

# Leptin Potentiates Thermogenic Sympathetic Responses to Hypothermia

## A Receptor-Mediated Effect

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**Leptin contributes to the regulation of thermogenesis. In rodents, sympathetic nerve activity efferent to interscapular brown adipose tissue (IBAT-SNA) is involved. On the basis of the hypotheses that 1) leptin acutely potentiates hypothermia-induced increases in IBAT-SNA; 2) this action of leptin is specific to IBAT-SNA, i.e., it does not occur with renal sympathetic nerve activity (R-SNA); and 3) this effect of leptin depends on intact and functional leptin receptors, we measured IBAT-SNA and R-SNA in anesthetized lean and diet-induced obese Sprague-Dawley and in obese Zucker rats, randomly assigned to low-dose leptin or vehicle. Before the start of leptin or vehicle and 5 min, 90 min, and 180 min after, hypothermia (30°C) was induced. Compared with vehicle, leptin did not significantly alter baseline R-SNA or IBAT-SNA. In lean Sprague-Dawley rats, hypothermia-induced increases in IBAT-SNA were significantly augmented by leptin but not by vehicle. In obese Sprague-Dawley rats, leptin did not potentiate hypothermia-induced increases in IBAT-SNA. In Zucker rats, IBAT-SNA did not increase with hypothermia and leptin was not able to induce sympathoactivation with cooling. Changes in R-SNA during hypothermia were not significantly modified by leptin in either group. Thus, low-dose leptin, although not altering baseline SNA, acutely enhances hypothermia-induced sympathetic outflow to IBAT in lean rats. This effect is specific for thermogenic SNA because leptin does not significantly alter the response of R-SNA to hypothermia. The effect depends on intact and functional leptin receptors because it occurs neither in rats with a leptin receptor defect nor in rats with acquired leptin resistance. *Diabetes* 51:2434–2440, 2002**

**L**eptin seems to have an important physiological role in the regulation of thermogenesis (1,2), via activation of the sympathetic nervous system (3). In rodents, this occurs by activation of sympathetic fibers efferent to brown adipose tissue (BAT) (4). Hypothermia is a major stimulus for thermogenesis; however, the effects of leptin on hypothermia-induced thermogenesis are not known. We hypothesized that leptin potentiates hypothermia-induced increases in sympathetic nerve activity to interscapular BAT (IBAT-SNA) in rats and that this effect should depend on intact and functional leptin receptors.

Indeed, obese Zucker rats with a genetically determined leptin receptor defect have blunted thermogenesis in response to hypothermia (5,6). However, the mechanisms underlying this phenomenon are not fully understood. Acquired resistance to many actions of leptin characterizes common diet-induced obesity (7). For example, thermogenesis is proposed to be attenuated in obesity (8), but few data exist on the effect of hypothermia on thermogenic SNA in diet-induced obesity (9), and the effects of leptin are not known.

Leptin has been shown to increase sympathetic nerve activity in various regions, i.e., activity of sympathetic fibers that serve circulatory or thermogenic or metabolic functions (10). Therefore, the effects of leptin on SNA responses to hypothermia may be regionally uniform or may be specific for thermogenic IBAT-SNA.

We tested the following hypotheses: First, leptin acutely potentiates hypothermia-induced increases in thermogenic IBAT-SNA in rats. Second, this action of leptin is specific for thermogenic SNA, i.e., it does not occur with SNA serving circulatory control. Third, this effect of leptin depends on intact and functional leptin receptors.

### RESEARCH DESIGN AND METHODS

**Animals and drugs.** Experiments were performed in 20 lean 3-month-old male Sprague-Dawley rats. Animals were allowed access to a standard diet containing 4% fat (Teklad Premier Laboratory Diets, Madison, WI) until anesthesia was induced. Also studied were 20 obese male Sprague-Dawley rats that were allowed access to a high-fat diet (57% fat rodent diet; Research Diets, New Brunswick, NY) for 12 weeks and that were 16 weeks of age when anesthesia was induced. Finally, experiments were conducted in 15 male 3-month-old Zucker rats that were fed a standard diet as described above.

Recombinant murine leptin, as previously described (10), was provided by Amgen Biologicals (Thousand Oaks, CA), methohexital was from Jones

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BAT, brown adipose tissue; IBAT-SNA, sympathetic nerve activity to interscapular BAT; MAP, mean arterial pressure.

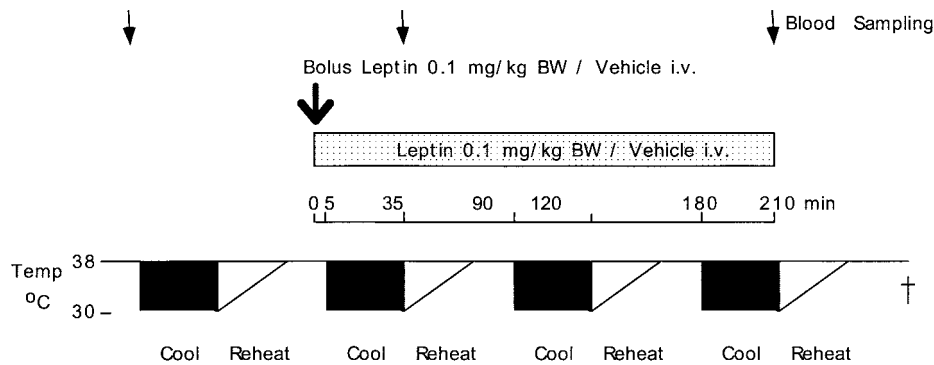


FIG. 1. The scheme of the study protocol.

Pharma (St. Louis, MO) and chloralose was from Sigma Chemicals (St. Louis, MO).

The University of Iowa and Iowa City Veterans Affairs Animal Research Committees approved all procedures.

#### Procedures

**General.** Anesthesia was induced using intraperitoneal methohexital sodium (50 mg/kg), and a catheter was inserted into the femoral vein for maintenance of anesthesia with intravenous chloralose ( $25 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ). For preventing upper respiratory tract obstruction and hypoxia, the trachea was cannulated for spontaneous respiration of  $\text{O}_2$ -enriched air. Rectal temperature was monitored continuously and varied between 38 and  $30^\circ\text{C}$  using a heated surgical table, lamps, and ice blocks. A catheter was inserted into the femoral artery for continuous arterial pressure measurement and blood sampling.

**SNA recordings.** SNA to BAT and kidney was measured by multifiber recording. The left kidney was exposed retroperitoneally through a left flank incision. With the use of a dissecting microscope, a nerve branch to the left kidney was carefully dissected and placed on a bipolar platinum-iridium electrode (Cooner Wire Company, Chatsworth, CA). IBAT was exposed through a nape incision. With the use of a dissecting microscope, sympathetic nerve fibers innervating IBAT were identified, cut distally, and attached to a bipolar platinum-iridium electrode. Electrodes were fixed in place with silicon gel (Sil-Gel 604; Wacker-Chemie) after an optimum recording of renal SNA was obtained.

Nerve electrodes were connected to a high-impedance probe (HIP-511; Grass Instruments), 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 1) to an oscilloscope (model 54501A; Hewlett-Packard) for monitoring, 2) to a MacLab analogue-digital converter (AD Instruments) for permanent recording of the neurogram on a Macintosh computer, and 3) to a nerve traffic analyzer (model 706C; University of Iowa Bioengineering) that counts action potentials above a threshold voltage level set just above background.

**Design.** Animals were allowed to stabilize for 60 min after placement of nerve electrodes. Baseline measurements of arterial pressure, heart rate, and renal and IBAT SNAs were made during 10 min. The protocol is depicted in Fig. 1.

During 30 min, rectal temperature was lowered continuously from 38 to  $30^\circ\text{C}$ ; thereafter, the animals' core temperature was restored to  $38^\circ\text{C}$ , which took another 30 min. A second 10-min baseline measurement was taken; thereafter, infusions of leptin or vehicle (phosphate-buffered 0.9% saline) were started. For leptin, a total dose of  $200 \mu\text{g}/\text{kg}$  body wt was chosen, which had been shown not to change baseline arterial pressure, heart rate, or SNA. Fifty percent of the total dose, i.e.,  $100 \mu\text{g}/\text{kg}$ , was given as a loading dose over 30 s, and the remainder of the dose ( $100 \mu\text{g}/\text{kg}$ ) was infused continuously at  $50 \mu\text{l}/\text{min}$  over 3.5 h.

Five minutes after infusion of leptin or vehicle had started, the animals' rectal temperature was again lowered from 38 to  $30^\circ\text{C}$  during 30 min and raised back to  $38^\circ\text{C}$ . Cooling was repeated from 90 to 120 min and from 180 to 210 min after start of leptin or vehicle infusions. Arterial blood samples for plasma leptin were collected at baseline, at minimum temperature 35 min after start of leptin or vehicle infusions, and at minimum temperature 210 min after start of infusions. At the end of the experiment, animals were killed by methohexital overdose ( $80 \text{ mg}/\text{kg}$  i.v.). Nerve activity that remained after methohexital administration provided an estimate of background noise, which was used to calculate specific nerve activity (see DATA ANALYSIS).

**Analytical.** Plasma leptin concentrations were measured as mouse leptin using a kit purchased from Linco. 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  $\pm$  SE. SNA measurements were corrected for background noise by subtracting postmortem measurements from the measurement obtained at each time point when the animals were alive.

In view of the interindividual variability of resting SNA, percentage change from baseline was calculated for SNA. For each cooling episode, the maximum change in SNA with hypothermia was calculated.

Differences in the responses to hypothermia between leptin-treated and control rats and between the different groups of animals were assessed using repeated-measures ANOVA; individual data points and trends were compared with post hoc tests. The model comprised two intersubject factors—treatment (leptin versus vehicle) and animal model (lean Sprague-Dawley versus obese Sprague-Dawley versus obese Zucker rats)—and two intrasubject factors—hypothermia ( $38 \rightarrow 30^\circ\text{C}$ ) and time (first, second, third, and fourth hypothermia episode). Statistical analysis was performed using Systat software for the Macintosh (version 5.2.1; SPSS, Chicago, IL).  $P < 0.05$  was considered statistically significant.

## RESULTS

Characteristics of the studied animals are presented in Table 1. Both obese Sprague-Dawley and Zucker rats were heavier than lean Sprague-Dawley rats. Obese Sprague-Dawley rats tended to be slightly heavier than obese Zucker rats ( $P = 0.03$  and  $P = 0.07$  for leptin- and vehicle-treated animals, respectively). Obese rats had increased baseline plasma leptin levels. With leptin but not vehicle infusion, plasma leptin increased significantly in all three groups, in obese more than in lean Sprague-Dawley rats.

**IBAT-SNA.** Baseline IBAT-SNA at normothermia did not change significantly with time in any of the three groups of animals, neither in leptin- nor in vehicle-treated rats (Figs. 2 and 3).

With hypothermia, there were consistent and substantial increases in IBAT-SNA in lean Sprague-Dawley and in obese Sprague-Dawley rats. Importantly, these hypothermia-induced increases in IBAT-SNA were significantly different from what was observed in obese Zucker rats, in which hypothermia failed to produce significant changes in IBAT-SNA. This was not modified by leptin or vehicle infusion in Zucker rats.

Vehicle infusion did not alter the hypothermia-induced increases in thermogenic SNA in lean and obese Sprague-Dawley rats. Indeed, increases in IBAT-SNA with hypothermia were similar during vehicle infusion in lean and obese Sprague-Dawley rats.

In lean but not in obese Sprague-Dawley rats, the hypothermia-induced increase in IBAT-SNA was substantially potentiated with leptin infusion (animal group  $\times$  treatment group  $\times$  time  $\times$  hypothermia interaction  $P <$

TABLE 1  
Characteristics of the studied animals

	Lean Sprague-Dawley	Obese Sprague-Dawley	Obese Zucker
Vehicle			
<i>n</i>	10	10	7
Weight (g)	336 ± 4	504 ± 9§	481 ± 7§
Plasma leptin (ng/ml), baseline	2.2 ± 0.4	12 ± 3§	19 ± 4§
Plasma leptin (ng/ml), end 2nd hypothermia	2.1 ± 0.2	7 ± 26	21 ± 17§
Plasma leptin (ng/ml), end 4th hypothermia	3.5 ± 0.5	9 ± 34	27 ± 15§
Leptin			
<i>n</i>	10	10	8
Weight (g)	339 ± 3	500 ± 9§	474 ± 8§
Plasma leptin (ng/ml), baseline	2.0 ± 0.3	6 ± 3	30 ± 4§
Plasma leptin (ng/ml), end 2nd hypothermia	39 ± 9*	218 ± 26*§	105 ± 16*
Plasma leptin (ng/ml), end 4th hypothermia	40 ± 20*‡	150 ± 34*†‡§	79 ± 14*‡

Data are means ± SD. \*Significantly different ( $P < 0.05$ ) from baseline. †Significantly different ( $P < 0.05$ ) from second hypothermia. ‡Significant ( $P < 0.05$ ) treatment × time interaction for plasma leptin. § $P < 0.05$  versus lean. Plasma leptin levels at baseline, at the end of the second hypothermia (35 min after start of leptin or vehicle infusion), and at the end of the 4th hypothermia (210 min after start of leptin or vehicle infusions).

0.05). A significant potentiation was observed during the second and third hypothermia episodes; potentiation of the hypothermia-induced increase in thermogenic SNA did not reach statistical significance at the final hypothermia episode.

**Renal SNA.** Baseline renal SNA (R-SNA) at normothermia increased with time in lean Sprague-Dawley rats ( $P < 0.05$ ), without a difference between leptin- or vehicle-treated animals. Baseline R-SNA at normothermia did not change significantly with time in obese Sprague-Dawley or obese Zucker rats. Hypothermia did not cause consistent significant changes in R-SNA in lean or obese Sprague-Dawley rats, and the responses to hypothermia were not altered by leptin or vehicle in these animals. In contrast, obese Zucker rats responded to hypothermia with consistent decreases in R-SNA, an effect significantly different from

what was observed in lean and obese Sprague-Dawley rats ( $P < 0.05$ ). However, the hypothermia-induced decrease in R-SNA in obese Zucker rats was not modified by leptin or vehicle treatment (Fig. 4).

**Mean arterial pressure and heart rate.** Baseline mean arterial pressure (MAP) did not differ significantly across groups in leptin-treated animals. Baseline values were slightly higher in the lean vehicle as compared with the obese vehicle ( $P = 0.005$ ) and the Zucker vehicle groups ( $P = 0.014$ ). MAP at normothermia decreased with time in vehicle-treated lean rats ( $P = 0.025$ ) and in vehicle-treated obese Sprague-Dawley rats ( $P = 0.007$ ) but did not change significantly in the other groups. However, trends did not differ significantly between leptin- and vehicle-treated animals. Hypothermia did not induce consistent changes in MAP in lean and obese Sprague-Dawley rats but tended to

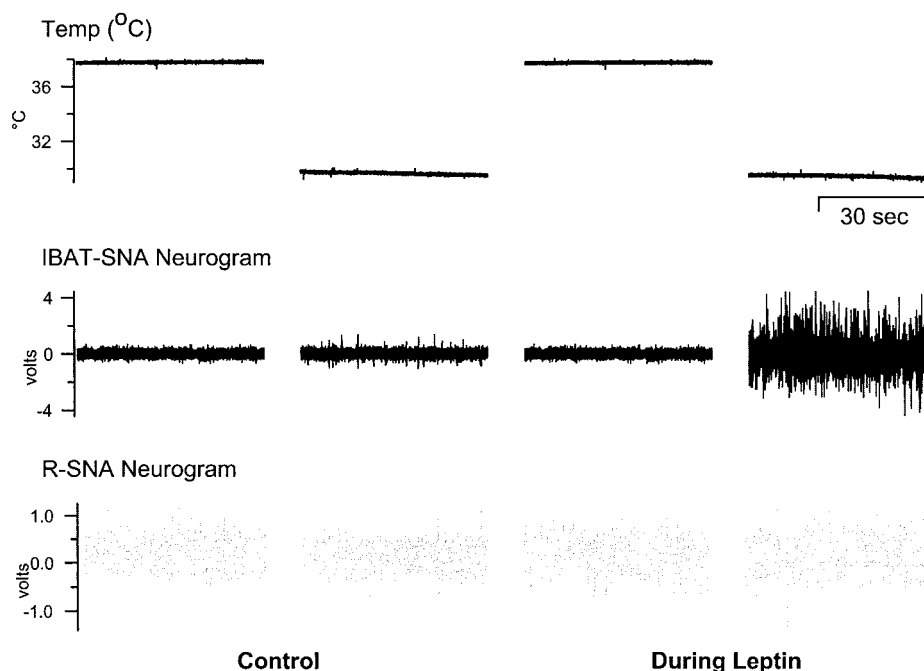


FIG. 2. Original tracings of rectal temperature, IBAT-SNA, and R-SNA in one lean Sprague-Dawley rat at normothermia and at hypothermia, before and after start of leptin infusion (start and end of first and second hypothermia). Before leptin, IBAT-SNA increased with hypothermia; during leptin, this response was potentiated. In contrast, leptin had no effect on the R-SNA response to hypothermia.

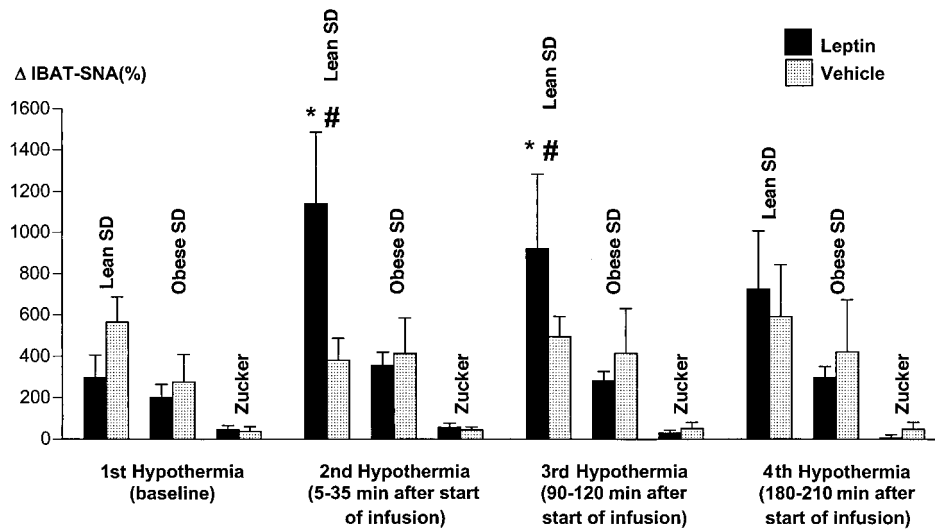


FIG. 3. Maximum hypothermia-induced changes in IBAT-SNA observed during the four hypothermia episodes in leptin- and vehicle-treated animals of all groups. In lean Sprague-Dawley (SD) rats, the increase in thermogenic SNA was potentiated with leptin, an effect that was observed during hypothermia from 5 to 35 min and from 90 to 120 min after start of leptin but that did not reach statistical significance during the last hypothermia episode. Obese Sprague-Dawley rats showed a sympathetic thermogenic response that was not significantly different from lean Sprague-Dawley rats at baseline; however, neither leptin nor vehicle had an effect in obese Sprague-Dawley rats. Note that the maximum changes in IBAT-SNA with hypothermia in obese Zucker rats were not significantly different from 0. \* $P < 0.05$  versus first hypothermia and vehicle (hypothermia-induced response); # $P < 0.05$  versus obese Sprague-Dawley and Zucker rats (hypothermia-induced response).

decrease MAP in obese Zucker rats (significant during the first two hypothermia episodes). The MAP responses to hypothermia were not consistently altered by leptin or vehicle in any group (Tables 2 and 3).

Vehicle-treated obese Sprague-Dawley rats had significantly lower baseline heart rates than vehicle-treated lean Sprague-Dawley ( $P = 0.002$ ) and obese Zucker rats ( $P = 0.02$ ). Baseline heart rate did not differ across leptin-treated animals. Heart rate at normothermia increased with time in lean and obese Sprague-Dawley rats ( $P < 0.05$ ) and decreased with time in obese Zucker rats ( $P < 0.05$ ). Trends differed significantly between Zucker rats and both groups of Sprague-Dawley rats ( $P < 0.05$ ); however, trends did not differ significantly between leptin- and vehicle-treated animals. With hypothermia, heart rate tended to decrease in lean Sprague-Dawley rats (statistically significant during the final hypothermia episodes)

and decreased significantly in obese Sprague-Dawley and obese Zucker rats. Hypothermia-induced decreases in heart rate were more pronounced in obese Sprague-Dawley and obese Zucker rats than in lean Sprague-Dawley rats ( $P < 0.05$ ), but these hypothermia-induced decreases in heart rate were not consistently modified by leptin or vehicle treatment.

#### DISCUSSION

The present study has the following major findings. First, leptin, at a low dose that does not alter baseline SNA, acutely enhances sympathetic outflow to BAT in response to hypothermia in lean Sprague-Dawley rats. Second, this effect is specific for thermogenic SNA and is not observed with R-SNA. Third, the potentiation of hypothermia-induced increases in thermogenic SNA depends on intact

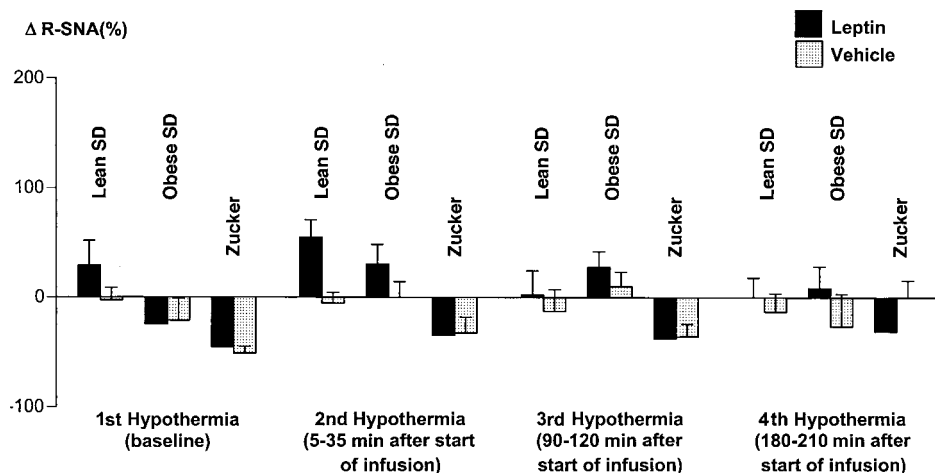


FIG. 4. Maximum hypothermia-induced changes in R-SNA observed during the four hypothermia episodes in leptin- and vehicle-treated animals of all groups. Note that only obese Zucker rats showed consistent decreases in R-SNA with hypothermia. Responses were not modified by leptin or vehicle treatment in either group.

TABLE 2  
Responses of MAP (mmHg) to hypothermia in the three groups of animals

	Before vehicle or leptin		During vehicle or leptin					
	1st hypothermia		2nd hypothermia		3rd hypothermia		4th hypothermia	
	38°C	30°C	38°C	30°C	38°C	30°C	38°C	30°C
Vehicle								
Lean SD	132 ± 4 <sup>†</sup>	123 ± 6*	134 ± 6	123 ± 6*	117 ± 6	119 ± 6	109 ± 6 <sup>‡</sup>	116 ± 5*
Obese SD	111 ± 5	95 ± 8*	109 ± 5	105 ± 6	95 ± 3	101 ± 7	89 ± 4 <sup>‡</sup>	87 ± 10
Zucker	115 ± 7	88 ± 7*	119 ± 3	106 ± 8*	116 ± 7	113 ± 9	105 ± 7	100 ± 9
Leptin								
Lean SD	122 ± 4	115 ± 5	120 ± 6	130 ± 4*	121 ± 4	120 ± 5	122 ± 4	120 ± 5
Obese SD	110 ± 3	96 ± 8*	104 ± 4	112 ± 5	101 ± 4	115 ± 3*	96 ± 6	113 ± 3*
Zucker	114 ± 7	92 ± 6*	119 ± 6	111 ± 7*	106 ± 4	117 ± 7*	105 ± 8	109 ± 13

Data are mean ± SE. Shown are values for MAP (mmHg) at the beginning and the end of the four experimental hypothermias in leptins and vehicle-treated rats of the three groups (lean Sprague-Dawley, obese Sprague-Dawley, and obese Zucker). \**P* < 0.05 vs. preceding normothermia; <sup>†</sup>*P* < 0.05 vs. obese Sprague-Dawley and obese Zucker rats (vehicle groups); <sup>‡</sup>*P* < 0.05 for trend vs. baseline before first hypothermia; for additional effects refer to text.

and functional leptin receptors because it occurs neither in rats with a leptin receptor defect nor in rats with acquired leptin resistance.

Leptin increases thermogenic metabolism and inhibits appetite (2,11). At high doses, leptin has been shown to increase thermogenic SNA. Collins et al. (3) observed increased norepinephrine turnover in BAT with leptin; our laboratory could confirm these results with direct microelectrode recordings of IBAT-SNA in Sprague-Dawley rats (10). It is interesting that at these doses, leptin increased not only thermogenic SNA but also SNA proposed to subservise circulatory functions such as renal, adrenal, and hindlimb SNA.

At low doses, leptin has not been demonstrated to cause sympathetic activation (10). Our current results, however, show that low doses of leptin that fail to alter baseline SNA potentiate the response of thermogenic SNA to hypothermia. It was demonstrated previously that experimental hypothermia produces regionally nonuniform alterations in sympathetic nerve traffic (12). This report, however, is the first to describe the effects of leptin on changes in SNA during hypothermia. A potentiating effect of leptin was seen only for thermogenic IBAT-SNA. Leptin did not modify the responses of R-SNA to hypothermia. Thus, at low doses, leptin specifically potentiates SNA that subserves thermogenic functions. The effect was observed

acutely, within 30 min of starting leptin. This is consistent with a rapid transport of leptin across the blood-brain barrier in normal rats (13).

Importantly, we demonstrated that the potentiation of thermogenic SNA responses to hypothermia is a receptor-mediated effect. In obese Zucker rats, which are characterized by a leptin receptor defect (14), leptin did not modify thermogenic sympathetic responses to hypothermia. Moreover, these animals did not show statistically significant increases in IBAT-SNA with hypothermia. This suggests that leptin has a fundamental physiological role to allow thermogenic SNA in response to hypothermia. Our results are consistent with the report of Bing et al. (15), who were able to link hypothermia-induced increases in BAT thermogenic activity in lean rats to decreased neuropeptide Y release in the paraventricular nucleus. In contrast, obese Zucker rats did not show hypothermia-induced increases in BAT thermogenic activity explained by sustained and nonsuppressible overactivity of hypothalamic neuropeptide Y neurons. Given that leptin is a major physiological suppressor of hypothalamic neuropeptide Y release (16), it is conceivable that basal leptin levels and intact leptin receptors are required to suppress central neuropeptide Y release and thereby facilitate increases in thermogenic SNA during hypothermia.

It could be argued that the defective thermogenic re-

TABLE 3  
Responses of heart rate (beats/min) to hypothermia in the three groups of animals

	Before vehicle or leptin		During vehicle or leptin					
	1st hypothermia		2nd hypothermia		3rd hypothermia		4th hypothermia	
	38°C	30°C	38°C	30°C	38°C	30°C	38°C	30°C
Vehicle								
Lean SD	391 ± 14	372 ± 22	407 ± 20	389 ± 15	427 ± 13	399 ± 16	446 ± 13 <sup>‡</sup>	384 ± 13*
Obese SD	337 ± 12 <sup>†</sup>	250 ± 18*	345 ± 11	284 ± 20*	353 ± 19	291 ± 23*	353 ± 19	281 ± 21*
Zucker	382 ± 12	239 ± 13*	346 ± 6	259 ± 10*	324 ± 18	269 ± 16*	312 ± 13 <sup>‡</sup>	230 ± 12*
Leptin								
Lean SD	365 ± 12	355 ± 18	379 ± 12	393 ± 14	421 ± 12	357 ± 10*	423 ± 12 <sup>‡</sup>	341 ± 10*
Obese SD	342 ± 12	266 ± 20*	354 ± 14	309 ± 21*	351 ± 13	310 ± 20*	375 ± 13 <sup>‡</sup>	307 ± 19*
Zucker	353 ± 12	241 ± 17*	324 ± 12	262 ± 13*	295 ± 11	242 ± 9*	307 ± 7 <sup>‡</sup>	273 ± 11*

Data are mean ± SE. Shown are values for heart rate (beats/min) at the beginning and the end of the four experimental hypothermias in leptin- and vehicle-treated rats of the three groups (lean Sprague-Dawley, obese Sprague-Dawley, and obese Zucker). \**P* < 0.05 vs. preceding normothermia; <sup>†</sup>*P* < 0.05 vs. lean Sprague-Dawley and obese Zucker rats (vehicle groups); <sup>‡</sup>*P* < 0.05 for trend vs. baseline before first hypothermia; for additional effects refer to text.

sponse seen in obese Zucker rats is not attributable to a specific leptin receptor defect but to obesity per se. However, the rats with diet-induced obesity showed preserved hypothermia-induced increases in thermogenic SNA. Moreover, the defective response to hypothermia in Zucker rats was specific for thermogenic SNA, i.e., circulatory responses to hypothermia such as decreases in heart rate and blood pressure were similar in obese Sprague-Dawley and obese Zucker rats.

Diet-induced obese Sprague-Dawley rats exhibited preserved increases in thermogenic IBAT-SNA during hypothermia. However, these responses were not potentiated by leptin contrasting with the effects seen in lean Sprague-Dawley rats. Resistance to leptin's action on body weight control generally characterizes diet-induced obesity (17). The present data support the concept that diet-induced obesity is characterized by resistance to the effects of leptin on thermogenic SNA.

We recognize several potential limitations of the present study. First, we studied only one dose of leptin that was selected not to alter baseline nerve activity. It is conceivable that with higher leptin doses, leptin may potentiate sympathetic thermogenic responses to hypothermia also in obese rats. However, obese Sprague-Dawley rats reached significantly higher plasma leptin concentrations than lean rats despite the same dose of leptin yet still lacked the potentiating effect of leptin observed in lean animals. Second, we cannot discern the nature of the observed leptin resistance in obese Sprague-Dawley rats because we applied leptin only intravenously. Conflicting results exist with the site of leptin resistance in animal models of diet-induced obesity. Some studies show resistance to intravenous but not to intracerebroventricular leptin (18), whereas other studies show resistance to both intravenous and intracerebroventricular leptin (19). It is conceivable that impaired transport of leptin across the blood-brain barrier contributes to the absent potentiation of hypothermia-induced thermogenic sympathoactivation in obese Sprague-Dawley rats. Third, the results in the studied animal model of diet-induced obesity may not extend to human common obesity. We failed to demonstrate reduced thermogenic SNA in response to hypothermia in obese Sprague-Dawley rats, contrasting with the results of Matsumoto et al. (9), who found reduced sympathetic thermogenic responsiveness to cold exposure in obese women. Fourth, our method of recording SNA does not allow us to compare absolute levels of SNA between different groups of animals. Holt and York (20) reported lower absolute levels of IBAT-SNA in obese as compared with lean Zucker rats at both normothermia and hypothermia. In contrast to our results, they observed similar increases in thermogenic SNA with hypothermia. However, they lowered the core temperature only to 36.6°C and not to 30°C as in the present study. Fifth, we did not measure thermogenesis or the expression of uncoupling proteins. IBAT-SNA is only one factor that influences these variables. The regulation of uncoupling proteins is complex (21,22), and a leptin receptor defect does not exclude increased expression of uncoupling proteins during hypothermia (23). Uncoupling protein 2 mRNA levels have even been reported to be normal in obese Zucker rats (24).

## CONCLUSIONS

Leptin, at a low dose that does not alter baseline SNA, acutely enhances sympathetic outflow to BAT in response to hypothermia in lean Sprague-Dawley rats. This effect is specific for thermogenic SNA because leptin does significantly alter the responses of renal SNA to hypothermia. The effect depends on intact and functional leptin receptors because it does not occur in rats with a leptin receptor defect or in rats with acquired leptin resistance. The absence of cooling-induced sympathoactivation in rats with a leptin receptor defect suggests that leptin may play a physiological role in hypothermia-induced increases in thermogenic SNA.

## ACKNOWLEDGMENTS

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