Chronic Cardiovascular and Renal Actions of Leptin
Role of Adrenergic Activity

Megan Carlyle, Oscar B. Jones, Jay J. Kuo, John E. Hall

Abstract—This study was designed to determine the role of changes in adrenergic activity in mediating the chronic cardiovascular, renal, and metabolic actions of leptin. Male Sprague-Dawley rats were implanted with catheters for mean arterial pressure (MAP) and heart rate (HR) measurements and IV infusions of either vehicle (n = 7) or α- and β-adrenergic receptor antagonists, terazosin and propranolol (10 mg/kg/d; n = 8) throughout the study. After control measurements, murine leptin was infused IV (1.0 μg/kg/min) for 7 days along with vehicle or adrenergic antagonists, followed by a 7-day recovery period. Leptin infusion significantly reduced food intake in control rats from 22.6 ± 0.8 to 10.6 ± 0.4 g/d and, in adrenergic blockade rats, from 22.6 ± 0.8 to 13.2 ± 0.8 g/d. Fasting plasma insulin decreased from 48 ± 10 to 5 ± 2 μU/mL in control rats and from 51 ± 9 to 9 ± 2 μU/mL in adrenergic blockade rats during leptin infusion. Leptin infusion did not significantly alter glomerular filtration rate in either group. MAP and HR increased by 6 ± 1 mm Hg and 23 ± 7 bpm after 7 days of leptin infusion in control rats. However, in adrenergic blockade rats, leptin infusion did not significantly alter MAP (−1 ± 1 mm Hg) and decreased, rather than increased, HR (−23 ± 8 bpm). These results indicate that leptin-induced increases in blood pressure and tachycardia are mediated by increased adrenergic activity and support the concept that leptin may be an important link between obesity, increased sympathetic activity, and hypertension. However, the chronic effects of leptin on insulin and glucose regulation do not appear to be altered by α- and β-adrenergic receptor blockade. (Hypertension. 2002;39[part 2]:496-501.)

Key Words: hypertension, obesity ■ obesity ■ nervous system, sympathetic ■ kidney ■ blood pressure.

The sympathetic nervous system appears to play a major role in mediating obesity-associated hypertension in experimental animals as well as in humans.1-2 Pharmacologic blockade of the sympathetic nervous system or renal denervation markedly attenuates sodium retention and hypertension associated with a high-fat diet in experimental animals,3,4 and pharmacologic blockade of adrenergic activity reduces blood pressure to a greater extent in obese than in lean hypertensive patients.5 However, the mechanisms that link obesity and increased sympathetic activity are still poorly understood.

Although the mechanisms that mediate sympathetic activation and hypertension in obesity have not been elucidated, recent studies suggest that hyperleptinemia is a possible candidate. Infusions of leptin, intravenous (IV) or intracerebroventricular (ICV), increased sympathetic activity in the kidneys, adrenals, and brown adipose tissue.5,7 Chronic leptin infusions in rats, at rates that increased plasma concentrations to about 94 ng/mL, raised mean arterial pressure (MAP) and heart rate (HR) despite marked reductions in food intake.8 Also, transgenic mice overexpressing leptin have higher blood pressures than control mice.9 Finally, obese mice that are leptin deficient are not hypertensive and have slightly lower blood pressures than lean control mice.10 These observations are all consistent with the hypothesis that hyperleptinemia may increase sympathetic activity and contribute to obesity hypertension. However, the importance of adrenergic activation in mediating the long-term cardiovascular actions of leptin has not been fully elucidated.

The main goal of this study was to determine the importance of changes in adrenergic activity in mediating the blood pressure, HR, renal, and metabolic responses to chronic hyperleptinemia. Our results indicate that leptin-induced increases in blood pressure and tachycardia are completely abolished by adrenergic blockade, but the chronic effects of leptin on insulin and glucose regulation are not altered by adrenergic blockade.

Methods

Animals and Surgery

The experimental procedures and protocols for these studies 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 Sprague-Dawley rats (Harlan Sprague-Dawley, Inc, Indianapolis, Ind) were anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg), and atropine sulfa (0.37 mg/kg) was
administered to prevent excessive airway secretions. A laparotomy was performed under aseptic conditions, and a nonocclusive catheter was inserted orthograde into the abdominal aorta, distal to the kidneys. A femoral vein catheter was also implanted through a separate incision, and the tip was advanced into the inferior vena cava. The incisions were infiltrated with penicillin G procaine and bupivacaine HCl, and the catheters were filled with heparin (1000 U.S.P. units/mL) and exteriorized through a stainless steel button that was implanted subcutaneously in the scapular region. After recovery from surgery, the rats were housed in individual metabolic cages in a quiet, air-conditioned room with a 12h/12h light/dark cycle. The arterial and venous catheters were connected to a dual channel infusion swivel (Instech) mounted above the cage and protected by a stainless steel spring. The venous catheter was connected, via the swivel, to a syringe pump for continuous infusions, and the arterial catheter was connected to a pressure transducer for continuous, 24-hour recordings of systolic, diastolic, and MAP using computerized techniques as previously described. The rats received food and water ad libitum throughout the study. Total sodium intake was maintained constant at about 3.1 mmol/d by a continuous infusion of 20 mL/d of 0.9% saline combined with a sodium-deficient rat chow (0.006 mmol sodium/g food, Teklab). All solutions were infused through a sterile filter (22 μm, Millipore), and the saline infusion was started immediately after placement of the rats in their metabolic cages. After an acclimation period of 4 to 7 days, control measurements were recorded.

**Experimental Protocols**

### Leptin Infusion in Control Rats (n=7)

After 10 days of control measurements in which saline vehicle was infused, an IV infusion of leptin was started at a rate of 1.0 μg/kg/min and continued for 7 days, followed by a 7 day recovery period after the leptin infusion was stopped. Mean arterial pressure (MAP), heart rate, urinary excretion of water and electrolytes, and intakes of food, sodium, and water were recorded daily. Blood samples (1.5 mL) were collected on the ninth day of control, the sixth day of leptin infusion, and the sixth day of the recovery period for measurements of plasma concentrations of insulin, glucose, and protein as well as plasma renin activity (PRA), hematocrit, and 125I-iothalamate for measurements of GFR. The blood samples were replaced with equal volumes of 0.9% saline.

### Leptin Infusion in Adrenergic Blockade Rats (n=8)

In a separate group of rats, control measurements were made for 5 days during vehicle infusion. Then, the α1-receptor antagonist, terazosin (Sigma Chemical Company), and the β-receptor antagonist, propranolol (Sigma), were added to the vehicle infusate to deliver 10 mg/kg/d IV of each of the antagonists throughout the experiment. After 5 days of α1- and β-antagonist infusion, leptin was added to the infusate to deliver 1.0 μg/kg/min. After 7 days of leptin plus α1- and β-receptor adrenergic blockade, the leptin infusion was stopped while infusion of the adrenergic antagonist was continued for 7 days of recovery. The effectiveness of α1- and β-receptor blockade were assessed in vehicle infused and adrenergic blockade rats before and after leptin infusion by analyzing the mean arterial pressure responses to bolus intra-arterial injections of phenylephrine (4 μg) and isoproterenol (0.7 μg).

### Analytical Methods

PRA and plasma insulin concentrations were measured by radioimmunoassay. Plasma protein concentration was measured by refractometry, and urine sodium and potassium concentrations were determined using ion-sensitive electrodes (NOVA). Plasma glucose was measured with an automatic analyzer by the glucose oxidase method (Beckman). GFR was determined from the clearance of 125I-iothalamate.

### Statistical Methods

Data are expressed as mean±SE and analyzed by using 2-factor ANOVA with repeated measures and Dunnett’s test for multiple comparisons, when appropriate. Statistical significance was accepted at *P*<0.05.

**Results**

### Effects of Leptin and Adrenergic Blockade on Food Intake and Hormones

Leptin infusion for 7 days in control rats decreased food intake from 20.8±0.8 to an average of 10.6±0.4 g/d (Figure 1). In adrenergic blockade rats, leptin infusion decreased food intake from 21.9±0.5 to 13.2±0.8 g/d, a response that was not significantly different from that observed in control rats. Fasting plasma insulin and glucose were not different between normal and adrenergic blockade rats. However, leptin infusion decreased fasting plasma insulin concentration from 48±10 to 5±2 μU/mL in control rats and from 51±9 to 9±2 μU/mL in adrenergic blockade rats. PRA did not change significantly in control or adrenergic blockade rats during leptin infusion. Body weight averaged 334±4 g and 334±4 g in the vehicle infused and adrenergic blockade rats before leptin infusion. Body weight was not measured during leptin infusion in either group, due to the fact that they were connected for 24-hour blood pressure recordings and continuous measurements throughout the experiment.

### Effects of Leptin and Adrenergic Blockade on MAP and HR

MAP increased from 94±2 to 101±2 mm Hg after 7 days of leptin infusion in control rats (Figures 2 and 3). MAP and HR, measured during 12 hours of daytime (6:30 AM to 6:30 PM), increased from 93±2 and 381±8 to 102±2 mm Hg and 417±6 b/min, respectively, after 7 days of leptin infusion. MAP and HR, measured during 12 hours of nighttime, increased from 95±2 and 412±7 to 101±2 mm Hg and 434±6 b/min, respectively, after 7 days of leptin infusion. Seven days after stopping leptin infusion, MAP returned to the initial control level, averaging 95±2 mm Hg. In contrast, leptin infusion in adrenergic blockade rats did not significantly alter MAP, which averaged 84±1 mm Hg during control and 83±1 mm Hg after 7 days of leptin infusion.
Thus, adrenergic blockade completely abolished the rise in MAP associated with chronic leptin infusion.

Adrenergic blockade also prevented the rise in HR during leptin infusion. In fact, in the adrenergic blockade rats there was a steady decline in heart rate during leptin infusion throughout the study. In control rats, HR increased from $397\pm7$ to $421\pm7$ bpm after 7 days of leptin infusion. When leptin infusion was stopped, HR declined gradually to $375\pm6$ bpm on the seventh recovery day. Combined $\alpha$- and $\beta$-adrenergic blockade for 5 days decreased HR from $407\pm4$ to $375\pm4$ bpm, and after 7 days of leptin infusion plus adrenergic blockade, HR decreased further to $349\pm7$ beats/min.

**Effects of Leptin and Adrenergic Blockade on Renal Function**

Leptin infusion caused no significant changes in urine volume or sodium excretion in control rats or in adrenergic blockade rats (Table 1). Potassium excretion, however, decreased significantly in both groups during leptin infusion, paralleling the decrease in intake of food and, therefore, the potassium intake. This resulted in a negative cumulative potassium balance of $-6.3\pm0.4$ mEq during 7 days of leptin infusion. Similar results were also observed during leptin infusion in adrenergic blockade rats, with cumulative potassium balance decreasing by $6.0\pm1.2$ mEq after 7 days.

In control rats, leptin infusion did not significantly alter GFR, which averaged $3.3\pm0.2$ and $3.5\pm0.4$ mL/min during control and the sixth day of leptin infusion, respectively. In adrenergic blockade rats, GFR averaged $4.6\pm1.1$ mL/min during control and $4.5\pm0.9$ mL/min after 6 days of leptin infusion, a change that was not significant.

**Effects of Leptin and Adrenergic Blockade on Pressor Response to $\alpha$- and $\beta$-Receptor Agonists**

The blood responses to phenylephrine and isoproterenol, assessed by measuring the area under the curve (AUC) for the MAP after bolus IV injection of the agonists, were markedly attenuated after adrenergic blockade, as shown in Figure 4. In control vehicle infused rats, leptin infusion did not significantly alter the pressor responses to phenylephrine but slightly increased the depressor response to isoproterenol. In adrenergic blockade rats, the responses to these agonists remained markedly attenuated during combined adrenergic blockade and leptin infusion.

**Discussion**

The most important finding of this study is that blockade of $\alpha$- and $\beta$-adrenergic activity completely abolished the increases in MAP and HR observed during 7 days of hyperleptinemia. These observations support the concept that increased adrenergic activity is an important mediator of the chronic cardiovascular actions of leptin and are consistent with the possibility that leptin might be an important link between obesity, increased adrenergic activity, and hypertension.

**Metabolic and Hormonal Effects of Leptin After Adrenergic Blockade**

We have previously shown that leptin infusion at the same rate used in the present study ($1.0 \mu g/kg/min$) raised plasma leptin concentrations to about $94$ ng/mL, a level comparable to that observed in severe human obesity.$^{2,8}$ This rate of leptin infusion markedly decreased fasting plasma insulin concentration without altering plasma glucose concentration. Although leptin has been suggested to decrease insulin release...
by stimulating α-adrenergic receptors in the pancreas, the results of the present study suggest that additional mechanisms may be involved. A primary decrease in insulin secretion should increase, rather than decrease, plasma glucose. Also, our finding that leptin’s chronic effects on insulin were not attenuated by α- and β-adrenergic blockade suggests that other mechanisms are responsible for the fall in plasma insulin concentration. Because decreased plasma insulin during leptin infusion was not associated with increased plasma glucose concentration (which actually tended to decrease slightly), the fall in insulin concentration may be related to increased glucose utilization or improvement of insulin sensitivity in peripheral tissues. These effects of leptin would enhance glucose disposal, suppress glucose output by the liver, and decrease plasma glucose concentration. Leptin may also increase glucose utilization in other tissues, including the brain and heart, by an insulin-independent mechanism. However, these metabolic effects of leptin do not appear to be mediated by changes in α- or β-adrenergic receptor activity because chronic leptin infusion reduced plasma insulin and glucose to the same extent after adrenergic receptor blockade as in control rats in the present study.

### Table 1: Results of the Study

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>GFR (mL/min)</th>
<th>Urine Volume (mL/day)</th>
<th>Water Intake (mL/day)</th>
<th>Plasma Insulin (μU/mL)</th>
<th>Glucose (mg/100 mL)</th>
<th>PRA (ng AI/mL/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>3.3±0.2</td>
<td>2.6±0.1</td>
<td>3.5±0.2</td>
<td>37±3</td>
<td>15±4</td>
<td>48.3±9.6</td>
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<tr>
<td>Leptin</td>
<td>3.5±0.4</td>
<td>2.8±0.1</td>
<td>2.2±0.1*</td>
<td>41±2</td>
<td>16±3</td>
<td>5.4±1.8*</td>
</tr>
<tr>
<td>Recovery</td>
<td>3.6±0.1</td>
<td>2.6±0.1</td>
<td>3.5±0.2</td>
<td>38±3</td>
<td>15±3</td>
<td>61.8±7.4</td>
</tr>
<tr>
<td>Adrenergic blockade group (n=8)</td>
<td></td>
<td></td>
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<tr>
<td>α/β blockade</td>
<td>4.6±1.1</td>
<td>2.4±0.1</td>
<td>3.7±0.1</td>
<td>32±1</td>
<td>9±1</td>
<td>50.5±8.9</td>
</tr>
<tr>
<td>α/β blockade + leptin</td>
<td>4.5±0.9</td>
<td>2.3±0.3</td>
<td>2.6±0.1*</td>
<td>36±1</td>
<td>9±1</td>
<td>9.0±1.5*</td>
</tr>
<tr>
<td>Recovery</td>
<td>5.2±1.1</td>
<td>2.4±0.3</td>
<td>3.3±0.1</td>
<td>37±2</td>
<td>11±2</td>
<td>53.2±10.9</td>
</tr>
</tbody>
</table>

GFR indicates glomerular filtration rate; Urine Volume, urinary sodium excretion; Water Intake, urinary potassium excretion; PRA, plasma renin activity. Values for Urine Volume, Water Intake, urine volume, and water intake are average values for the 5-day control periods, 7 days of leptin infusion, and 7 days of recovery after stopping leptin infusion. *p<0.05.

### Figure 4: Area under the curve (AUC) of the changes in mean arterial pressure after bolus IV injections of phenylephrine (4 μg) or isoproterenol (0.7 μg) in control rats infused with vehicle and in adrenergic blockade rats infused with the α- and β-adrenergic receptor antagonists, terazosin (10 mg/kg/day), and propranolol (10 mg/kg/day). Responses were measured under control conditions and after 5 days of leptin infusion.
tion, and hypertension. However, this hypothesis is complicated by the possibility that obesity may induce “resistance” to the effects of leptin on the hypothalamus. For example, leptin’s effect of suppressing appetite is diminished in obese, compared with lean, animals. Because basal sympathetic activity is elevated in obese animals, possibly due to high circulating leptin, infusion of exogenous leptin to produce pharmacological levels may not cause further sympathetic stimulation. Also, obesity may induce “selective leptin resistance” in which the sympathoexcitatory effects are preserved despite resistance to the metabolic actions of leptin. Therefore, it is still uncertain whether diet-induced obesity causes resistance to the chronic sympathoexcitatory effects of endogenous leptin.

**Renal Responses to Leptin After Adrenergic Blockade**

Previous studies have suggested that leptin may have natriuretic as well as antinatriuretic actions. Evidence in support of a natriuretic effect of leptin comes mainly from acute studies showing that injection or infusion of large amounts of leptin may cause natriuresis and diuresis. In our previous studies and in the present study, we found no significant changes in sodium excretion or urine volume during chronic infusion of leptin at levels at rates that raised plasma concentrations to physiological or pathophysiologic levels. It is possible that a natriuretic effect of leptin could be attenuated by increased renal adrenergic activity, secondary to activation of the sympathetic nervous system. However, in the present study we found no significant changes in urine volume or sodium excretion during 7 days of leptin infusion even after adrenergic blockade. Leptin infusions did decrease urinary excretion of potassium, although this was probably due mainly to decreased potassium intake secondary to the reduction in food intake. Although sodium intake in our study was held relatively constant by continuous IV infusion of most of the daily sodium intake, all of the potassium intake was derived from the food.

The absence of significant changes in sodium excretion during leptin infusion in control rats or after adrenergic blockade does not necessarily imply that leptin has no significant effect on renal function. In fact, the observation that leptin did not increase sodium excretion, despite higher arterial pressure, suggests that leptin shifted the renal-pressure natriuresis relationship to higher blood pressures. In the absence of impaired pressure natriuresis, a rise in blood pressure would tend to increase renal sodium and water excretion. Because adrenergic blockade completely prevented the increase in arterial pressure, the effect of leptin of shifting pressure natriuresis appears to be mediated mainly by increased adrenergic activity.

The effect of leptin to alter renal-pressure natriuresis does not appear to be caused by renal vasoconstriction or decreased GFR, because GFR did not decrease significantly during chronic leptin infusion. In fact, there was a slight increase in GFR during hyperleptinemia in control rats and in adrenergic blockade rats. However, the precise mechanisms by which chronic hyperleptinemia alters renal function are still uncertain.

In summary, our results indicate that chronic hyperleptinemia, comparable to that found in severe human obesity, increases MAP and HR and that these changes are mediated mainly by increased adrenergic activity. However, increased adrenergic activity does not appear to mediate the chronic metabolic effects of leptin, including reduced plasma insulin and glucose concentrations, and enhanced insulin sensitivity. Although our observations are consistent with the possibility that hyperleptinemia may be a link among obesity, increased adrenergic activity, and hypertension, further studies are needed to determine whether obesity is characterized by resistance to the chronic sympathoexcitatory effects of leptin.

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**References**


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