The Concept of Selective Leptin Resistance
Evidence From Agouti Yellow Obese Mice

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Leptin, a hormone secreted by adipose tissue, acts to inhibit appetite and promote metabolism, thereby reducing body weight. Leptin also increases sympathetic activity and arterial pressure. Several murine models of obesity, including agouti obese mice, exhibit resistance to the anorexic and weight-reducing effects of leptin. Hypertension in agouti mice has been attributed to hyperleptinemia. These observations pose a seeming paradox. If these mice are leptin-resistant, then how can leptin contribute to hypertension? We tested the novel hypothesis that these mice have selective leptin resistance, with preservation of the sympathoexcitatory action despite resistance to the weight-reducing actions. Leptin-induced decreases in food intake and body weight were less in agouti obese mice than in lean littermates. In contrast, leptin-induced increases in sympathetic nerve activity did not differ in obese and lean mice. These findings support the concept of selective leptin resistance, with resistance to the metabolic actions of leptin but preservation of the sympathoexcitatory actions. This finding may have potential implications for human obesity, which is associated with elevated plasma leptin and is thought to be a leptin-resistant state. If leptin resistance is selective in obese humans, then leptin could contribute to sympathetic overactivity and its adverse consequences in human obesity. *Diabetes* 51:439–442, 2002

Leptin suppresses appetite and promotes weight loss (1,2). In addition, leptin increases sympathetic nerve activity (3) and arterial pressure (4,5).

Agouti yellow obese (Ay) mice develop obesity because of blockade of hypothalamic melanocortin-4 receptors secondary to ectopic expression of agouti peptide (6–9). The agouti mice are resistant to the satiety and weight-reducing actions of leptin (2), even though they do not have mutations in the leptin receptor gene.

Ay mice have higher arterial pressure than their lean littermates (10). The agouti mice have hyperleptinemia (2,5), and a recent study indicated that the elevated leptin contributes to regulation of arterial pressure (5).

This brings us to a seeming paradox and to the focus of this study. How can leptin contribute to regulation of arterial pressure in agouti mice if these mice are resistant to the effects of leptin? In this study, we tested the hypothesis that Ay mice have selective leptin resistance, with preservation of the sympathetic actions of leptin despite resistance to the metabolic actions.

RESEARCH DESIGN AND METHODS

We tested the effects of exogenous leptin on body weight, food intake, and sympathetic nerve activity in agouti yellow obese (C57BL/6J-A^y) and lean littermates (C57BL/6Ja/a) aged 12–14 weeks, which were purchased from Jackson Laboratories. All procedures were approved by the University of Iowa Animal Research Committee.

Recording of renal sympathetic nerve activity. To measure direct multifiber renal sympathetic nerve activity (SNA) in anesthetized mice, we made a left retroperitoneal incision and carefully isolated a nerve fascicle to the left kidney. A bipolar platinum-iridium electrode (Cooner Wire) was suspended under the nerve and secured with silicone gel (Sil-Gel 604; Wacker-Chemie). The electrode was attached to a high-impedance probe (HIP-511; Grass Instruments), and the nerve signal was amplified 10 times with a Grass P5 AC preamplifier. After amplification, the nerve signal was filtered at a 100- and 1,000-Hz cutoff with a nerve traffic analysis system (model 706C; University of Iowa Bioengineering). Subsequently, the amplified and filtered nerve signal was routed to an oscilloscope (model 54501A; Hewlett-Packard), whose cursor was positioned precisely above the background noise. The nerve traffic analyzer counted the action potentials that exceeded this threshold voltage. Both the counted action potentials and the renal neurogram were routed to a MacLab analogue-digital converter (model 8S, AD Instruments) for permanent recording and data analysis on a Macintosh 9500 computer.

Design. Each mouse was housed individually in a Plexiglas cage, under a 12-h light/dark cycle with lights on at 6 A.M. and off at 6 P.M. Animals had free access to water and regular mouse diet. The environmental temperature was maintained at 23°C. Mice were allowed to adapt to the animal care unit for at least 7 days before the study.

Groups of lean and obese mice were assigned to receive murine leptin (Amgen) at 30, 60, or 100 μg or 0.9% NaCl (3 μl) intraperitoneally twice daily. Body weight and food intake were measured daily at 8–10:00 A.M. for 3 consecutive days before and again during leptin or vehicle.

On the fourth day of leptin or vehicle, mice were anesthetized with an intraperitoneal injection of ketamine (91 mg/kg) and xylazine (9.1 mg/kg). Catheters were introduced into a carotid artery and jugular vein. Subsequently, the renal nerve was dissected through a retroperitoneal incision. The renal nerve was attached to an electrode for measurements of renal SNA as described previously. Arterial pressure and heart rate were continuously monitored through a pressure transducer connected to the carotid artery catheter. Body temperature was measured throughout the study. Hemodynamic and nerve signals were digitalized in a MacLab converter and displayed, stored, and analyzed on a Macintosh 9500 computer.

Baseline renal SNA and hemodynamic variables were recorded for 20 min. Leptin (30, 60, or 100 μg) or 30 μl of vehicle was then administered intravenously over 5 min. Renal SNA, heart rate, and arterial pressure responses to leptin or vehicle were recorded continuously for 150 to 240 min. Anesthesia was sustained with the administration of chloralose (25 mg · kg^{-1} · h^{-1}) intravenously. Body temperature was maintained at 37.5°C with the assistance of a lamp and a heating pad. At the end of the study, blood samples

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SNA, sympathetic nerve activity.

TABLE 1
Body weight, food intake, and plasma leptin

	n	Baseline		Cumulative Δ food intake (g)	Day 4 plasma leptin (ng/ml)
		Weight (g)	Food intake (g)		
Lean					
Vehicle	9	27 ± 1	9.7 ± 0.7	−0.2 ± 0.5	3 ± 1
Leptin (μg b.i.d.)					
30	8	27 ± 1	12.9 ± 0.9	−2.5 ± 0.7*	38 ± 10*
60	11	27 ± 1	8.8 ± 1.0	−2.6 ± 0.7*	67 ± 12*
100	11	28 ± 1	12.1 ± 0.6	−3.5 ± 0.3*	142 ± 35*
Obese					
Vehicle	9	35 ± 2†	11.4 ± 0.6	−2.0 ± 0.7	20 ± 7†
Leptin (μg b.i.d.)					
30	8	30 ± 2	12.8 ± 0.8	−1.3 ± 0.4	52 ± 9*
60	12	32 ± 1	4.8 ± 0.4	1.2 ± 0.5†	72 ± 12*
100	11	30 ± 1	11.8 ± 0.6	−1.9 ± 0.5†	118 ± 26*

Data are means ± SE. *$P < 0.05$ versus saline; †$P < 0.05$ versus lean.

were collected for plasma leptin measurements. Animals were killed with a lethal dose of methohexital.

Data analyses. Results are expressed as means ± SE. Sympathetic nerve firing rate was corrected for background noise by subtracting postmortem measurements from the measurement obtained at each time point during the experiment. Values from three separate baseline measurements did not differ significantly and were therefore averaged for each animal. Because there is significant interindividual variation in baseline renal SNA, the data for renal SNA are expressed as percentage change from baseline. Differences between leptin-treated and control mice were assessed by use of Student's t test or repeated-measures ANOVA, with statistical testing by Schaffe's F test. Statistical analysis was performed using StatView software for Macintosh (Version 4; Abacus Concepts). $P < 0.05$ was considered to be statistically significant.

RESULTS

Leptin caused dose-dependent suppression of food intake ($P = 0.0001$; ANOVA linear trend) and weight loss ($P < 0.0001$) in lean animals. As expected, obese animals were substantially resistant to the anorexic effects of leptin, with no significant change in food intake ($P = 0.77$) or body weight ($P = 0.19$) after leptin administration (Table 1, Fig. 1*A*). Leptin produced significantly less effect on body weight ($P = 0.006$) and food intake ($P = 0.0001$) (Table 1, Fig. 1*A*) in obese mice compared with lean mice.

Leptin caused substantial increases in SNA to the kidney in both obese ($P = 0.0047$) and lean mice ($P = 0.0001$). There was no significant difference in effects of leptin on SNA between lean and obese mice ($P = 0.17$) (Fig. 1*B*).

In anesthetized mice, arterial pressure and core temperature did not differ between leptin and saline groups. Basal plasma leptin levels were substantially higher in obese than in lean mice after saline ($P = 0.03$), consistent with chronic leptin resistance in the obese animals. Intravenous leptin increased plasma leptin levels identically in lean and obese mice (Table 1).

Because it was possible that pretreatment with leptin might have altered sympathetic responses to subsequent administration of leptin, we performed additional experiments with a single dose of intravenous leptin (100 μg) in previously untreated lean ($n = 8$) and obese animals ($n = 10$). The leptin-induced increases in renal SNA again did not differ between lean (125 ± 33%) and obese animals (154 ± 31%; $P = 0.5$).

DISCUSSION

The major finding from this study is that Ay mice have preservation of the sympathoexcitatory action of leptin despite resistance to the metabolic actions on appetite and

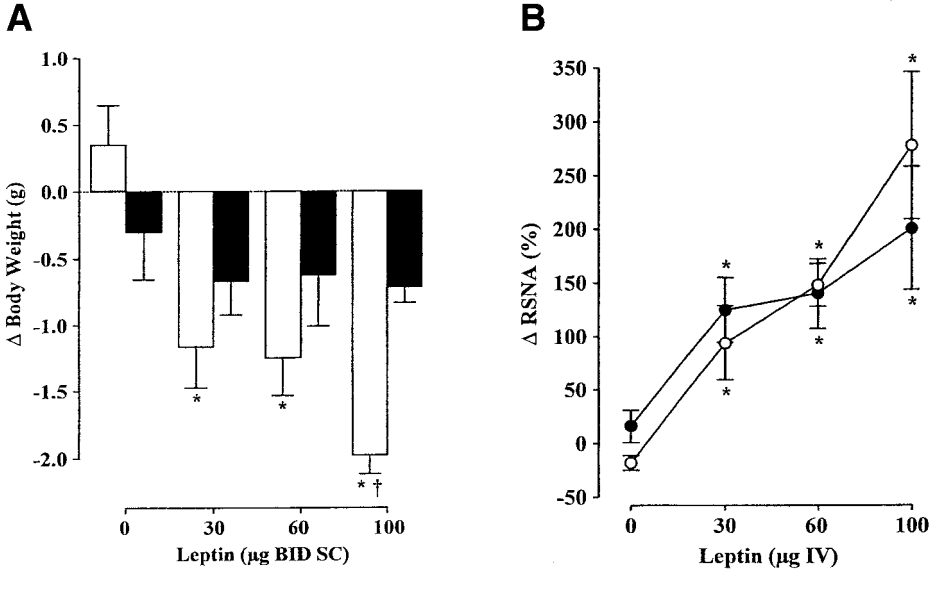

FIG. 1. *A*: Change in body weight after varying doses of intraperitoneal leptin (twice daily for 3 days) in lean (□) and obese (■) Ay mice. There was a significant difference between lean and obese mice in the effects of leptin on body weight ($P = 0.006$). *$P < 0.05$ versus saline; †$P < 0.05$ versus obese. *B*: Effect of bolus intravenous administration of leptin on SNA to kidney in anesthetized lean (○) and obese (●) Ay mice. There was no significant difference between lean and obese mice in the effects of leptin on sympathetic traffic.

body weight. This finding supports the novel concept of selective leptin resistance.

There are several strengths to this study. The sympathetic and metabolic responses were studied in the same mice. Several doses of leptin were used. We assessed both the sympathetic and metabolic actions using physiologic end points rather than some physiologic and some molecular biologic end points.

We recognize several potential limitations of the study. First, the metabolic actions of leptin were assessed over several days in conscious mice, whereas the sympathetic actions were assessed over several hours in anesthetized mice. However, the sympathetic responses were robust, and it seems unlikely that anesthesia would explain preservation of the sympathetic responses in the Ay mice. Second, the study did not identify the neurophysiologic and molecular biologic mechanisms of the selective leptin resistance. Third, the exogenous administration of leptin was superimposed on different endogenous levels of leptin in the agouti obese and the lean wild-type mice. It is unlikely that this would explain a dissociation of the sympathetic and metabolic responses in the same mice over a range of doses of leptin.

We speculate that the phenomenon of selective leptin resistance reflects alterations in hypothalamic signaling pathways downstream from leptin receptors, i.e., from central neural alterations. The metabolic effects of leptin originate in the hypothalamus (2), and we previously demonstrated that the sympathetic actions also emanate from the hypothalamus (11). Brain cerebroventricular administration of leptin reproduces the sympathetic effects of systemic leptin (11). In addition, lesioning of the arcuate nucleus abolishes the sympathetic nerve responses to systemic leptin (11).

However, other investigators have reported that leptin injected into white adipose tissue can increase sympathetic nerve activity, presumably by activating sensory afferents in white adipose tissue (12–14). Therefore, in contrast to our speculation, one could suggest that selective leptin resistance reflects preservation of peripherally originating sympathetic actions of leptin with attenuation of central neural metabolic actions because transport of leptin across the blood-brain barrier is attenuated in agouti obese mice. We believe that this alternative explanation is unlikely because we have preliminary evidence that selective leptin resistance also pertains to brain cerebroventricular administration of leptin in agouti obese mice (K.R., D.A., W.G.H., A.L.M., unpublished data).

The preservation of the sympathetic actions of leptin in the presence of compensatory hyperleptinemia in agouti obese mice might explain how leptin contributes to hypertension in agouti mice despite metabolic leptin resistance. Leptin did not increase arterial pressure in either lean or obese mice in this study. This might be explained by effects of anesthesia. Alternatively, it might reflect the study of acute responses to leptin. Several studies have demonstrated that acute administration of leptin does not increase arterial pressure, whereas increases in pressure are observed with chronic administration. The increases in arterial pressure with chronic elevation of circulating leptin are reversed by alpha adrenergic blockade (5). This indicates the importance of sympathetic activation in the pressor action of leptin. The renal sympathetic nerves seem to increase arterial pressure substantially by increasing renal tubular sodium reabsorption (15). This might explain a delay between leptin-induced increases in renal sympathetic nerve activity and leptin-induced increases in arterial pressure.

We (16) and other investigators (17) have reported that hypothalamic melanocortin pathways mediate at least partly the metabolic and sympathetic responses to leptin. These studies predict that the sympathetic and blood pressure effects of leptin are attenuated in agouti obese mice with blockade of melanocortin receptors; however, we found preservation of the renal sympathetic responses in this study, and Aizawa-Abe et al. (5) observed preservation of the pressor action of leptin in agouti mice.

The concept of selective leptin resistance has potential implications for human obesity, which is commonly associated with elevated plasma leptin and is thought to be a state of partial leptin resistance (18). Human obesity is associated with increased sympathetic outflow (19,20). If leptin resistance is selective in humans, then leptin could contribute to sympathetic overactivity and its adverse consequences in human obesity.

In summary, we demonstrated that the inhibitory effects of leptin on appetite and weight can be dissociated from its stimulation of sympathetic nerve traffic in agouti obese mice. These data support the novel concept of selective leptin resistance.

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