Melanocortin-4 Receptor Mediates Chronic Cardiovascular and Metabolic Actions of Leptin

Lakshmi S. Tallam, Alexandre A. da Silva, John E. Hall

Abstract—This study tested whether the melanocortin 4-receptor (MC4R) is essential for the chronic cardiovascular and metabolic actions of leptin. Twenty- to 22-week-old male wild-type (WT) C57BL/6J and obese MC4R (−/−) mice (N=5 to 6 per group) were implanted with radiotelemetric transmitters and catheters for measuring mean arterial pressure (MAP) and heart rate 24 hours per day and intravenous infusions. After a 3-day stable control period, leptin was infused (2 µg/kg per minute IV) for 7 days in WT, obese ad libitum–fed MC4R (−/−), and nonobese pair–fed MC4R (−/−) mice. WT mice receiving vehicle for 7 days served as controls. MC4 (−/−) mice were 30% heavier and had 4- and 11-fold increases in plasma insulin and leptin levels, respectively, compared with WT mice. Despite obesity, MAP and heart rate tended to be lower in MC4R (−/−) mice compared with WT mice. Chronic leptin infusion in the different groups increased plasma leptin levels to 45 to 65 ng/mL. Seven-day leptin infusion in WT mice increased MAP by 12±3 mm Hg despite a 35% reduction in food intake and an 8% reduction in body weight. Leptin did not alter plasma glucose but reduced plasma insulin in WT mice (5.9±1.0 versus 3.0±0.5 µU/mL). These cardiovascular and metabolic actions of leptin were abolished in obese and nonobese MC4R (−/−) mice. These data suggest that MC4R deficiency, and not obesity-induced leptin resistance, abolished the cardiovascular and metabolic actions of leptin in obese MC4R (−/−) mice. Thus, a functional MC4R is essential for the chronic cardiovascular and metabolic actions of leptin. (Hypertension. 2006;48:58-64.)

Key Words: hypertension ■ arterial pressure ■ heart rate ■ insulin ■ insulin resistance ■ obesity ■ hypothalamus ■ sympathetic nervous system

Leptin, a 167-amino–acid peptide released from adipocytes, acts on the central nervous system (CNS) to suppress appetite and increase energy expenditure by increasing sympathetic nervous system (SNS) activity to thermogenic tissues, such as the brown adipose tissue.1,2 Leptin also increases sympathetic activity to nonthermogenic organs, such as the kidneys and adrenal glands.2 Moreover, chronic leptin infusion in rats increases arterial pressure, and this effect can be inhibited by combined α- and β-adrenergic receptor blockade, suggesting a role for the SNS in the pressor actions of leptin.3 Neuroanatomical and functional studies indicate an important role for the hypothalamic pro-opiomelanocortin (POMC) neurons in mediating some of the actions of leptin. POMC neurons are principal sites of leptin receptor expression, and leptin increases POMC neuronal firing and POMC protein expression.4,5 Conditional POMC neuron-specific leptin receptor deletion in mice leads to a modest increase in body weight.6 One of the byproducts of hypothalamic POMC post-translational processing, α-melanocyte–stimulating hormone (α-MSH), is considered to be an important mediator of some of the action of leptin to reduce food intake. α-MSH binds the hypothalamic melanocortin 3/4 receptors (MC3/4R) to elicit metabolic actions similar to those elicited by leptin.7 Furthermore, acute studies have shown that treatment with an MC3/4R antagonist abolishes the effects of leptin on food intake and renal SNS activity.8,9 We have shown that MC3/4R antagonists also abolish the chronic cardiovascular actions of leptin.10 These data suggest that α-MSH and the hypothalamic MC3/4R may play a pivotal role in mediating some of the cardiovascular and metabolic actions of leptin.

Previous studies implicate both the MC3R and the MC4R in regulating cardiovascular and metabolic function. γ-MSH, a specific MC3R agonist, increases arterial pressure when administered into the CNS in rodents.11,12 Adult MC3R-deficient (−/−) mice have increased adipose mass despite hypophagia and normal metabolic rates but do not have increased body weight compared with wild-type (WT) mice.13 In contrast, adult MC4R (−/−) mice are hyperphagic and are much heavier than WT mice despite severe hyperleptinemia.14 Although the phenotype of MC4R (−/−) mice supports the view that the MC4R may be important for the full expression of the metabolic effects of leptin, acute studies have also demonstrated that the anorexic actions of leptin are maintained in young, nonobese MC4R (−/−) mice.15 Thus, it is still unclear whether activation of the MC4R plays an essential role in mediating the long-term metabolic actions of leptin.

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The specific role of the MC4R in regulating cardiovascular function is also not fully understood. We have shown that MC4R (−/−) mice are not hypertensive despite hyperinsulinemia, visceral adiposity, and obesity, suggesting that the MC4R may be an important link between obesity and hypertension. The acute actions of leptin to raise renal sympathetic activity are also abolished in MC4R (−/−) mice, suggesting that the MC4R may mediate the sympathoexcitatory actions of leptin. However, there have been no previous studies, to our knowledge, that have examined the specific role of the MC4R in the long-term actions of leptin on arterial pressure, renal function, and metabolism, especially at circulating leptin levels that are found under physiological or pathophysiological conditions. The main goal of these studies was, therefore, to determine the role of the MC4R in mediating the chronic cardiovascular, renal, and metabolic responses to leptin at levels that might be encountered in obesity using WT and MC4R (−/−) mice.

Methods

All of the experimental procedures and protocols conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center. Male WT C57Bl/6J mice and MC4R (−/−) mice (22 weeks old) maintained on the C57Bl/6J background for 10 generations were used in our studies. The MC4R (−/−) mice were bred in our transgenic facility and genotyped as described previously. Whereas the C57Bl/6J WT mice were obtained from Jackson Laboratories (Bar Harbor, ME).

Surgical Protocols

Implantation of the Telemetry Transmitter

Under anesthesia with isoflurane, mice were implanted with a telemetry pressure transmitter in the carotid artery (Model TA11PAC40, DSI) to monitor mean arterial pressure (MAP) and heart rate (HR) 24 hours per day as described previously.

Implantation of Femoral Arterial and Venous Catheters

One week after transmitter implantation, mice were implanted with femoral arterial and venous catheters for the collection of blood samples and for constant infusion of saline or drug solutions, respectively. Under isoflurane anesthesia, using aseptic techniques, a sterile catheter (microenathane tubing, tip diameter of ~200 μm, Braintree Scientific, Inc) was introduced into the vena cava through a femoral vein incision, sealed using a pin, and secured using silk suture and tissue adhesive. Similarly, through a femoral arterial incision, another sterile catheter (renapulse tubing, tip diameter of ~300 μm, Braintree Scientific) was introduced into the aorta, sealed using a pin, and secured. Catheters were tunneled subcutaneously and exteriorized between the scapulae and passed through a vinyl tubing with a flared button end, which was later sutured to the muscle between the scapula. The vinyl tubing was used to hook the mice to a swivel and arm system (Instech) mounted on a metabolic cage specially designed for mice.

Mice were moved to metabolic cages 4 to 5 hours after recovery from surgery, and the venous catheter was connected through a sterile filter to a syringe pump for continuous saline infusion (3 mL per day). Mice received food and water ad libitum throughout the study. A normal sodium intake of ~462 μeq per day was maintained via a constant saline infusion combined with sodium-deficient chow (0.006 mmol sodium/g food, Teklad). Mice were allowed to recover for 7 to 10 days before control measurements were initiated. To prevent the femoral arterial catheters from clotting, catheters were flushed daily with 5% dextrose solution and filled with 250 U/mL of heparin until 3 days after surgery at which the catheters were filled with 1000 U/mL of heparin.

Experimental Design

Four groups of mice were used in this study. After a 3-day control period, ad libitum–fed male WT (n=6), obese MC4R (−/−) mice (n=6), and pair-fed nonobese MC4R (−/−) mice (n=6) received an intravenous infusion of leptin (2 μg/kg per minute) for 7 days, beginning after the third day of control measurements. WT mice (n=6) receiving isotonic saline intravenous infusion served as time controls. Pair-fed nonobese MC4R (−/−) mice were included in the study to test whether obesity-induced leptin resistance might contribute to the observed attenuation of the responses of leptin in the obese ad libitum–fed MC4R (−/−) mice. Pair-fed MC4R (−/−) mice were prevented from becoming obese by feeding them the same amount of food as WT mice for 10 to 12 weeks starting at 8 to 10 weeks of age and continuing until they were 22 weeks of age. The rate of leptin infusion, 2 μg/kg per minute, was selected from our preliminary studies demonstrating this dose to be the lowest that yielded pressor responses and marked hypophagia while producing plasma leptin levels similar to that found in obesity.

Twenty-four–hour telemetered arterial pressure and HR, urine volume, urinary sodium, and potassium excretion, and food and water intake were recorded daily. Average nighttime (6:00 PM to 6:00 am) and daytime (6:00 AM to 6:00 pm) were also determined. Six- to 8-hour fasting blood samples (100 μL) were collected once during the control (day 3) and during leptin infusion (day 7) for measurements of glomerular filtration rate (GFR) and plasma insulin, glucose, and leptin concentrations. The blood samples were replaced with 100 μL of 0.9% saline.

Analytical Methods

Plasma insulin and leptin concentrations were determined by ELISA (Linco Insulin ELISA kit and R&D leptin ELISA kit), and plasma glucose concentrations were determined using the glucose oxidation method (Beckman glucose analyzer 2). Urinary sodium and potassium concentrations were measured using ion-sensitive electrodes (NOVA electrolyte analyzer 1+). GFR was calculated from average 24-hour clearance of [125I]-iothalamate after a 24-hour intravenous infusion as described previously.

Statistical Methods

The data are expressed as mean±SEM. All of the data obtained were analyzed by paired t test or 1-way ANOVA with repeated measures followed by Dunnett’s post hoc test for comparison between control and experimental values within each group where appropriate. Comparisons between different groups were done by unpaired t test or 1-way ANOVA followed by Dunnett’s post hoc test where appropriate. Statistical significance was accepted at a level of P<0.05.

Results

Body Weights, Visceral Adiposity, Food Intake, and Hormones

Body weights of the ad libitum–fed MC4R (−/−) mice, before leptin infusion, were 30% greater than WT mice (40±2 versus 31±1 g). Food intake of ad libitum–fed MC4R (−/−) mice was 23% greater than WT mice. Pair feeding the MC4R (−/−) mice the same amount of food eaten by WT mice for 10 weeks resulted in body weights (31±1 g) that were not different than those of WT mice. Also, pair feeding significantly reduced the visceral fat pad weights in MC4R (−/−) mice to a level not significantly different than in WT mice (Table 1).

Leptin infusion for 7 days significantly decreased food intake in WT mice by 35%, from an average value of 4.1±0.2 g per
day in the control period to 2.7±0.2 g per day, whereas vehicle infusion did not significantly alter food intake (Figures 1 and 2). Associated with the decrease in food intake, chronic leptin infusion in WT mice decreased body weight from 31±1 g to 28±1 g by the end of the 7-day experimental period. Chronic hyperleptinemia also significantly reduced visceral epididymal and retroperitoneal fat pad weights in WT mice to 17% to 30% of the values observed in vehicle-infused WT mice (Table 1). The effects of leptin on food intake and body weight were abolished in obese ad libitum and nonobese pair-fed MC4R (/−−/) mice (Table 1).

Plasma leptin levels in obese MC4R (/−−/) mice were several-fold greater than in WT mice (Table 1). Pair feeding restored the plasma leptin levels of MC4R (/−−/) mice to levels that were not significantly different than in WT mice. Leptin infusion for 7 days in the different groups of mice raised plasma leptin levels to 45 to 65 ng/mL, values similar to those found in human obesity. Plasma leptin levels were not significantly altered in the vehicle-infused WT mice (Table 1). Chronic leptin infusion did not significantly alter plasma glucose levels in any of the groups of mice. Fasting plasma glucose levels were slightly greater in obese MC4R (/−−/) mice as compared with WT mice (Table 1). However, obese MC4R (/−−/) mice had several-fold greater plasma insulin levels compared with WT mice. Chronic leptin infusion decreased plasma insulin levels in WT mice, but this effect was abolished in both groups of MC4R (/−−/) mice (Table 1). Pair feeding reduced the plasma insulin level in MC4R (/−−/) mice but did not restore it to normal as in WT mice (Table 1).

**Hemodynamics**

There were no significant differences in 24-hour MAP between MC4R (/−−/) mice and WT mice during the control period, although the MC4R (/−−/) mice tended to have lower MAPs compared with WT mice (WT, 114±3; MC4R [−−−], 111±1; pair-fed MC4R [−−−], 108±4 mm Hg). Also, the average daytime and nighttime MAPs were also not significantly different in MC4R (/−−/) and WT mice during the control period (Table 2). Leptin infusion for 7 days in WT mice significantly increased 24-hour MAP despite a 35% decrease in food intake and significant decrease in body weight. The peak increase in MAP during 7 days of leptin infusion at daytime and nighttime was 13 and 10 mm Hg, respectively. This effect of leptin on MAP was abolished in both ad libitum and pair-fed MC4R (/−−/) mice (Figures 1 and 2 and Table 2). There were no significant changes in MAP in the vehicle-infused mice. Basal 24-hour HR in the MC4R (/−−/) mice also tended to be lower as compared with WT mice (WT, 615±40; MC4 [−−−], 589±40; pair-fed MC4R [−−−], 556±47 bpm). Although daytime HRs were not significantly different between groups, at nighttime, MC4R (/−−/) mice had significantly lower HR compared with WT mice (Table 2). Chronic hyperleptinemia did not alter HR in any of the groups of mice at daytime or nighttime (Figure 1 and Table 2).

**Renal Function**

Twenty-four-hour urine volumes and water intake were significantly greater in the MC4R (/−−/) mice compared with WT mice (Table 1). Urine volume and sodium excretion rates were not significantly altered by vehicle or leptin infusion in any of the groups of mice (Table 1). Leptin infusion did not significantly alter GFR in WT or MC4R (/−−/) mice (Table 1).

**Discussion**

The most significant finding of the present study is that the chronic effects of hyperleptinemia on arterial pressure, food intake, and plasma insulin levels are abolished in obese ad libitum–fed and pair-fed nonobese MC4R (/−−/) mice. These observations suggest that a functional MC4R is necessary for the chronic actions of leptin to raise arterial pressure, to reduce food intake, and to reduce plasma insulin levels. Moreover, obesity-induced leptin resistance did not seem to contribute to the attenuation of the actions of leptin in the obese MC4R (/−−/) mice, because the effects of leptin were abolished even in nonobese MC4R (/−−/) mice.

### TABLE 1. Effect of Chronic Hyperleptinemia on Body Weight, Visceral Fat Weights, Renal Function, and Circulating Hormones

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Weight, g</th>
<th>Epi. Fat Pad wt., g</th>
<th>Ret. Fat Pad wt., g</th>
<th>Glucose, mg/dL</th>
<th>Insulin, μU/mL</th>
<th>Leptin, ng/mL</th>
<th>UV, mL/d</th>
<th>UNaV, mmol/d</th>
<th>GFR, mL/min</th>
<th>Water Intake, mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT-Vehicle</td>
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<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Control day 3</td>
<td>29.7±1</td>
<td></td>
<td></td>
<td></td>
<td>163±17</td>
<td>5.4±1.5</td>
<td>2.4±0.5</td>
<td>2.2±0.1</td>
<td>0.28±0.03</td>
<td>0.39±0.02</td>
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<tr>
<td>Experimental day 7</td>
<td>30.1±1</td>
<td>0.4±0.06</td>
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<td>177±50</td>
<td>4.1±1.0</td>
<td>2.7±0.5</td>
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<td>0.31±0.01</td>
<td>1.4±0.3</td>
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<tr>
<td>WT-Leptin</td>
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</tr>
<tr>
<td>Control day 3</td>
<td>31.2±1</td>
<td></td>
<td></td>
<td></td>
<td>165±21</td>
<td>5.9±1.0</td>
<td>3.1±0.3</td>
<td>2.5±0.2</td>
<td>0.36±0.02</td>
<td>0.47±0.07</td>
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<td>0.07±0.02†</td>
<td>0.03±0.00†</td>
<td>127±24</td>
<td>3.0±0.5†</td>
<td>54.6±6.9†</td>
<td>2.8±0.3</td>
<td>0.30±0.05</td>
<td>0.39±0.05</td>
<td>2.2±0.2</td>
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<td>MC4R(/−−)–Leptin</td>
<td></td>
<td></td>
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<tr>
<td>Control day 3</td>
<td>40.4±2†</td>
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<td></td>
<td></td>
<td>231±53</td>
<td>21.0±3.4†</td>
<td>26.2±3.4†</td>
<td>3.9±0.6†</td>
<td>0.42±0.03</td>
<td>2.8±0.2†</td>
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<tr>
<td>Experimental day 7</td>
<td>42.6±2†</td>
<td>1.5±0.12†</td>
<td>0.6±0.06†</td>
<td>187±37</td>
<td>19.1±6.4†</td>
<td>44.9±11.2†</td>
<td>4.2±0.4†</td>
<td>0.39±0.01</td>
<td>3.3±0.1†</td>
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<tr>
<td>MC4R(/−−)–PF-Leptin</td>
<td></td>
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</tr>
<tr>
<td>Control day 3</td>
<td>31.5±1</td>
<td></td>
<td></td>
<td></td>
<td>168±28</td>
<td>11.0±1.9†</td>
<td>3.1±1.8</td>
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<td>31.5±2</td>
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<td>0.13±0.07</td>
<td>211±36</td>
<td>10.2±3.4†</td>
<td>65.5±0.4†</td>
<td>3.9±0.5†</td>
<td>0.43±0.02</td>
<td>0.47±0.09</td>
<td>2.8±0.5†</td>
</tr>
</tbody>
</table>

UnA NaV indicates average urinary sodium excretion during each period; UV, average urine volume during each period; Epi. fat pad wt, epididymal fat pad weights; Ret. fat pad wt, retroperitoneal fat pad weights; PF, pair-fed.

*P<0.05 vs control values of same group; †P<0.05 vs corresponding values in WT-Vehicle group.
Metabolic and Hormonal Responses to Chronic Hyperleptinemia

The main goal of these studies was to ascertain the role of the MC4R in mediating the chronic responses to leptin at levels that might be encountered in physiological or pathophysiological conditions. Most previous studies have assessed mainly the acute response to leptin, often using concentrations that are supraphysiological. In the present study, chronic hyperleptinemia for 7 days in mice raised plasma leptin levels to 45 to 65 ng/mL, values that are similar to those found in severe obesity. These levels of leptin significantly decreased food intake and body weight in WT mice, but these effects were abolished in ad libitum–fed obese MC4R (−/−; n=6) mice, suggesting that the MC4R may be necessary for the inhibitory actions of chronic hyperleptinemia on feeding.

We also tested the possibility that the anorexic actions of leptin were eliminated in the obese MC4R (−/−) mice because of obesity-induced leptin resistance. Previous studies suggest that obesity can cause leptin resistance by desensitization of the MC4R.

**Figure 1.** Effect of leptin (2 μg/kg per minute) or isotonic saline vehicle infusion for 7 days on 24-hour average food intake, MAP, and HR in WT (n=6), MC4R (−/−; n=6), and pair-fed MC4R (−/−; n=6) mice. WT-Lep indicates WT mice infused leptin; WT-Veh, WT mice infused vehicle; MC4R (−/−)-Lep, obese MC4R (−/−) mice infused leptin; MC4R (−/−)-PF-Lep, nonobese pair-fed MC4R (−/−) mice infused leptin; C1–C3, control days 1 to 3; E1–E7, experimental days 1 to 7.

**Figure 2.** Change in 24-hour average food intake and mean arterial pressure (ΔMAP) from control values with leptin (2 μg/kg per minute) or vehicle infusion for 7 days in WT (n=6), MC4R (−/−; n=6), and pair-fed MC4R (−/−; n=6) mice. WT-Lep indicates WT mice infused leptin; WT-Veh, WT mice infused vehicle; MC4R (−/−)-Lep, obese MC4R (−/−) mice infused leptin; MC4R (−/−)-PF-Lep, nonobese pair-fed MC4R (−/−) mice infused leptin; C1–C3, control days 1 to 3; E1–E7, experimental days 1 to 7.

**Table 2.** Effect of Chronic Hyperleptinemia on Daytime and Nighttime MAP and HR

<table>
<thead>
<tr>
<th>Groups</th>
<th>MAP, AM</th>
<th>MAP, PM</th>
<th>HR, AM</th>
<th>HR, PM</th>
</tr>
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<tbody>
<tr>
<td>WT-Vehicle</td>
<td></td>
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<tr>
<td>Control period</td>
<td>108±3</td>
<td>116±1</td>
<td>613±12</td>
<td>640±8</td>
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<tr>
<td>Experimental period</td>
<td>107±2</td>
<td>118±1</td>
<td>584±9</td>
<td>631±11</td>
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<td>WT-Leptin</td>
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<tr>
<td>Control period</td>
<td>111±4</td>
<td>116±3</td>
<td>608±27</td>
<td>623±23</td>
</tr>
<tr>
<td>Experimental period</td>
<td>123±4∗</td>
<td>126±4∗</td>
<td>634±27</td>
<td>637±26</td>
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<tr>
<td>MC4R (−/−)-Leptin</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control period</td>
<td>108±1</td>
<td>114±1</td>
<td>579±15</td>
<td>574±7†</td>
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<tr>
<td>Experimental period</td>
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<td>566±11</td>
<td>600±10†</td>
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<tr>
<td>MC4R (−/−)-PF-Leptin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control period</td>
<td>104±3</td>
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<tr>
<td>Experimental period</td>
<td>105±1</td>
<td>113±3</td>
<td>576±39</td>
<td>567±33†</td>
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</table>

∗P<0.05 vs control values of same group; †P<0.05 vs corresponding values in WT-Vehicle group.
tizing leptin receptor signaling or attenuating transport of leptin across the blood–brain barrier, thereby restricting its access to the hypothalamic leptin receptors. However, we found that the anorexic actions of leptin were also abolished in pair-fed, nonobese MC4R (−/−) mice, suggesting that leptin resistance did not contribute to the attenuation of the responses of leptin in the obese MC4R (−/−) mice and that MC4R signaling is necessary for the chronic anorectic actions of leptin regardless of whether the mice are obese or nonobese.

The results of the present study are consistent with our previous finding that central blockade of the MC3/4R completely eliminated the anorectic actions of chronic hyperleptinemia. However, those studies did not determine the separate roles of the MC3R and MC4R in mediating the actions of leptin. Studies by Marsh et al reported that, although the acute anorectic effects of leptin were abolished in adult obese MC4R (−/−) mice, these actions were only blunted in young nonobese MC4R (−/−) mice and pair-fed nonobese MC4R (−/−) mice compared with WT mice suggesting that leptin may suppress appetite acutely by mechanisms that are independent of the MC4R. However, Marsh et al administered leptin systemically at a dose that was ~3 times greater than the dose used in the present study, producing leptin levels that substantially exceed those found in obesity. It is possible that increasing the dose of leptin to pharmacological levels in the present study would have chronically suppressed appetite by non-MC4R pathways in the MC4R (−/−) mice. However, the physiological relevance of these effects is unclear. Zhang et al found recently that anorectic effects after daily bolus injections of leptin intraperitoneally or intracerebroventricularly for 3 days were abolished in MC3R (−/−) but not the MC4R (−/−) mice, suggesting that the MC3R but not the MC4R mediates the anorectic actions of leptin. However, in their study too, leptin may have been present at supraphysiological levels because of high-dose acute bolus injections. Also, MC3R (−/−) mice are not hyperphagic as would be expected if MC3R mediates a major part of the effects of leptin on appetite.

Inhibition of other orexigenic pathways, including neuropeptide Y (NPY) and agouti-related peptide (AGRP), have also been implicated in the actions of leptin. In fact, NPY deficiency attenuates obesity in leptin-deficient ob/ob mice. However, NPY (−/−), AGRP (−/−), and double-knockout (NPY(−/−)AGRP(−/−)) mice have normal feeding and body weight regulation suggesting that the NPY/AGRP pathway is not required for normal energy homeostasis and may be important mainly when leptin production, leptin receptor function, or anorexyc pathways are compromised. Therefore, although MC3R and other orexigenic pathways, such as NPY/AGRP, may mediate part of the anorexyc actions of leptin, these mechanisms may not be tonically active at physiological concentrations of leptin. Our results suggest that an intact MC4R is essential for expression of most, if not all, of the chronic physiological effects of leptin on appetite.

Our experiments also shed some light on the etiology of obesity when the MC4R is defective. Ad libitum–fed MC4R (−/−) mice at 20 to 22 weeks of age were ~30% heavier and had severe visceral adiposity and hyperleptinemia compared with age-matched WT mice. However, MC4R (−/−) mice that were pair-fed had body weights, visceral adiposity, and plasma leptin levels that were indistinguishable from those in WT mice. This suggests that increased food intake and not hypometabolism contributed to most of the excess weight gain and visceral adiposity of the MC4R (−/−) mice in our study. Similar conclusions were reached by Weide et al, who studied cumulative food intake and energy expenditure in 3- to 8-week–old MC4R (−/−) mice and showed that hyperphagia and not hypometabolism contributed to the excess fat deposition and early onset obesity in MC4R (−/−) mice. However, Ste Marie et al reported that pair-fed MC4R (−/−) mice had greater visceral fat accumulation compared with WT mice despite similar body weights. Thus, MC4R (−/−) deficiency may promote visceral adiposity to some extent even when hyperphagia is prevented, possibly as a result of mild reduction in energy expenditure. However, the present study suggests that hyperphagia may play a dominant role in causing excess weight gain and visceral adiposity in the MC4R (−/−) mice, at least up to 22 weeks of age.

In addition to its effects on food intake and visceral adiposity, leptin also has important effects on glucose homeostasis that may largely be via activation of the MC4R. In the present study, plasma glucose levels were not substantially altered in any of the groups of mice during chronic hyperleptinemia, although plasma insulin levels were reduced in WT mice suggesting improved glucose use. This effect of leptin to reduce plasma insulin was abolished in both obese and nonobese MC4R (−/−) mice suggesting that the MC4R is also crucial for the chronic glucose regulatory actions of leptin. Although we and others have shown previously that central MC3/4R antagonist attenuates the ability of leptin to decrease plasma insulin levels in rats, the importance of the MC4R in mediating the actions of leptin was not assessed in these studies. Previous acute studies in both rats and mice also demonstrate that central MC3/4R agonist administration increases insulin-mediated glucose disposal during an acute euglycemic-hyperinsulinemic clamp, which suggests an important role for the MC3/4R in glucose use independent of changes in food intake and body weight. In the present study, although pair feeding prevented obesity and hyperleptinemia in MC4R (−/−) mice, plasma levels of insulin in the nonobese, pair-fed MC4R (−/−) mice were ≥2-fold greater compared with WT mice, suggesting that MC4R deficiency may contribute to insulin resistance independent of hyperphagy and obesity. Supporting this view, Fan et al have reported development of insulin resistance and hyperinsulinemia in young lean MC4R (−/−) mice before the onset of detectable hyperphagy or obesity. However, the mechanisms by which leptin and MC4R activation increase peripheral glucose use and reduce plasma insulin levels are not known and remain a promising area for further research.
Cardiovascular Responses to Chronic Hyperleptinemia

In the present study, as observed in our previous study,\textsuperscript{16} 24-hour MAP measured by telemetry was not increased in the ad libitum-fed, obese MC4R (\(-/-\)) mice compared with WT mice. In fact, obese MC4R (\(-/-\)) mice had slightly lower MAP than WT mice despite the coexistence of hyperphagia, obesity, visceral adiposity, and hyperinsulinemia that normally tend to raise arterial pressure. When the MC4R (\(-/-\)) mice were pair fed to prevent obesity, MAP was further reduced. These observations suggest that an intact MC4R may be an important link between obesity and hypertension.

The MC4R also seems to be essential for most, if not all, of the chronic effects of leptin on blood pressure. Chronic elevation in plasma leptin to pathophysiological levels in WT mice increased 24-hour MAP by \(\approx 12\) mm Hg despite significant reductions in food intake and body weight. The hypertensive effect of chronic hyperleptinemia observed in WT mice was abolished in obese and nonobese MC4R (\(-/-\)) mice, suggesting that MC4R signaling is necessary for the hypertensive actions of leptin and that obesity-induced leptin resistance, per se, did not contribute to the elimination of the pressor actions of leptin in the obese MC4R (\(-/-\)) mice.

It could be contended that compensatory adaptations associated with MC4R deficiency played a role in attenuating the actions of leptin. Previous studies have found increased hypothalamic NPY mRNA expression in MC4R (\(-/-\)) mice.\textsuperscript{30} However, it is still not clear whether these elevations in NPY are large enough to alter metabolic and cardiovascular function in MC4R (\(-/-\)) mice. Correia et al\textsuperscript{31} found that although chronic central infusion of NPY caused mild obesity, it did not alter arterial pressure in rats. Therefore, the elimination of the hypertensive actions of leptin in MC4R (\(-/-\)) mice is unlikely to be caused by increases in hypothalamic NPY. Our results are most consistent with the hypothesis that the MC4R is necessary for the chronic cardiovascular actions of leptin.

One observation that seems to conflict with our results is that mice overexpressing agouti protein, which are believed to be obese because of agouti-mediated hypothalamic MC4R antagonism, have elevated rather than reduced arterial pressures, which is attenuated by fasting and restored by leptin administration.\textsuperscript{32,33} Agouti mice are, therefore, not resistant to the appetite suppression or renal sympathoexcitatory actions of leptin.\textsuperscript{34} The mechanisms for the increased blood pressure and leptin sensitivity in agouti mice are unclear, but leptin may stimulate MC4R-independent hypothalamic cocaine-amphetamine regulated transcript pathway, which is upregulated in the agouti mice and has pressor actions.\textsuperscript{34,35} Also, agouti mice have only partial antagonism of the MC4R, as evidenced by the ability of MT-II (MC3/4R agonist) to suppress food intake in agouti mice and the inability of the MC3R to mediate appetite suppression.\textsuperscript{36} We have shown previously that chronic intracerebroventricular infusion of AGRP, a peptide that is highly homologous to agouti protein, decreased arterial pressure and HR in rats despite increased food intake and weight gain.\textsuperscript{37} These responses are almost precisely what we have observed with pharmacological inhibition of the MC3/4R and in MC4R (\(-/-\)) mice. These observations indicate that in the agouti mouse there are additional factors that oppose the depressor actions of MC4R blockade and raise arterial pressure.

The mechanisms by which MC4R deficiency attenuates the chronic pressor actions of leptin were not investigated in the present study, but previous evidence suggests a role for impaired sympathoexcitation by leptin with MC4R deficiency. Rahmouni et al\textsuperscript{17} showed that leptin-induced increases in renal sympathetic nerve activity were abolished in obese homozygous MC4R (\(-/-\)) mice and attenuated in heterozygous MC4R (\(-/-\)) mice suggesting that the MC4R is important for the renal sympathoexcitatory action of leptin. However, the importance of sympathoexcitation in mediating the chronic effects of leptin and MC4R activation on blood pressure in mice have, to our knowledge, not been reported previously.

In the present study, 24-hour HR was lower in obese and nonobese MC4R (\(-/-\)) mice compared with WT mice, suggesting that endogenous activity of the MC4R may contribute to HR regulation in mice. We also found previously that chronic CNS infusion of the MC3/4R antagonist SHU9119 markedly decreased HR in both ad libitum and pair-fed rats, supporting the concept that MC4R activation may contribute to HR regulation.\textsuperscript{38}

Renal Response to Chronic Hyperleptinemia

Chronic hyperleptinemia did not produce significant changes in urinary sodium excretion or urine volume in any of the groups. The absence of changes in sodium excretion despite a 12-mm Hg increase in arterial pressure during chronic leptin infusion in WT mice suggests that the renal pressure–natriuresis relationship was shifted to higher pressures in the WT mice. Absence of this shift in pressure–natriuresis during chronic leptin infusion in MC4R (\(-/-\)) mice is consistent with the possibility that activation of the MC4R may be essential for chronic hyperleptinemia to induce renal sympathoexcitation. This would also be consistent with studies demonstrating that acute leptin infusion failed to increase renal sympathetic activity in MC4R-deficient mice.\textsuperscript{17} However, further studies are needed to assess the chronic effects of leptin and MC4R activation on renal sympathetic activity.

Perspectives

The present study emphasizes the role of the MC4R in the tonic regulation of appetite, glucose metabolism, and cardiovascular function and in mediating the chronic cardiovascular and metabolic actions of leptin. The separate roles of the MC3R and the MC4R in regulating energy expenditure were not addressed in the present study and need further evaluation. Although MC4R is expressed peripherally in metabolically important tissues, such as the adipose tissue, the physiological significance of these receptors remains unclear. Whether MC4R activation also contributes to the cardiovascular and metabolic actions of other hypothalamic signals unrelated to leptin also requires further investigation. Understanding these mechanisms may provide important targets for treatment of obesity and related metabolic disorders while preventing unwanted cardiovascular effects, such as increased blood pressure.
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Melanocortin-4 Receptor Mediates Chronic Cardiovascular and Metabolic Actions of Leptin

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