Selective Resistance to Central Neural Administration of Leptin in Agouti Obese Mice

Kamal Rahmouni, William G. Haynes, Donald A. Morgan, Allyn L. Mark

Abstract—We recently demonstrated that in a rodent model of obesity (agouti yellow mice), there is a selective leptin resistance with preservation of the sympathetic actions despite loss of appetite and weight-reducing actions of systemic leptin. Here, we examined whether selective leptin resistance exists in agouti mice during central neural administration of leptin. In agouti obese mice and lean controls, we tested the effects of single intracerebroventricular (ICV) injection of leptin on vehicle on food intake and body weight in the conscious state and on renal sympathetic nerve activity during anesthesia. Agouti obese mice had higher (P<0.0001) mean arterial pressure (100±2 mm Hg) than lean controls (90±2 mm Hg). In lean controls (n=9 to 10), ICV leptin caused a dose-dependent decrease in body weight (P<0.001) and food intake (P<0.001). For example, ICV leptin (3 μg) decreased food intake and body weight, respectively, by 3.3±0.3 g (P<0.001) and 2.6±0.3 g (P<0.001) in lean mice. However, in agouti obese mice (n=9 to 10), ICV leptin did not significantly decrease food intake or body weight. ICV leptin caused in RSNA a significant and dose-dependent increase in renal sympathetic nerve activity that was of the same magnitude in the lean and agouti obese mice. The rise in renal sympathetic nerve activity induced by ICV leptin (3 μg) was 274±67% (P<0.001) in lean controls and 275±46% (P<0.001) in the agouti obese mice. In summary, this study indicates that selective leptin resistance in agouti obese mice occurs with central neural administration of leptin, suggesting that selective leptin resistance in this model is not due to a defect in leptin transport across the blood brain barrier. It seems to result instead from alterations in the central neural pathways mediating effects of leptin. (Hypertension. 2002;39[part 2]:486-490.)

Key Words: obesity ■ hypertension, obesity ■ sympathetic nervous system ■ mice

Leptin is an adipocyte-derived hormone that promotes weight loss by reducing appetite and food intake and by increasing energy expenditure through sympathetic stimulation to brown adipose tissue.1 Leptin also produces sympathoactivation to kidneys, hindlimb, and adrenal glands.2 This suggest that leptin contributes not only in the regulation of energy homeostasis but also in the control of cardiovascular function. This has been confirmed by chronic infusion of leptin that increases arterial blood pressure and heart rate in conscious rats.3 Furthermore, transgenic mice overexpressing leptin develop elevations of arterial pressure.4 In contrast, leptin-deficient mice, ob/ob mice, have reduced arterial pressure.5 These sympathetic and cardiovascular actions of leptin might contribute to the link between obesity and hypertension. In humans, it has been reported that plasma leptin concentration correlates significantly with arterial pressure6 and with muscle sympathetic nerve activity.7

Agouti yellow obese mice are a rodent model of obesity caused by ubiquitous overexpression of agouti protein that blocks hypothalamic melanocortin receptors.8 The obesity observed in these mice is associated with high levels of circulating leptin9 and elevated arterial pressure.5 These mice are resistant to the appetite- and weight-reducing effect of leptin.9 Nevertheless, leptin contributes importantly to regulation of arterial pressure in these mice.4 How can leptin contribute to the hypertension if the mice are leptin resistant? To explain this seeming paradox, we recently demonstrated that agouti mice have selective leptin resistance with preservation of the sympathetic action despite loss of the metabolic (appetite- and weight-reducing) actions of systemic leptin. The effects of systemic administration of murine leptin on food intake and body weight were significantly less in agouti obese mice than in lean controls, whereas the increase in renal sympathetic nerve activity (RSNA) was not different in agouti obese and lean mice.10

Several mechanisms may contribute to selectivity in leptin resistance. One potential mechanism involves downstream signaling pathways in the hypothalamus.11,12 Another possible explanation for selective leptin resistance relates to peripheral versus central actions of leptin. For example, Tanida et al13 showed that local injection of leptin in white adipose tissue can activate sympathetic nerve activity to the kidney. If the RSNA effects of leptin result from peripheral action, then selective leptin resistance could result from central neural resistance to metabolic effects and preservation of peripheral sympathetic action of leptin. However,
Haynes,14 from our laboratory showed that the sympathoexcitatory effects of leptin result from an action of this hormone in the central nervous system because intracerebroventricular (ICV) injection of leptin increases sympathetic nerve activity in rats, and lesions of the arcuate nucleus in the hypothalamus prevent the sympathetic responses to intravenous administration of leptin.14

In the present study, to determine whether central neural mechanisms explain selective leptin resistance, we examined whether selective leptin resistance exists in agouti mice with central neural administration of leptin. For this, we compared the metabolic and sympathetic effects of ICV administration of leptin in agouti obese mice and lean controls.

**Methods**

**Animals**

We studied 13- to 16-week-old male agouti yellow obese mice (C57BL/6J-Ay) and their wild-type controls C57BL/6J a/a (Jackson Laboratories). Animals were housed in a temperature-, humidity-, and light-controlled room (light/dark cycle of 12 hours each) with free access to food and tap water. The protocols were approved by the University of Iowa Animal Research Committee.

**Experimental Protocol**

To study the metabolic and sympathoexcitatory effects of ICV administration of murine leptin (Amgen, Inc) in agouti obese and lean mice, we performed 2 protocols. In protocol 1, we examined the effect of ICV injection of different doses of leptin or vehicle (0.9% NaCl) on food intake and body weight in conscious lean mice and agouti obese mice. In protocol 2, we examined the effect of the same doses of leptin or vehicle on RSNA in anesthetized lean mice and agouti obese mice.

At least 1 week before experimentation, each mouse received an ICV cannula as described.15 Briefly, animals were anesthetized with 91 mg/kg ketamine and 9.1 mg/kg xylazine intraperitoneally and placed in a stereotaxic apparatus (Kopf Instruments). A 25-G stainless steel guide cannula was implanted in the lateral ventricle of the brain with the following coordinates: 0.3 mm posterior and 1 mm lateral relative to bregma, and 3 mm down the skull surface. The proper position of the ICV cannula was verified at the end of each protocol by injection of methylene blue staining.

**Protocol 1: Study of the Metabolic Effects of Leptin**

In the groups of lean and obese mice assigned to study the metabolic effect of leptin, the animals were housed in individual cages 1 week before we started measuring daily food intake and body weight during 3 consecutive days. Then, each mouse received 1 ICV injection, given between 11 AM and 12 PM, in a volume of 2 μL over 1 minute. The mice received leptin at doses of 0.3, 1, 3, and 10 μg or vehicle. To perform the ICV injections, we placed the mice in plastic restraint cages. The feeding and weight responses to ICV leptin were assessed by measuring the changes in body weight and food intake during the 24-hour period after the ICV injection. The mice were then anesthetized, and blood samples were collected for plasma leptin and insulin measurements. As a more sensitive measure of adiposity, we determined the weights of 3 fat pads at euthanization. These included interscapular brown adipose tissue (BAT), epididymal fat, and renal fat.

**Protocol 2: Study of the Effect of Leptin on RSNA**

Other groups of lean and obese mice were assigned to study the effect of leptin on RSNA. Anesthesia was induced with ketamine/xylazine cocktail and sustained with α-chloralose (25 mg/kg per h). A jugular vein and carotid artery were cannulated for infusion of anesthetic and for hemodynamic recording (arterial pressure and heart rate), respectively. The trachea was also cannulated, and each mouse was allowed to breathe oxygen-enriched air spontaneously. Body temperature was maintained near 37.5°C using a temperature-controlled surgical table. For direct multifer recording of RSNA, a retroperitoneal incision was made and a nerve branch to the left kidney was carefully dissected free and placed on a bipolar 36-G platinum-iridium electrode (Cooner Wire). After an optimum recording of multifer RSNA was obtained, the electrode was fixed in place using silicone gel (Sil-Gel 604, Walker-Chimie). Nerve signals were amplified, filtered, and counted as described previously.2

After 10 to 20 minutes of stabilization, baseline RSNA and hemodynamic parameters were recorded for 10 minutes. An average of 3 separate measurements during the 10-minute control period was taken as baseline value for each animal. Each mouse received 1 ICV injection of leptin at doses of 0.3, 1, 3, or 10 μg or vehicle over 1 minute. Effects of leptin or vehicle on RSNA, arterial pressure, and heart rate were continuously recorded for 240 minutes. At the end of the protocol, blood samples were collected for plasma leptin and insulin assay. Animals were euthanized with a lethal dose of methohexital. Postmortem measurement of RSNA, considered as a background noise, was subtracted from the measurement obtained at each time point during the protocol. Plasma concentration of murine leptin and insulin were measured by radioimmunoassay using commercially available kits (both from Linco).

**Data Analysis**

Results are expressed as mean±SEM. Because of the animal-to-animal variability in baseline RSNA, the data for RSNA are expressed as percentage change (mean of the last hour of recording) from baseline. Data were analyzed using 1-way or 2-way analysis of variance. When analysis of variance reached significance, the Bonferroni test was used to compare the mean values among the different levels of mice type and treatment. A value of P<0.05 was considered significant.

**Results**

**Metabolic Effect of Leptin in Lean and Obese Mice**

At baseline, agouti obese mice had a higher (P<0.0001) body weight (31±0.5 g, n=47) compared with the lean controls (27±0.3 g, n=47), consistent with previous reports of moderate obesity in yellow agouti mice. The obese mice consumed less (P<0.0001) food (2.8±0.1 g) than lean controls (3.8±0.1 g).

As shown in Figures 1A and 1B, in lean controls, ICV injection of leptin caused a dose-dependent decrease in body weight (P<0.001) and food intake (P<0.001) measured 24 hours after the ICV injection. However, in agouti obese mice, ICV leptin did not significantly affect body weight or food intake, as compared with the vehicle. In lean mice, the decrease in body weight induced by ICV leptin was associated with a decrease (P=0.0009) in BAT measured 24 hours after the ICV injection (Table). The BAT mass decreased in a dose-dependent manner. Epididymal fat and renal fat also tended to decrease, but these changes were not statistically significant compared with the vehicle group (Table). In the obese mice, fat pads were not affected by ICV leptin as compared with the vehicle (Table).

As previously reported, the plasma levels of leptin and insulin were significantly higher (P<0.001) in the obese animals compared with the lean controls. ICV injection of leptin did not affect plasma levels of these 2 hormones in either lean or obese mice (Table).

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Sympathetic Effect of Leptin in Lean and Obese Mice

The agouti obese mice had higher (P<0.0001) basal mean arterial pressure (100±2 mm Hg, n=39) than lean controls (90±2 mm Hg, n=36). As these baseline values were obtained under anesthesia, we confirmed in awake state with an indwelling arterial catheter that mean arterial pressure was higher (P<0.01) in the obese mice (124±5 mm Hg, n=6) than in wild-type littermates (92±6 mm Hg, n=8). Heart rate (bpm) and RSNA (volts/second per minute) did not differ significantly between obese (respectively, 444±47 and 1.60±0.24) and lean mice (412±98 and 1.55±0.15).

As depicted in Figure 2, ICV leptin caused a significant (P<0.001) and dose-dependent increase in RSNA. The rise in RSNA induced by each dose of ICV leptin was of the same magnitude in the lean and obese mice (P=0.76). ICV leptin did not affect significantly mean arterial pressure and heart rate in both lean and obese mice (data not shown).

Compared with the lean controls, the obese mice had substantially higher levels of plasma leptin (respectively, 19±2.3 and 38±4 ng/mL; P=0.0002) and insulin (1.1±0.1 and 4.8±1.2 ng/mL; P=0.003). Plasma levels of these 2 hormones (measured here 4 hours after ICV injection) also were not affected by ICV leptin in lean and obese mice (data not shown).

### Table 1: Plasma Leptin and Insulin, and Fat Pad Weight 24 Hours after ICV Administration of Leptin and Vehicle in Lean Controls and Agouti Obese Mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle (µg)</th>
<th>0.3</th>
<th>1</th>
<th>3</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agouti lean mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma leptin, ng/ml</td>
<td>4.6±1.2</td>
<td>5.0±1.0</td>
<td>5.8±1.3</td>
<td>6.0±0.9</td>
<td>5.6±2.0</td>
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<tr>
<td>Plasma insulin, ng/ml</td>
<td>0.8±0.2</td>
<td>0.7±0.1</td>
<td>0.7±0.2</td>
<td>0.5±0.1</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>BAT, g</td>
<td>0.093±0.008</td>
<td>0.066±0.003*</td>
<td>0.065±0.004*</td>
<td>0.062±0.006*</td>
<td>0.055±0.008*</td>
</tr>
<tr>
<td>Epididymal fat, g</td>
<td>0.40±0.06</td>
<td>0.36±0.04</td>
<td>0.26±0.06</td>
<td>0.33±0.04</td>
<td>0.24±0.04</td>
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<tr>
<td>Renal fat, g</td>
<td>0.09±0.02</td>
<td>0.08±0.01</td>
<td>0.07±0.02</td>
<td>0.07±0.01</td>
<td>0.05±0.01</td>
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<tr>
<td>Agouti obese mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma leptin, ng/ml</td>
<td>19.0±2.7†</td>
<td>24.0±0.8†</td>
<td>23.0±0.7†</td>
<td>22.0±3.7†</td>
<td>21.0±0.3†</td>
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<tr>
<td>Plasma insulin, ng/ml</td>
<td>2.0±0.3†</td>
<td>1.3±0.3†</td>
<td>1.3±0.5†</td>
<td>1.3±0.3†</td>
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</tr>
<tr>
<td>BAT, g</td>
<td>0.106±0.006</td>
<td>0.081±0.011</td>
<td>0.083±0.005</td>
<td>0.083±0.008</td>
<td>0.077±0.004</td>
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<td>Epididymal fat, g</td>
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<td>1.42±0.17</td>
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<td>Renal fat, g</td>
<td>0.34±0.08</td>
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<td>0.30±0.04</td>
<td>0.20±0.04</td>
</tr>
</tbody>
</table>

Data are mean±SEM of 5 to 9 animals per group. †P<0.05 compared with saline; †P<0.05 compared with agouti lean.
Discussion

The primary new finding from the present study was that selective leptin resistance in agouti obese mice occurs with central neural as well as with systemic administration of leptin. In a previous study, we demonstrated preservation of the sympathetic action despite loss of the metabolic actions of systemic leptin in agouti mice. The present findings extend our previous report in 2 ways. First, in agouti obese mice, direct administration of 1 dose of leptin in the lateral ventricle of the brain increased sympathetic nerve activity to the kidney in a dose-dependent manner but failed to affect body weight, food intake, and BAT mass. Second, the increase in RSNA occurred without any change in plasma levels of leptin and insulin.

The effects of leptin on metabolic function and sympathetic nerve activity were studied in different mice. One potential limitation of the present study is that the metabolic effects of leptin were studied in conscious mice during a period of 24 hours, whereas sympathetic actions of leptin were assessed in anesthetized mice during 4 hours. However, anesthesia does not affect the sympathetic responses to different stimuli, including baroreflex activation and hemorrhage. We demonstrate here that the phenomenon of selective leptin resistance in the agouti obese mice is not due to a defect in leptin transport across the blood brain barrier. Leptin from the plasma is transported to the central nervous system by a saturable, unidirectional system that involves binding of leptin to the short form of the leptin receptor located at the endothelium of the vasculature and the epithelium of choroid plexus. Saturation in the transport of leptin into the central nervous system represents one potential mechanism of leptin resistance in obesity, in which the high-circulating endogenous leptin fails to decrease body weight. In support of this idea is the observation of decreased cerebrospinal fluid-serum ratio for leptin with increasing obesity in humans and the ability of leptin to decrease body weight in mice with diet-induced obesity if given centrally but not peripherally. However, data from the present study as well as from a previous report show that resistance to the appetite- and weight-reducing actions of leptin in agouti obese mice does not result from absence of functional transport system to the brain for leptin, because short-term or long-term ICV administration of leptin failed to affect food intake and body weight in these mice. Furthermore, absence of changes in circulating levels of leptin suggests that the increase in RSNA after ICV administration of leptin was caused by an action of this hormone in the brain.

Selective leptin resistance may help explain how high levels of circulating leptin in agouti mice contribute to hypertension despite resistance to its metabolic effects. Long-term renal sympathetic stimulation by leptin could raise arterial pressure by causing peripheral vasoconstriction and by increasing renal tubular sodium reabsorption. This would predict increased RSNA in agouti mice. Using direct nerve recording, we failed to see a difference in RSNA at baseline between lean and obese mice. However, this method of sympathetic nerve recording is not reliable to measure absolute levels of sympathetic activity but is best for measuring responses to a short-term stimulus.

The hypophagia in agouti obese mice during the control period and the reduction in body weight and food intake observed in these obese mice after ICV administration of vehicle were also intriguing. The hypophagia could be explained by the isolation of the animals for 1 week and the handling (to measure the body weight) during the control period. The decrease in food intake and body weight after ICV vehicle could be due to restraint stress to which these mice were submitted during the ICV injection. Such effects of isolation and restraint stress on feeding and body weight have been documented in agouti mice and transgenic mice overexpressing agouti protein. De Souza et al reported that isolation decreased food intake in agouti obese mice but not in lean controls. Furthermore, restraint stress caused a more marked decrease in body weight and food intake in agouti obese mice and transgenic mice overexpressing agouti protein compared with their controls.

In conclusion, we have demonstrated that acute ICV administration of leptin in agouti obese mice increases sympathetic nerve activity to the kidney in a dose-dependent manner, without effecting body weight, food intake, and BAT mass. Thus, selective leptin resistance in these obese mice cannot be attributed to a defect in leptin transport across the blood brain barrier. It seems to result instead from alterations in leptin signaling pathways in the brain.

Acknowledgments

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References


