Central Leptin Infusion Attenuates the Cardiovascular and Metabolic Effects of Fasting in Rats

James Michael Overton, Todd Dennis Williams, James Bradley Chambers, Michael Edward Rashotte

Abstract—The role of reduced leptin signaling in the regulation of cardiovascular responses to negative energy balance is not known. We tested the hypothesis that central infusion of leptin would attenuate the cardiovascular and metabolic responses to fasting. Male Sprague-Dawley rats, instrumented with telemetry devices and intracerebroventricular cannulas, were housed in metabolic chambers for continuous (24 hours) measurement of dark-phase (active) and light-phase (inactive) mean arterial pressure, heart rate, oxygen consumption, and respiratory quotient. Rats received central infusions of either saline (0.5 μL/h) or leptin (42 ng/h) for 6 days through osmotic pumps and were either fed ad libitum or were fasted for 48 hours followed by refeeding for 4 days. In ad lib animals, continuous intracerebroventricular leptin infusion significantly reduced caloric intake, body weight, and respiratory quotient compared with saline controls while having no effect on mean arterial pressure or heart rate. Fasting reduced mean arterial pressure, heart rate, oxygen consumption, and respiratory quotient in rats receiving saline infusions. Fasting-induced reductions in mean arterial pressure were specific to the active phase and were not attenuated by central leptin infusion. In contrast, intracerebroventricular leptin, at a dose that had no cardiovascular effects in ad lib control animals, completely prevented fasting-induced decreases in light-phase heart rate and oxygen consumption and blunted fasting-induced reductions in dark-phase heart rate and oxygen consumption. The results are consistent with the hypothesis that reductions in central leptin signaling contribute to the integrated cardiovascular and metabolic responses to acute caloric deprivation. (Hypertension. 2001;37[part 2]:663-669.)

Key Words: diet ■ rats ■ neuroregulators ■ metabolism

Several lines of evidence support the concept that leptin is an important neuroendocrine signal involved in the regulation of the autonomic nervous system and of cardiovascular function. Central or peripheral leptin administration increases lumbar, brown adipose, and renal efferent sympathetic nerve activity.1–4 Acute peripheral leptin administration has generally produced minimal cardiovascular effects in conscious or anesthetized rats;5,6 however, central and chronic peripheral administration of leptin increases heart rate (HR) and blood pressure (BP) in conscious animals.7,8 In addition, transgenic skinny mice that have elevated plasma leptin exhibit increases in tail-cuff systolic BP, HR, and urinary norepinephrine.9 Taken together, these observations suggest that elevated leptin levels, which are evident in obesity, could increase sympathetic activity, HR, and BP.10,11

Caloric deprivation produces concurrent reductions in metabolic rate, sympathetic activity, HR, and BP.12–14 Decreased serum leptin levels and subsequent decreased signaling within the hypothalamus may be crucial mediators of many of the neuroendocrine and hypothalamic responses to fasting.15,16 However, it is not yet known if the cardiovascular responses to caloric deprivation are regulated by leptin-dependent mechanisms. The goal of this study was to determine if reduced endogenous leptin signaling is involved in the cardiovascular and metabolic responses to caloric deprivation. To accomplish this goal, we examined the cardiovascular and metabolic responses to fasting in animals that received continuous central leptin infusion during fasting. Specifically, we tested the hypothesis that central leptin infusion would attenuate the cardiovascular and metabolic responses to 48 hours of fasting in normotensive rats.

Methods

The procedures and protocols described below are in accord with the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee at The Florida State University. Male Sprague-Dawley rats (Charles River) were anesthetized (sodium pentobarbital, 50 mg/kg IP) for stereotaxic surgery to implant a guide cannula (3220P/SPC; Plastics One) in the lateral ventricle (Coordinates from bregma: 1.4 mm lateral, 0.9 mm posterior, 3.7 mm ventral). Two to 3 days after surgery, placement of the guide cannula was verified by the presence of a vigorous drinking response (>5 mL/1 h) to intracerebroventricular (ICV) injection of angiotensin II (10 ng/10 μL). Rats with accurate guide cannula placements were then anesthetized (sodium pentobarbital, 50 mg/kg IP) and instrumented with a catheter in the descending aorta coupled.
Baseline Values

<table>
<thead>
<tr>
<th></th>
<th>Adlib-Saline (n=5)</th>
<th>Adlib-Leptin (n=5)</th>
<th>Fast-Saline (n=6)</th>
<th>Fast-Leptin (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>400±18</td>
<td>404±8</td>
<td>458±23</td>
<td>431±11</td>
</tr>
<tr>
<td>Food intake, kcal</td>
<td>126±18</td>
<td>143±16</td>
<td>125±10</td>
<td>111±7</td>
</tr>
<tr>
<td>Fluid intake, mL</td>
<td>37±2</td>
<td>37±1</td>
<td>42±4</td>
<td>36±1</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Day</th>
<th>Night</th>
<th>Day</th>
<th>Night</th>
<th>Day</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>112±5</td>
<td>118±5</td>
<td>120±2</td>
<td>125±3</td>
<td>109±4</td>
<td>114±4</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>322±14</td>
<td>365±12</td>
<td>329±5</td>
<td>377±6</td>
<td>317±6</td>
<td>366±5</td>
</tr>
<tr>
<td>SDIBI, ms</td>
<td>7.2±1.4</td>
<td>6.2±0.7</td>
<td>4.9±0.6</td>
<td>4.6±0.4</td>
<td>5.6±0.3</td>
<td>5.5±0.3</td>
</tr>
<tr>
<td>V̇O₂, mL/min per kg</td>
<td>15.0±0.8</td>
<td>20.8±0.5</td>
<td>15.5±0.6</td>
<td>21.3±0.5</td>
<td>14.7±0.6</td>
<td>20.1±0.3</td>
</tr>
<tr>
<td>RO, V̇CO₂/V̇O₂</td>
<td>0.97±0.01</td>
<td>0.99±0.02</td>
<td>0.93±0.01</td>
<td>0.95±0.02</td>
<td>0.88±0.01</td>
<td>0.92±0.03</td>
</tr>
<tr>
<td>Activity, m</td>
<td>30 (n=1)</td>
<td>199 (n=1)</td>
<td>42±4 (n=3)</td>
<td>174±17 (n=3)</td>
<td>46±6 (n=4)</td>
<td>160±8 (n=4)</td>
</tr>
</tbody>
</table>

Values are mean±SEM for the last 3 days of baseline data for experimental groups of Sprague-Dawley rats that were fed ad libitum (Adlib) or were fasted for 48 hours (Fast) during continuous ICV infusion of saline (0.5 µL/h) or leptin (42 ng/h; 1 µg/d). Subject numbers for activity measures are listed immediately below values.

with a sensor and transmitter (TA11PA-C40; Data Sciences) for telemetric monitoring of BP. During recovery from surgery (>10 days), rats were housed individually with ad libitum access to powdered rodent chow (Purina 5001; caloric value = 3.3 kcal/g) and deionized water in acclimation cages as described previously.14 Rats were housed in an ambient temperature of 23±1°C and maintained on a 12-hour light/dark schedule.

After recovery from surgery, the rats were transferred to metabolic chambers, where they remained for the duration of the study. The chambers have been described in detail and consist of a small room calorimeter and a telemetry receiver (RPC-1; Data Sciences) for continuous determination of cardiovascular, metabolic, and behavioral variables.14 Food and water intake and body mass of each rat were determined during a daily maintenance period that occurred 1 to 2 hours before lights off.

Cardiovascular Variables

Telemetry signals were sampled continuously at 500 Hz. Mean arterial BP (MAP), HR, and the standard deviation of the interbeat interval (SDIBI) were calculated and stored for offline analysis as described previously.14

Metabolic Variables

Oxygen consumption (V̇O₂) and carbon dioxide production (V̇CO₂) were measured every 2.5 minutes by open-circuit respirometry as described previously.14 V̇O₂ was adjusted for mass (mL/min per kg0.75). Respiratory quotient (RQ) was calculated as V̇CO₂/V̇O₂.

Locomotor Activity

Two of the metabolic chambers available for this study were instrumented to record locomotor activity in meters. Thus, for some animals, locomotor activity was accumulated in 30-second periods and stored with a 1-mm resolution, as described previously.14

Experimental Design

After 3 days of baseline measurement, the rats were assigned to 1 of 4 groups (n=5 to 6/group): (1) ICV saline–ad libitum, (2) ICV leptin–ad libitum, (3) ICV saline–fasting/refeeding, and (4) ICV leptin–fasting/refeeding. Rats were briefly (5 minutes) anesthetized at the onset of the light phase with 2% halothane in oxygen for attachment of 7-day osmotic pumps (Alzet, 1007D; flow rate = 12 µL/24 h) containing either PBS (Sigma-Aldrich) or murine leptin (Peptide) dissolved in PBS at a concentration of 0.083 µg/µL, resulting in a delivery of 1 µg/24 h or 42 ng/h. The pumps were filled at least 8 hours before implantation and primed by soaking in warm (37°C) saline. The dose was selected on the basis of dose-response data for leptin effects on body mass in mice as well as our prior experience with acute ICV administration of leptin.2 The goal was to infuse a dose of leptin that would reduce food intake but might have minimal cardiovascular effects in ad lib–fed animals. Animals were returned to their metabolic chambers within 1 hour after osmotic pump implantation.

For animals assigned to ad lib conditions, the cardiovascular, metabolic, and behavioral effects of saline and leptin infusion were determined for the next 6 days. For animals assigned to fasting conditions, food was removed ~10 hours after pump implantation, during the normal daily maintenance period, and was returned 48 hours later just before lights off to ensure that the rats would resume food consumption at a time point consistent with normal circadian feeding behavior. The cardiovascular, metabolic, and behavioral effects of leptin infusion were monitored during the refeeding period for an additional 4 days, at which time the experiment was concluded.

Data Analysis and Statistics

The final 2 hours of the light phase (during which daily chamber maintenance procedures were performed) were excluded from analysis, resulting in 12-hour averages for the dark phase and 10-hour averages for the light phase. The effects of ICV leptin infusion in ad libitum–fed and fasted groups were evaluated with 2-way (treatment×time) ANOVA (Sigma-Stat, version 2.0, SPSS). Tukey post hoc tests were used to determine significant differences between means. In addition, the magnitude of the physiological responses to the 48-hour fast in control and leptin-treated animals were compared by means of Student’s t tests. Significance levels of P<0.05 were accepted.

Results

Ad Libitum Experiment

Group baseline values were generally similar and are provided in the Table. For clarity, subsequent results are presented as changes from baseline. Immediately after anesthesia and pump implantation, ad libitum–fed control animals receiving ICV saline infusion displayed a transient, nonsignificant reduction in food intake (Figure 1A), which had no effect on body weight (Figure 1B). Leptin-treated rats displayed significant reductions in food intake for the first 4 days of infusion (Figure 1A) and sustained reductions in body weight that were statistically significant during the last 4 days of infusion (Figure 1B).
Figure 1. Influence of ICV infusion of saline (12 μL per day, circles) or leptin (1 μg per day, triangles) on A, caloric intake; B, body weight; C, MAP; D, HR; E, SDIBI; F, $\dot{V}_O_2$; G, locomotor activity; and H, RQ. Graphs depict mean changes from baseline that were calculated as average of last 3 days of baseline period. For C through H, light-phase data (open symbols) and dark-phase data (closed symbols) are plotted sequentially within day. Dashed vertical line indicates beginning of infusion of either saline or leptin. Rats had free access to food during 6-day infusion period. *Significant difference between saline-treated and leptin-treated values.
Figure 2. Influence of ICV infusion saline (12 μL per day, circles) or leptin (1 μg per day, triangles) on A, caloric intake; B, body weight; C, MAP; D, HR; E, SDIBI; F, \( \dot{V}O_2 \); G, locomotor activity; and H, RQ. Graphs depict changes from baseline calculated as average of last 3 days of baseline period. For C through H, light-phase data (open symbols) and dark-phase data (closed symbols) are plotted sequentially within day. Dashed vertical line indicates beginning of infusion of either saline or leptin. Rats were fasted for 48 hours (FAST, dark shading), beginning ~10 hours after pump implantation, and were then given free access to food during last 4 days of experiment (REFEED). *Significant difference between saline-treated and leptin-treated values.
Transient increases in MAP (Figure 1C) and HR (Figure 1D) were observed during the first 12 hours after pump implantation in both saline- and leptin-treated groups. In contrast, transient decreases in HR variability (SDIBI; Figure 1E) were observed in both groups after pump implantation. There were no significant differences in MAP, HR, and SDIBI between saline- and leptin-treated rats across the 6-day experimental period.

Transient dark-phase reductions in VO2 (Figure 1F) and locomotor activity (Figure 1G) were observed after pump implantation in both saline- and leptin-treated animals. There was no evidence of a significant effect of leptin infusion on either VO2 or locomotor activity. Leptin-treated animals exhibited significant reductions in RQ, indicating increased oxidation of fat (Figure 1H).

**Fasting Experiment**

Leptin infusion significantly reduced both caloric intake (Figure 2A) and body weight recovery (Figure 2B) during the postfast refeeding period.

Fasting produced a dark-phase–specific reduction in MAP in saline-treated animals that was not influenced by leptin infusion (Figure 2C). One day of refeeding was adequate to return MAP to levels not different from baseline controls. During the refeeding period, leptin infusion had no effect on MAP. In rats treated with saline, fasting significantly reduced HR during both the light and dark phases (Figure 2D). When these rats were refed, HR recovered partially but remained below control levels for 4 days (Figure 2D). Leptin infusion completely prevented fasting-induced bradycardia during the light phase and significantly attenuated fasting bradycardia during the dark phase (Figure 2D). During the postfast refeeding period, rats receiving leptin infusions displayed HR that was slightly greater than baseline levels and significantly greater than in saline-treated rats.

Similar to the pattern observed in the ad lib groups (Figure 1E), pump implantation transiently reduced SDIBI (Figure 2E). ANOVA revealed no differences in the SDIBI response to fasting and refeeding between saline-treated and leptin-treated rats (Figure 2E).

Leptin treatment significantly attenuated fasting-induced reductions in VO2 during both the second light-phase period and during both the first and second dark-phase periods (Figure 2F). It is clear that significant reductions in dark phase VO2 were still evident during fasting in leptin-treated animals, but the magnitude of this reduction was attenuated by leptin. Pump implantation transiently reduced dark-phase locomotor activity in both groups (Figure 2G). Although the locomotor activity data are somewhat variable, there is no suggestion that leptin infusion significantly altered locomotor activity during either fasting or refeeding (Figure 2G).

Leptin treatment did not modulate fasting-induced reductions in RQ (Figure 2H) but did sustain a significantly lower RQ into the refeeding period.

**Discussion**

The primary new finding of this study is that continuous ICV leptin infusion strongly attenuates fasting-induced bradycardia in normotensive rats. Leptin infusion, at a low dose that did not significantly increase MAP, HR, or VO2 in ad lib–fed animals, completely prevented fasting-induced bradycardia during the inactive light phase and blunted fasting bradycardia during the dark phase. Leptin also attenuated fasting-induced reductions in VO2 in a pattern similar to HR. The effects of leptin infusion on the cardiovascular and metabolic responses to fasting were observed in the absence of any significant alterations in locomotor activity. In contrast to the effects on HR, fasting-induced reductions in MAP, which were modest, were only evident during the dark phase and were not modulated by leptin. Taken together, the results are consistent with a prominent role for leptin in the concurrent regulation of HR and VO2 in response to negative energy balance.

The effects of leptin on the cardiovascular and metabolic responses to fasting were examined using a relatively low ICV dose (42 ng/h) that we predicted would influence appetite but might not have cardiovascular-excitatory effects. Indeed, leptin infusion at this dose reduced food intake and body weight but had no significant effects on MAP, HR, or VO2 in rats with free access to food. This finding tends to contrast with previous reports that exogenous leptin increases MAP,1,3,7,8 HR, 3,7,8 and VO2.18 Transgenic skinny mice, which overexpress the ob gene, also exhibit elevated tail-cuff blood pressure and HR.9 In general, these excitatory cardiovascular and metabolic effects of leptin have been obtained with high peripheral levels or higher doses of central leptin injection. The observation that a lower ICV level of leptin can decrease food intake without overtly increasing MAP, HR, and VO2 suggests that the threshold for the anorexie effects of leptin may occur at a lower level than required for the autonomic/cardiovascular effects of leptin.

An important caveat to the conclusions concerning the cardiovascular effects of leptin in the ad libitum group is the reduced food intake produced by central leptin infusion and the lack of an additional pair-fed control group. Although not matched to the exact pattern of caloric restriction produced by leptin administration in this study, we have recently observed that 1 week of caloric restriction to 60% of control intake produces a modest but significant bradycardia (≈25 bpm), with a nonsignificant decrease in MAP in Sprague-Dawley rats (Overton et al; unpublished results). In the current study, leptin-treated animals exhibited decreased food intake with no significant change in HR. Thus, we speculate that leptin infusion served to blunt the modest bradycardia that accompanies reduced caloric intake. Indeed, this idea is analogous with reports indicating that leptin administration also prevents the reduction in metabolic rate observed in pair-fed animals.17,19,20

Fasting reduced MAP, HR, VO2, and RQ while increasing HR variability in normotensive rats. The most striking effects of leptin infusion during fasting were the prevention of both bradycardia and reduced VO2 during the light phase. Leptin infusion blunted but did not prevent reductions in dark-phase HR and VO2. At the same time, leptin infusion also tended to attenuate the increase in HR variability produced by fasting. It must be noted that we observed clear effects of the brief osmotic pump implantation procedure that may have influenced the physiological responses to subsequent fasting.
These effects of halothane anesthesia included increased MAP, HR, and \( V_O^2 \) and decreased HR variability during the first 12 hours and a substantial decrease in locomotor activity and \( V_O^2 \) during the subsequent 12- to 24-hour dark phase. The early light-phase responses are in opposition to fasting effects. For example, the consistent decrease in HR variability observed with pump implantation (Figure 1G and Figure 2G) may have reduced subsequent fasting-induced increases in HR variability. Nonetheless, the effects of pump implantation were transient, and the cardiovascular and metabolic responses to fasting were clearly evident during the second day of fasting. The results of this study are consistent with a growing body of evidence indicating that the neuroendocrine and hypothalamic responses to fasting can be attenuated or prevented by administration of exogenous leptin.

It is clear that the cardiovascular and metabolic responses to fasting are accompanied by reduced sympathetic activity.\(^{12,21,25}\) However, direct evidence that fasting-induced bradycardia is mediated by reduced sympathetic activity is lacking. We have recently observed that rats receiving chronic atenolol treatment continue to display significant fasting-induced bradycardia (Overton et al, unpublished results). Thus, it is likely that fasting either increases parasympathetic tone or decreases intrinsic HR, in addition to reducing sympathetic outflow. Weight loss in humans has been shown to increase parasympathetic control of HR.\(^{26}\) Given the very potent effects of leptin infusion on fasting-induced bradycardia, additional information is needed concerning the potential role of leptin in the modulation of parasympathetic control of HR.

The specific pathways by which leptin may modulate food intake, pituitary function, and the autonomic nervous system have been recently reviewed.\(^{24,25}\) Ahima and colleagues\(^{15}\) have convincingly demonstrated that physiological leptin replacement attenuates fasting-induced increases in hypothalamic neuropeptide Y (NPY) gene expression and decreases in pro-opiomelanocortin and cocaine- and amphetamine-related transcript gene expression. At present, it is not clear if these pathways are responsible for the metabolic and cardiovascular actions of fasting and/or leptin. In fact, rats and mice that lack leptin or are severely leptin resistant continue to display appropriate compensatory physiological\(^{26–28}\) and hypothalamic gene expression\(^{29,30}\) responses to negative energy balance. The hormonal and/or metabolic cues that signal negative energy balance in the absence of leptin, which are poorly understood at this time, may be crucial components of the mechanisms integrating energy balance and cardiovascular function.

Conclusions

We have demonstrated that continuous ICV infusion of low levels of leptin strongly attenuates fasting-induced reductions in HR and \( V_O^2 \). The effects were observed with a dose of leptin that clearly reduced food intake and body weight but did not increase MAP or HR above resting levels. The findings are consistent with a key role for reduced leptin levels in the integrated regulation of the cardiovascular and metabolic responses to reduced energy intake. An important area for future investigation is the precise central nervous system mechanisms by which leptin and other metabolic/neuroendocrine cues, which are influenced by energy balance, regulate the cardiovascular system.

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References


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