptin receptor, were resistant to the sympathoexcitatory effects of leptin, despite higher achieved plasma leptin concentrations. These data demonstrate that leptin increases thermogenic sympathetic nerve activity and reveal an unexpected stimulatory effect of leptin on overall sympathetic nerve traffic. (*J. Clin. Invest.* 1997. 100:270–278.) Key words: autonomic nervous system • renal • neural • thermogenic tissues • obesity**

## Introduction

Recent studies of monogenic animal models of obesity have implicated several novel molecular mechanisms responsible for body weight control. One important hormonal factor is the protein leptin, which was isolated and synthesized following the positional cloning of the gene responsible for obesity in the *ob/ob* mouse strain (1). Leptin is synthesized and secreted by adipose tissue and circulates to the brain where it binds to at least one receptor (2). Leptin acts to decrease weight and adipose tissue mass through decreases in appetite and food intake (3–5). In addition, leptin-treated mice lose more weight than pair-fed vehicle-treated animals, implying that leptin also increases energy expenditure (4). Furthermore, leptin-treated animals have higher core temperatures and metabolic rates than controls (3). Leptin has been shown to increase norepinephrine turnover, in interscapular brown adipose tissue (BAT),[1] suggesting increased sympathetic outflow to this thermogenic organ (6). However, leptin's role in increasing sympathetic outflow to BAT has not been confirmed by direct measurements of sympathetic nerve activity (SNA). In addition, it is not known whether leptin selectively alters sympathetic activity to BAT alone or has a more generalized sympathoexcitatory effect.

In this study, we assessed the effect of intravenous leptin on directly measured SNA to BAT, kidney, hindlimb, and adrenal gland in lean Sprague-Dawley rats. Several doses of leptin were used, and plasma leptin concentrations were measured to delineate a concentration–response relationship. Obese Zucker rats, known to possess a mutation in the gene for the leptin receptor (7, 8), were also studied to test whether the sympathetic effects of leptin were mediated through the leptin receptor.

## Methods

### *Animals and drugs*

Experiments were performed in 3-mo-old male Sprague-Dawley, and lean and obese Zucker (*fa*) rats from Harlan Sprague-Dawley (Indianapolis, IN). Rats were allowed access to a standard diet containing 4% fat (Teklad Premier Laboratory Diets, Madison, WI) until anesthesia was induced. All procedures were approved by the University of Iowa and Iowa City Veterans Affairs Animal Research Committees. Recombinant murine leptin was kindly provided by Amgen Biologicals (Thousand Oaks, CA). This batch of murine leptin from Amgen was biologically active in Sprague-Dawley rats, with weight decreasing by 15±3 g during 5 d of subcutaneous infusion of leptin by osmotic minipump at doses between 0.1 and 1 μg/h (mean daily dose = 44 μg/kg per d; $n = 4$), as compared to an increase in weight of 20±3 g in saline-treated animals ($n = 5$; $P < 0.001$ vs. leptin).

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*Received for publication 27 February 1997 and accepted in revised form 21 April 1997.*

The Journal of Clinical Investigation
Volume 100, Number 2, July 1997, 270–278

1. *Abbreviations used in this paper:* A-SNA, adrenal sympathetic nerve activity; BAT, brown adipose tissue; BAT-SNA, brown adipose tissue sympathetic nerve activity; HR, heart rate; L-SNA, lumbar sympathetic nerve activity; R-SNA, renal sympathetic nerve activity; SNA, sympathetic nerve activity.

### Procedures

*General.* Anesthesia was induced using intraperitoneal methohexital sodium (50 mg/kg) and a catheter inserted into the femoral vein for maintenance of anesthesia with intravenous chloralose (50 mg/kg initially then 25 mg/kg per h). To prevent upper respiratory tract obstruction and hypoxia, the trachea was cannulated for spontaneous respiration of $O_2$-enriched air. Sodium bicarbonate (0.1 mmol) was administered intravenously every 60 min. Rectal temperature was monitored continuously and maintained at 37.5°C using a heated surgical table and lamps. A catheter was inserted into the femoral artery for continuous arterial pressure measurement.

*SNA recordings.* SNA to BAT, kidney, hindlimb, and adrenal gland was measured by multifiber recording. The left kidney was exposed retroperitoneally through a left flank incision. Using a dissecting microscope, a nerve branch to the left kidney was carefully dissected and placed on a bipolar platinum-iridium electrode (Cooner Wire Co., Chatsworth, CA) (9). In some studies, this nerve was transected distal to the recording site to exclude renal afferent signals. A similar surgical approach was used to identify and isolate a sympathetic nerve to the adrenal gland (9). Sympathetic nerves to the hindlimb were identified from the L2 and L3 roots of the lumbar plexus, using an anterior abdominal incision (9). Interscapular BAT was exposed through a nape incision. Using a dissecting microscope, sympathetic nerve fibers innervating BAT were identified, cut distally, and attached to a bipolar platinum-iridium electrode. Electrodes were fixed in place using silicon gel (Sil-Gel 604; Wacker-Chemie, Munich, Germany) after an optimum recording of renal sympathetic nerve activity (R-SNA) was obtained.

Nerve electrodes were connected to a high impedance probe (HIP-511; Grass Instrument Co., Quincy, MA), amplified by $10^5$, and filtered at low- and high-frequency cutoffs of 100 and 1,000 Hz with a nerve traffic analysis system (model 662-C; University of Iowa Bioengineering, Iowa City, IA). The filtered, amplified nerve signal was routed: (*a*) to an oscilloscope (model 54501A; Hewlett-Packard Co., Palo Alto, CA) for monitoring; (*b*) to a MacLab analogue-digital converter (AD Instruments Castle Hill, New South Wales, Australia) for permanent recording of the neurogram on a Macintosh 9500 computer; and (*c*) to a nerve traffic analyzer (model 706C; University of Iowa Bioengineering) which counts action potentials above a threshold voltage level set just above background.

### Design

*General.* Animals were allowed to stabilize for 45 min after placement of nerve electrodes. Baseline measurements of arterial pressure, heart rate (HR), R-SNA, and BAT sympathetic nerve activities (BAT-SNA) were made for 5 min on three occasions. An arterial blood sample was obtained for measurement of blood glucose concentration. After infusion of leptin or vehicle had started, measurements of arterial pressure, HR, and R- and BAT-SNA were made every 5 min. Blood glucose concentrations were measured every 30 min. After 3 h, arterial blood was obtained for assay of rat insulin and mouse leptin concentrations. The ganglion blocker chlorisondamine was administered intravenously (30 mg/kg) to assess efferent SNA to BAT and kidney. Animals were then killed by methohexital overdose (80 mg/kg intravenously). Nerve activity which remained after methohexital provided an estimate of background noise, which was used to calculate specific nerve activity (see *Data analysis*).

*R- and BAT-SNA.* Animals were instrumented for measurement of arterial pressure and SNA to the kidney and BAT. Five separate groups of rats ($n = 8$ in each) received one of four doses of leptin or 0.9% saline intravenously over 3 h. Leptin was dissolved in phosphate-buffered 0.9% saline, which was used as the control infusion. The doses of leptin were 10, 100, 500, and 1,000 μg/kg, with 50% of the total dose being given as a loading dose over 30 s (i.e., 5 μg/kg loading dose in the 10 μg/kg group) and the remainder of the dose infused continuously at 50 μl/min over 3 h (i.e., 1.67 μg/kg per h in the 10 μg group). Because mouse leptin is manufactured by expression of recombinant DNA in *Escherichia coli*, it contains a small amount (< 4%) of bacterial lipopolysaccharide. To exclude an effect of lipopolysaccharide on hemodynamic and sympathetic parameters, a separate series of control experiments was performed in six rats by infusing lipopolysaccharide in a dose chosen to represent the maximum amount which could have been infused at the highest dose of leptin (total dose of lipopolysaccharide = 40 μg/kg).

*Lumbar sympathetic nerve activity (L-SNA).* Animals were instrumented for measurement of arterial pressure and L-SNA. Separate groups of rats received leptin ($n = 8$) or 0.9% saline ($n = 6$) intravenously over 3 h. The total dose of leptin was 1,000 μg/kg, using the same regimen as in the initial study.

*Adrenal sympathetic nerve activity (A-SNA).* Animals were instrumented for measurement of arterial pressure and SNA to the adrenal

*Table I. Hemodynamic, BAT- and R-SNA, and Endocrine Data Obtained From Sprague-Dawley Rats at Baseline and in the Third Hour of Experimental Infusion of Leptin (10–1,000 μg/kg), Saline, or Lipopolysaccharide*

| | | SBP | DBP | HR | BAT-SNA | R-SNA | Glucose | Insulin | Leptin |
|---|---|---|---|---|---|---|---|---|---|
| | | *mmHg* | *mmHg* | *bpm* | *sp/s* | *sp/s* | *mg/dl* | *ng/ml* | *ng/ml* |
| Saline | Basal | 154±7 | 86±4 | 385±12 | 85±11 | 93±6 | 60±4 | | |
| | 3 h | 149±9 | 89±8 | 417±7* | 79±17 | 117±23 | 72±3* | 2.2±0.2 | 3.1±0.3 |
| 0.4% LPS | Basal | 154±11 | 88±10 | 357±13 | 77±11 | 105±9 | 61±4 | | |
| | 3 h | 136±7* | 81±9 | 371±10* | 85±25 | 96±18 | 60±3 | 1.4±0.2 | 5.5±0.6 |
| Leptin 10 μg/kg | Basal | 165±6 | 98±5 | 404±9 | 64±8 | 85±7 | 60±3 | | |
| | 3 h | 160±8 | 95±9 | 422±11* | 104±13* | 128±18* | 70±4* | 2.2±0.3 | 2.8±0.3 |
| Leptin 100 μg/kg | Basal | 147±10 | 80±8 | 382±9 | 74±9 | 78±7 | 62±5 | | |
| | 3 h | 149±8 | 84±8 | 409±9* | 112±21 | 132±17* | 76±4* | 1.8±0.1 | 5.0±1.3 |
| Leptin 500 μg/kg | Basal | 178±5 | 111±6 | 378±14 | 42±5 | 86±17 | 56±5 | | |
| | 3 h | 163±9 | 97±10 | 405±9 | 143±54* | 201±27* | 87±8* | 2.8±0.6 | 85±27 |
| Leptin 1,000 μg/kg | Basal | 151±6 | 89±3 | 355±12 | 48±11 | 77±8 | 62±4 | | |
| | 3 h | 149±6 | 87±6 | 408±9* | 154±25* | 225±28* | 77±4* | 1.7±0.2 | 149±92 |

*Significantly different ($P < 0.05$) from baseline. *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *bpm*, beats per minute; *sp/s*, spikes per second.

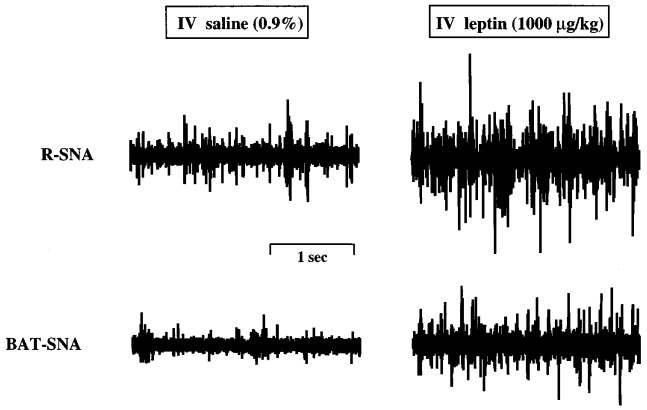



*Figure 1.* Examples of SNA to the kidney and interscapular BAT before and after administration of leptin (1,000 μg/kg over 3 h) in a Sprague-Dawley rat.

gland. Separate groups of rats received leptin ($n$ = 4) or 0.9% saline ($n$ = 4) intravenously over 3 h. The total dose of leptin was 1,000 μg/kg, using the same regimen as used previously.

*Sympathetic effects of leptin in Zucker lean and obese rats.* Animals were instrumented for measurement of arterial pressure and SNA to the kidney and BAT. Leptin was administered intravenously (1,000 μg/kg) to lean ($n$ = 6) and obese Zucker rats ($n$ = 8) using the same regimen as used previously. Separate groups of lean ($n$ = 6) and obese Zucker rats ($n$ = 5) received 0.9% saline. Plasma samples for assay of leptin concentrations were obtained at baseline, and at 30, 60, 120, and 180 min after starting leptin infusion.

### *Analytical*

Blood glucose concentrations were measured using a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin concentrations were measured by RIA, using a kit purchased from Linco Inc. (St. Charles, MO). Interassay coefficient of variation in our laboratory is 2% at 0.5 ng/ml and sensitivity is 0.1 ng/ml. Plasma leptin concentrations were measured as mouse leptin using a kit purchased from Linco Inc. Interassay coefficient of variation in our laboratory is 2.7% at 1.18 ng/ml and the sensitivity is 0.2 ng/ml.

### *Data analysis*

Results are expressed as mean±SEM. SNA measurements were corrected for background noise by subtracting postmortem measurements from the measurement obtained at each time point when alive. Values from the three separate baseline measurements did not differ significantly for any parameter and were therefore averaged for each animal. Data collected in the final hour of leptin infusion were averaged. In view of the interindividual variability of resting SNA, percentage change from baseline was calculated for SNA. Plasma leptin concentrations were not normally distributed and were therefore log transformed before statistical analysis. Baseline values in the control and leptin-treated rats were compared by Student's unpaired $t$ test and changes from baseline by Student's paired $t$ test. Differences between leptin-treated and control rats were assessed using a repeated measures ANOVA, with statistical testing by Scheffe's F-test. Dose-dependency was tested by a factorial ANOVA using linear trend testing. Statistical analysis was performed using StatView software for the Macintosh (v. 4; Abacus Concepts Inc., Berkeley, CA). $P < 0.05$ was considered statistically significant.

## Results

### *Hemodynamics*

There was no significant difference between rats treated with saline and leptin in baseline systolic or diastolic arterial pressure or HR (see Tables I–III). Arterial pressure did not change significantly in any group after leptin or saline administration. In all groups, there was a tendency for HR to increase during the study, but the magnitude of the increase did not differ significantly between leptin and control groups. Infusion of lipopolysaccharide did not alter diastolic arterial pressure or HR,

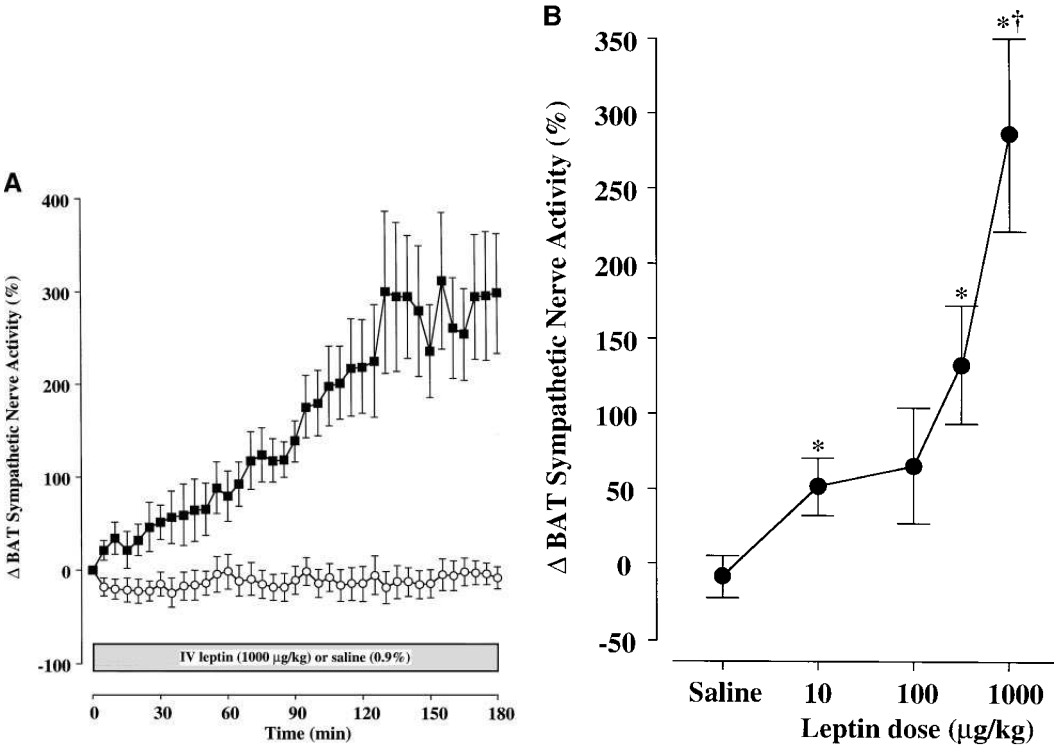



*Figure 2.* (*A*) Percent change from baseline in SNA to BAT during infusion of saline (0.9%; ○) and leptin (1,000 μg/kg; ■). Infusion of leptin produced a slow onset and substantial increase in SNA to BAT. Values are mean±SE. (*B*) Dose–response curve to show percent change from baseline in SNA to BAT in the last hour of infusion of 0.9% saline and four doses of leptin. *$P < 0.05$ vs. baseline on Student's $t$ test. †$P < 0.05$ vs. saline on ANOVA. Values are mean±SE.

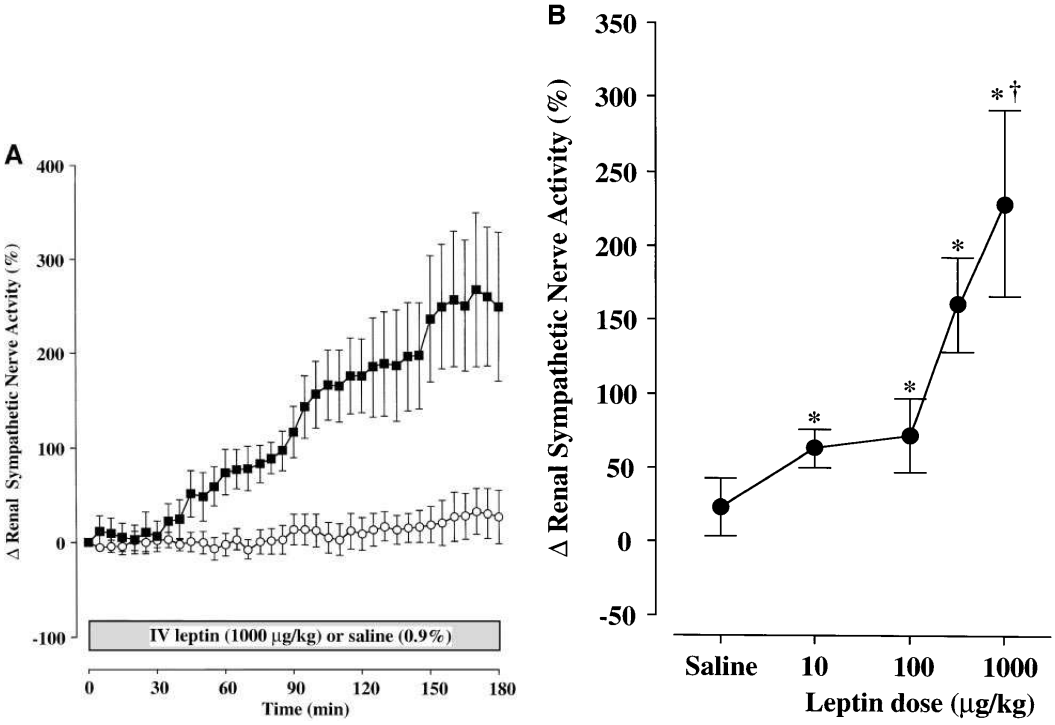



*Figure 3.* (*A*) Percent change from baseline in R-SNA during infusion of saline (0.9%; ○) and leptin (1,000 μg/kg; ■). Infusion of leptin produced a slow onset and substantial increase in SNA to kidney. Values are mean±SE. (*B*) Dose–response curve to show percent change from baseline in SNA to kidney in the last hour of infusion of 0.9% saline and four doses of leptin. $*P < 0.05$ vs. baseline on Student's *t* test. $^{\dagger}P < 0.05$ vs. saline on ANOVA. Values are mean±SE.

but there was a significant decrease in systolic arterial pressure (Table I).

*Sympathetic effects of leptin*

*R- and BAT-SNA.* Intravenous administration of leptin increased SNA to BAT, with a 286±64% increase in the third hour after the 1,000 μg/kg dose ($P = 0.002$ vs. control; Figs. 1 and 2). This effect was slow in onset, taking ~ 2 h to reach maximum (Fig. 2 *A*). Lower doses of leptin produced more modest increases in SNA to BAT (Table I; Fig. 2 *B*), which were significantly different from baseline, but not from saline control. The effect of leptin on SNA to BAT was significantly dose-dependent on linear trend testing ($P = 0.0001$; Fig. 2 *B*). Leptin also produced a slow-onset increase in R-SNA, with a 228±63% increase in the third hour at the highest dose ($P = 0.0008$; Figs. 1 and 3 *A*). Again, there was significant dose-dependency demonstrated on linear trend testing ($P = 0.0006$; Table I; Fig. 3 *B*). Ganglion blockade with chlorisondamine 3 h after administration of leptin markedly decreased SNA to both BAT (by 88±5%) and kidney (by 94±1%). In three animals, renal sympathoactivation to leptin was not altered by transection of the renal nerve distal to the recording site, with a mean increase in R-SNA of 329±29% in the third hour of infusion. SNA to BAT and kidney did not change significantly during administration of saline (Figs. 2 and 3). Infusion of lipopolysaccharide did not significantly alter SNA to BAT (+7±25%

*Table II. Hemodynamic, L- and A-SNA, and Endocrine Data Obtained from Sprague-Dawley Rats at Baseline and in the Third Hour of Experimental Infusion of Leptin (1,000 μg/kg) or Saline*

| | | SBP | DBP | HR | L-SNA | A-SNA | Glucose |
|---|---|---|---|---|---|---|---|
| | | *mmHg* | *mmHg* | *bpm* | *sp/s* | *sp/s* | *mg/dl* |
| L-SNA group | | | | | | | |
| Saline | Basal | 178±10 | 111±9 | 419±16 | 102±21 | | 62±6 |
| | 3 h | 176±5 | 107±6 | 407±11 | 143±41 | | 67±6 |
| Leptin 1,000 μg/kg | Basal | 183±7 | 112±6 | 400±17 | 48±13 | | 68±3 |
| | 3 h | 174±7 | 103±4 | 404±14 | 149±28* | | 71±4 |
| A-SNA group | | | | | | | |
| Saline | Basal | 195±11 | 122±11 | 351±17 | | 67±12 | 59±3 |
| | 3 h | 176±10 | 98±4 | 363±14 | | 87±18 | 63±3 |
| Leptin 1,000 μg/kg | Basal | 203±6 | 110±2 | 388±19 | | 32±6 | 55±4 |
| | 3 h | 185±13 | 99±8 | 413±10 | | 100±28* | 68±5* |

*Significantly different ($P < 0.05$) from baseline. *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *bpm*, beats per minute; *sp/s*, spikes per second.

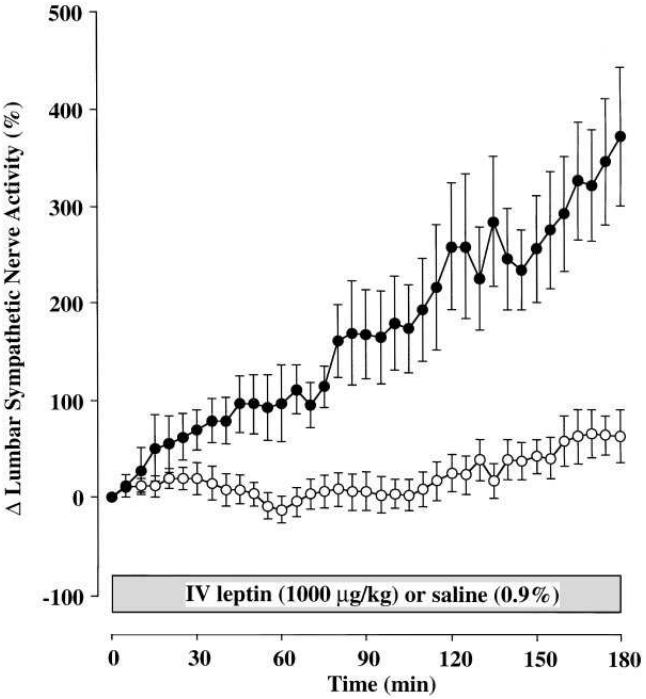



*Figure 4.* Percent change from baseline in L-SNA to hindlimb during infusion of saline (0.9%; ○) and leptin (1,000 μg/kg; ●). Infusion of leptin produced a slow onset and substantial increase in L-SNA. Values are mean±SE.

in the third hour) or the kidney (−9±20% in the third hour) (Table I).

*L-SNA.* Intravenous administration of leptin (1,000 μg/kg) increased L-SNA to the hindlimb, with a 287±60% increase from baseline in the third hour ($P = 0.0016$ vs. saline control; Table II; Fig. 4). As with R- and BAT-SNA, this effect was slow in onset, taking ~ 2 h to reach maximum (Fig. 4). L-SNA did not change significantly during saline infusion (Fig. 4).

*A-SNA.* Intravenous administration of leptin (1,000 μg/kg) slowly increased SNA to the adrenal gland, with a 388±171% increase from baseline in the third hour ($P = 0.04$ vs. saline control; Table II). A-SNA did not change significantly during saline infusion (+34±26% in the third hour; Table II).

*Effects of leptin in Zucker lean and obese rats.* Body mass was higher in obese (641±28 g) than lean Zucker rats (453±11 g). Obese rats also had significantly higher systolic arterial pressure and R-SNA ($P = 0.01$; Table III). In lean Zucker rats, intravenous infusion of leptin (1,000 μg/kg) caused significant increases in sympathetic nerve traffic to both kidney (+90± 27% in the third hour; $P = 0.005$ vs. saline) and BAT (+293± 40% in the third hour; $P = 0.0001$ vs. saline; Figs. 5 and 6). In contrast, obese Zucker rats exhibited markedly blunted renal (+1±13% in the third hour; $P = 0.9$ vs. saline; $P = 0.01$ vs. lean Zucker rats) and BAT-SNA responses to leptin (+79± 27% in the third hour; $P = 0.1$ vs. saline; $P = 0.001$ vs. lean Zucker rats; Figs. 5 and 6).

*Insulin, glucose, and leptin assays*

Blood glucose concentrations increased modestly after both saline and leptin administration (Tables I–III). Plasma insulin concentrations were no different between saline-, lipopolysaccharide-, and leptin-treated animals (Tables I–III). Circulating concentrations of immunoreactive murine leptin after 3 h of infusion increased dose-dependently; this achieved statistical significance in the 500 ($P = 0.0001$ vs. saline) and 1,000 μg/kg groups ($P = 0.0001$ vs. saline; Table I; Fig. 7). Plasma murine leptin concentrations were significantly elevated from baseline 30 min after starting the 1,000 μg/kg infusion regimen of leptin in both lean and obese Zucker rats ($P = 0.0001$; Fig. 8). Murine leptin concentrations tended to increase further over the next 150 min of maintenance infusion, but were not significantly different from the 30-min values in either obese or lean Zucker rats (Fig. 8). Plasma murine leptin concentrations during infusion of leptin were significantly greater in obese Zucker rats than in lean animals ($P = 0.03$ on ANOVA).

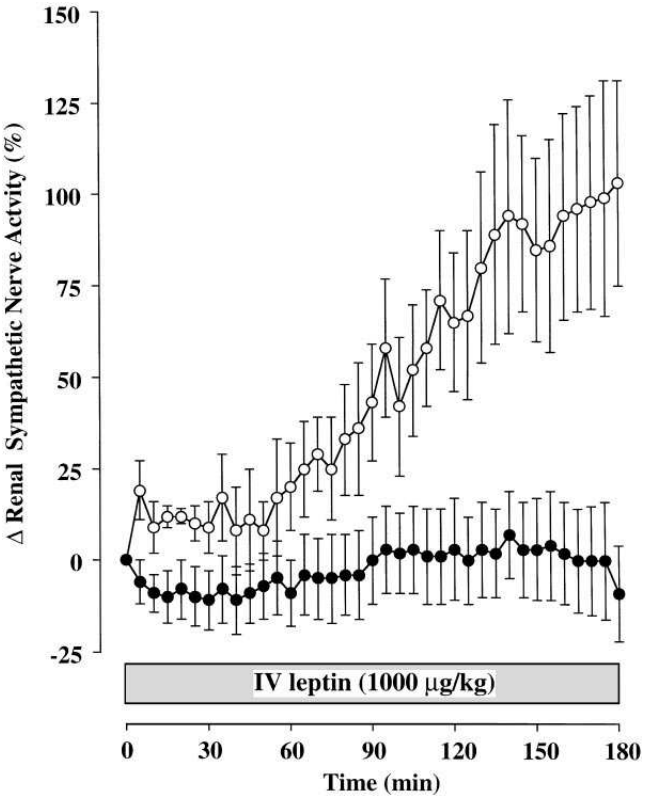
## Discussion

Our data show that infusion of murine leptin increases SNA to BAT, kidney, hindlimb, and the adrenal gland in lean Sprague-Dawley rats. These sympathetic actions of leptin are dose dependent, although they do not closely track plasma leptin concentrations, and are slow in onset. The effect on SNA to BAT

*Table III. Hemodynamic and BAT- and R-SNA Data Obtained from Lean and Obese Zucker Rats at Baseline and in the Third Hour of Experimental Infusion of Leptin (10–1,000 μg/kg) or Saline*

| | | SBP | DBP | HR | BAT-SNA | R-SNA | Glucose |
|---|---|---|---|---|---|---|---|
| | | *mmHg* | *mmHg* | *bpm* | *sp/s* | *sp/s* | *mg/dl* |
| Zucker lean rats | | | | | | | |
| Saline | Basal | 160±5 | 92±5 | 365±12 | 56±7 | 87±15 | 55±2 |
| | 3 h | 154±3 | 80±3 | 378±15 | 59±14 | 70±14 | 54±4 |
| Leptin 1,000 μg/kg | Basal | 138±3 | 73±3 | 387±16 | 54±3 | 149±33 | 62±3 |
| | 3 h | 148±3 | 77±4 | 469±10 | 213±29* | 267±60* | 78±5* |
| Zucker obese rats | | | | | | | |
| Saline | Basal | 173±10 | 112±5 | 374±8 | 87±7 | 244±65 | 65±3 |
| | 3 h | 171±11 | 102±5 | 372±21 | 65±11 | 283±105 | 76±7* |
| Leptin 1,000 μg/kg | Basal | 167±7 | 84±7 | 413±13 | 77±9 | 251±35 | 72±3 |
| | 3 h | 170±8 | 78±4 | 417±11 | 124±13 | 266±56 | 69±3 |

*Significantly different ($P < 0.05$) from baseline. *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *bpm*, beats per minute; *sp/s*, spikes per second.




is consistent with a report that leptin increases norepinephrine turnover in this tissue (6). Because sympathetic activation of BAT would be expected to enhance thermogenesis, our data support the concept that leptin regulation of body fat extends beyond control of food intake alone. The increase in SNA to other tissues after leptin infusion was unexpected and has not been reported previously. Sympathoactivation to leptin was absent in obese Zucker rats, suggesting that this effect of leptin is mediated by the intact leptin receptor.

There are several potential limitations of this study which need to be addressed. First, we used mouse leptin in the rat and the effects of rat leptin may be different. However, we have shown that murine leptin given to Sprague-Dawley rats causes weight loss, so it is presumably biologically active. Second, animals were anesthetized and it could be argued that different results would have been obtained in conscious rats. However, we have demonstrated previously that the anesthesia regimen used here does not alter efferent renal and lumbar sympathetic responses to baroreflex stimuli or hemorrhage (10). Third, our technique does not identify precisely whether recordings are obtained from afferent or efferent sympathetic nerve fibers.

*Figure 5.* Percent change from baseline in R-SNA during infusion of leptin (1,000 μg/kg) in lean (○) and obese Zucker rats (●). Infusion of leptin increased R-SNA only in lean Zucker rats. Values are mean±SE.

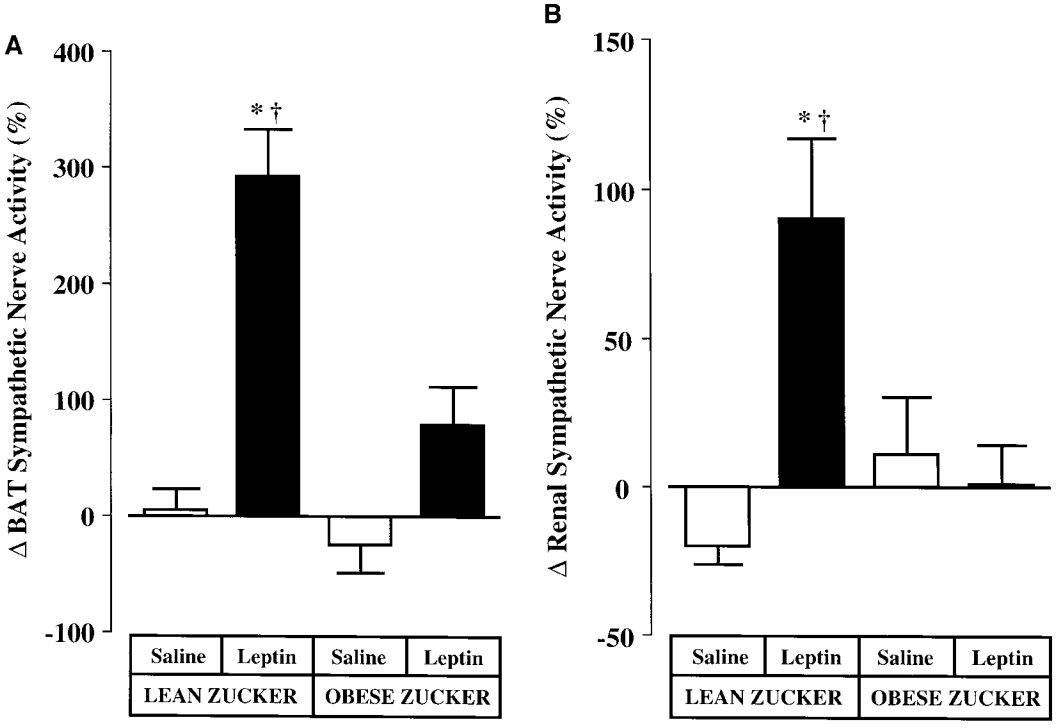



*Figure 6.* (*A*) Percent change from baseline in SNA to BAT during infusion of saline (0.9%; *open columns*) and leptin (1,000 μg/kg; *solid columns*) in Zucker lean and obese rats. Results represent mean change in the third hour of infusion. Infusion of leptin increased SNA to BAT only in lean Zucker rats. $^{*}P < 0.05$ vs. baseline on Student's *t* test. $^{\dagger}P < 0.05$ vs. obese rats on Student's *t* test. Values are mean±SE. (*B*) Percent change from baseline in R-SNA during infusion of saline (0.9%; *open columns*) and leptin (1,000 μg/kg; *solid columns*) in Zucker lean and obese rats. Results represent mean of recordings taken in the third hour of infusion. Infusion of leptin increased SNA to kidney only in lean Zucker rats. $^{*}P < 0.05$ vs. baseline on Student's *t* test. $^{\dagger}P < 0.05$ vs. obese rats on Student's *t* test. Values are mean±SE.

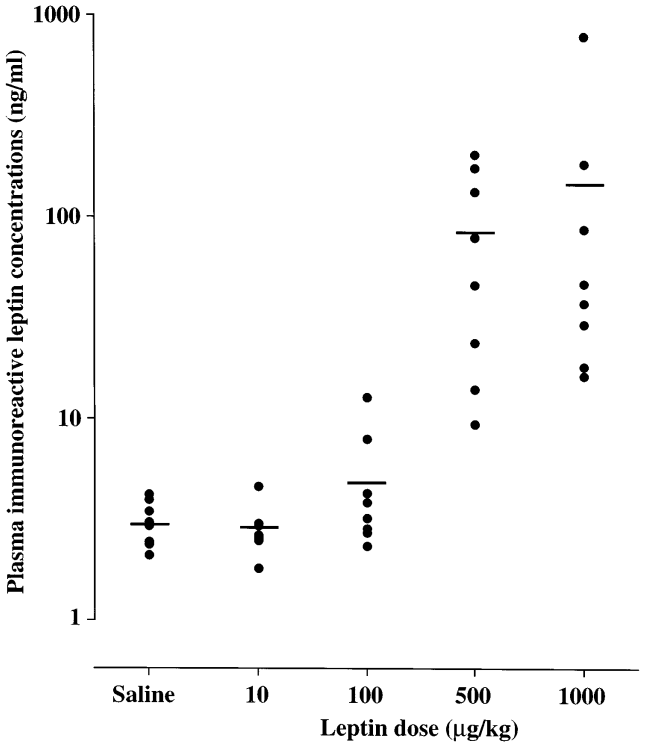



*Figure 7.* Individual values for plasma immunoreactive mouse leptin concentrations in rats infused with saline and the four different doses of mouse leptin. Concentrations are shown on a log scale because of the nonparametric nature of the data. The solid bar indicates mean values for each group.

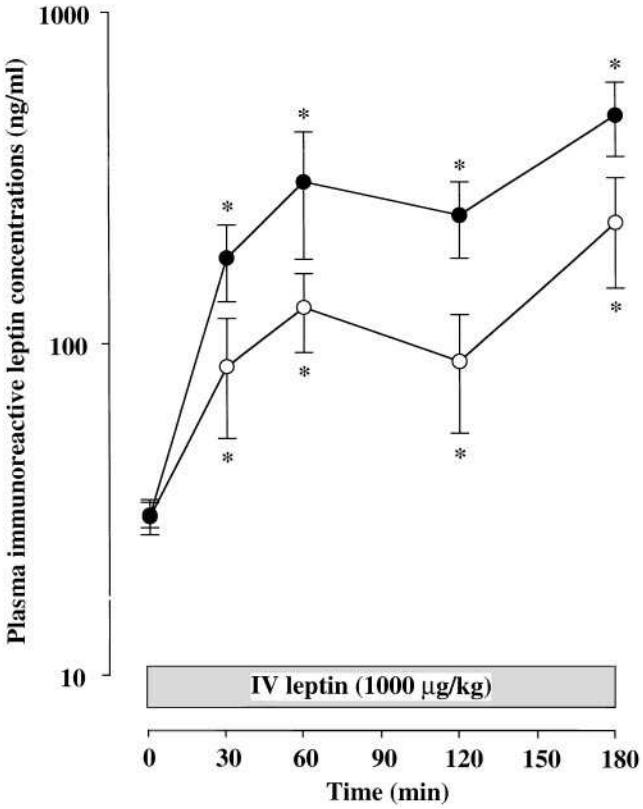



*Figure 8.* Time course of plasma immunoreactive leptin concentrations during intravenous administration of leptin (total dose: 1,000 μg/kg) in lean (○) and obese Zucker rats (●). Leptin was administered as a loading dose (500 μg/kg over 30 s) followed by a continuous infusion at 167 μg/kg per h for 3 h. Infusion of leptin significantly increased leptin concentrations in both lean and obese Zucker rats. Obese Zucker rats achieved significantly higher leptin concentrations during infusion than did lean Zucker rats ($P = 0.03$). Concentrations are shown on a log scale because of the nonparametric nature of the data. *$P < 0.05$ vs. baseline on Student's *t* test.

However, given that ganglion blockade abolished the effects of leptin and that distal transection of the sympathetic nerves did not alter them, it is highly likely that leptin stimulated efferent rather than afferent SNA. Fourth, the dose of leptin at which substantial sympathetic activation occurred was one that increased circulating leptin concentrations to a supraphysiologic range. However, these responses may still be of physiological and clinical relevance. The threshold concentration of leptin for an effect on SNA was 5 ng/ml, within the physiological range. In addition, the effects of leptin on regulation of the sympathetic nervous system may be more important when superimposed with other factors, such as hypertension or hyperinsulinemia, which are also associated with increased sympathetic outflow (11, 12).

The mechanism(s) through which leptin caused sympathoactivation are not clear. However, our data suggest a central nervous system receptor-mediated effect. A central neural effect is supported by the fact that sympathoactivation was not clearly related to achieved plasma concentrations of leptin, with significant increases in BAT- and R-SNA at doses of leptin (10 and 100 μg/kg) that failed to significantly increase plasma leptin. There was also a dissociation between the time course of plasma leptin concentrations and SNA during infusion of leptin, with delayed increases in SNA (Figs. 5 and 8). The implication of these data is that plasma leptin does not closely track leptin concentrations at the site where it activates SNA. Given that leptin is transported into cerebrospinal fluid by a specific saturable transport process (13, 14), the central nervous system is a plausible site for the actions of leptin on sympathetic nerve traffic. However, further studies will be necessary to prove this. A receptor-mediated effect is supported by substantially decreased sympathoactivation in obese Zucker rats, known to possess a mutation in the gene for the leptin receptor (7, 8). There was a tendency for SNA to BAT to increase in obese Zucker rats after leptin, albeit nonsignificantly. This might reflect activation of mutated leptin receptors or a partial nonreceptor-mediated effect of leptin on sympathetic nerve traffic to BAT. In this regard, it has been reported that intracerebroventricular injection of leptin is able to exert biological effects in obese Zucker rats, albeit only at much higher concentrations than in lean rats (15). Sympathoactivation to leptin was present in lean Zucker rats, proving that the *fa* mutation in the leptin receptor is the cause of diminished responses in obese Zucker rats. Interestingly, the degree of renal sympathoactivation to leptin in lean Zucker rats was less than that observed in lean Sprague-Dawley rats, suggesting that genetic background may influence this effect of leptin. The appetite-suppressing effects of leptin are mediated through activation of STAT proteins to ultimately suppress central neuropeptide Y, and increase corticotrophin releasing factor, expression (16, 17). It is tempting to speculate that sympatho-

activation to leptin occurs via the same central neural pathways.

Arterial pressure and HR were not altered by leptin, although there was a very substantial increase in overall efferent SNA. There are three possibilities that may explain this apparent contradiction. First, in the control studies using lipopolysaccharide, there was a significant decrease in systolic, though not diastolic, arterial pressure. Thus, it is possible that a moderate pressor effect of leptin may have been counteracted by an hypotensive effect of lipopolysaccharide contamination of the leptin preparation. However, the pressor effect of increased SNA would be expected to also alter diastolic arterial pressure, which was unaffected by lipopolysaccharide. Second, we do not know the functional consequences of sympathoexcitatory responses to leptin in different tissues. It is possible that this sympathoactivation represents increases mainly in nerve fibers that subserve metabolic functions, without altering vascular tone, although a renal metabolic effect appears unlikely. Third, leptin may have other effects which offset the expected vasoconstrictor effects of increased sympathetic outflow. One group has reported recently that leptin increases renal tubular sodium and water excretion (18), which could oppose the pressor effects of sympathoactivation. These latter two possibilities require further study.

Given the absence of a change in HR and arterial pressure, is sympathoactivation to leptin 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 reabsorbtion and vascular smooth muscle growth (19, 20). Thus, increases in sympathetic nervous system activity may be functionally and pathophysiologically relevant in the absence of acute changes in arterial pressure. This is particularly likely when increases in sympathetic nerve traffic to several tissues are observed, as was the case here.

Human obesity is associated with circulating hyperleptinemia, probably due to deficient penetration into the central nervous system or resistance to its actions (21–23). Our finding that leptin increases thermogenic nerve activity further supports the concept that leptin resistance may contribute to obesity through deficient thermogenesis, as well as increases in appetite. Increased SNA to kidney and muscle has been reported in obese Zucker rats and humans, respectively (24, 25). Given that we found that leptin-induced renal sympathoactivation was absent in obese Zucker rats, leptin appears unlikely to underlie the renal sympathoactivation observed in these animals. It is possible that humans, who are not thought to have primary leptin resistance, may exhibit preservation of the effects of leptin on sympathetic nerve traffic to nonthermogenic tissues. If so, it is possible that our findings are pertinent to the high cardiovascular morbidity and mortality of obesity (26). In any case, our data suggest that future therapeutic trials of leptin in obese human subjects should carefully monitor indices of sympathetic function, particularly because high doses of leptin may be required to treat leptin-resistant obesity. These sympathetic effects of leptin may also be of relevance to the physiological changes of starvation, where both circulating leptin concentrations and thermogenic SNA decrease (27–29).

In conclusion, we have demonstrated that leptin increases sympathetic outflow both to thermogenic BAT and other tissues. These effects occur at similar concentrations of leptin and suggest a novel role for leptin in neural control of the circulation. Sympathoactivation to leptin may offer an explanation for some of the deleterious cardiovascular effects of obesity, and may be clinically relevant to the potential therapeutic use of leptin and synthetic analogues in obesity.

## Acknowledgments

The authors are grateful to Shannon Powell for technical assistance.

Research was supported by grants HL44546 and HL43514 from the National Heart, Lung and Blood Institute, grant DK25295 from the National Institute of Diabetes, Digestive and Kidney Diseases, and by funds from the Department of Veterans Affairs. William G. Haynes was the recipient of a Wellcome Trust Advanced Training Fellowship (No. 04215/114).

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