Chronic Leptin Infusion Increases Arterial Pressure

Eugene W Shek, Michael W. Brands, John E. Hall

Abstract—Plasma leptin concentration is increased in hypertensive obese humans, but whether leptin contributes to the increased arterial pressure in obesity is not known. In this study, we tested whether chronic increases in leptin, to levels comparable to those in obesity, could cause a sustained increase in arterial pressure and also the importance of central nervous system (CNS) versus systemic mechanisms. Five male Sprague-Dawley rats were implanted with chronic nonoccluding catheters in the abdominal aorta and both carotid arteries for CNS infusion, and five other rats were implanted with an abdominal aorta catheter and femoral vein catheter for intravenous (IV) infusion. After 7 days of control, leptin was infused into the carotid arteries or femoral vein at 0.1 μg/kg/min for 5 days and 1 μg/kg/min for 7 days, followed by a 7-day recovery period. The carotid artery and IV infusions of leptin at 1 μg/kg/min significantly increased plasma leptin levels, from 1.2±0.4 ng/mL to 91±5 ng/mL and from 0.9±0.1 ng/mL to 94±9 ng/mL, respectively, but there was no significant increase in either group at the low dose. Food intake also did not change at the low dose but decreased by approximately 65% in the carotid group and 69% in the IV group after 7 days of the 1 μg/kg/min infusion. Mean arterial pressure (MAP) increased slightly at the low dose only in the carotid group, but this was not statistically significant. At the higher dose, however, MAP increased significantly from 86±1 mm Hg to 94±1 mm Hg in the carotid group and from 87±1 mm Hg to 93±1 mm Hg in the IV group. Heart rate also increased significantly in both groups at 1 μg/kg/min leptin infusion. Fasting blood glucose and insulin levels decreased significantly at 1 μg/kg/min in both the carotid artery group (−10.5% and −82.5%, respectively) and the IV group (−13.6% and −80.4%, respectively). All variables returned to control levels after leptin infusion was stopped. These results indicate that chronic increases in circulating leptin cause sustained increases in arterial pressure and heart rate and are consistent with a possible role for leptin in obesity hypertension. (Hypertension. 1998;31[part 2]:409-414.)

Key Words: leptin • hypertension • sympathetic nervous system • blood pressure • heart rate • food intake

Increased circulating leptin, the peptide product of the “obese gene,” has been suggested to contribute to increased blood pressure in obesity by activation of the sympathetic nervous system. Leptin production is increased in obese animals and humans and correlates positively with body mass index. Because leptin binding sites have been found in regions of the brain that are also important in cardiovascular control, there is reason to suspect that leptin could affect cardiovascular function through its CNS effects. Supporting this possibility is the observation by Haynes and colleagues, who recently demonstrated that acute intravenous (IV) infusion of leptin increased sympathetic nerve activity to the kidney, the adrenal, and the brown adipose tissue in anesthetized Sprague-Dawley rats. However, leptin has also been shown to cause nausea and dizziness following bolus IV infusion. Thus, leptin may have multiple actions on the SNS and the kidney that, in some circumstances, could have opposite effects on arterial pressure regulation. Moreover, it is not known which of these effects, if any, can be sustained and exert long-term effects on cardiovascular and renal function.

The aim of this study, therefore, was to determine whether increases in circulating leptin, to levels similar to those found in obesity, cause sustained changes in cardiovascular, renal, and neurohumoral function. Furthermore, we investigated the role of CNS versus systemic mechanisms by comparing the chronic effects of carotid artery and IV leptin infusion on control of arterial pressure and renal function.

Methods

Animal Surgery

Male Sprague-Dawley rats (n=10) weighing approximately 350 to 400 g were used in this study. The protocol was approved by the Institutional Animal Care and Use Committee of University of Mississippi Medical Center and conformed to the National Institute of Health Guide for the Care and Use of Laboratory Animals. Under pentobarbital sodium (50 mg/kg, intraperitoneal), anesthesia and aseptic conditions, a laparotomy was performed, and a nonocclusive polyvinyl catheter was inserted into the abdominal aorta, distal to the kidneys, through a puncture made with a 20-gauge needle tip. The insertion point was sealed with cyanoacrylate adhesive, and the catheter was exterized through the lateral abdominal wall. In the carotid artery infusion group (n=5), nonocclusive polyvinyl catheters were placed orthograde in both common carotid arteries via a midventral neck incision for continuous intracarotid infusion of vehicle or leptin into the cerebral circulation. In the rat, the carotid arteries provide most of the blood supply to the hypothalamus and
Experimental Protocol

After 7 days of control measurements, murine leptin (Amgen Inc.) was infused into the carotid arteries or femoral vein at 0.1 µg/kg/min for 5 days and at 1 µg/kg/min for 7 days, followed by a 7-day recovery period. Mean arterial pressure (MAP), heart rate (HR), urine volume, urinary sodium excretion, and food and water intake were recorded daily. Blood samples (2 mL) were collected on the fifth day of the control, leptin infusion, and recovery periods for the measurement of plasma insulin, leptin, aldosterone, corticosterone, and protein concentration, blood glucose, hematoctit, PRA, GFR, and RPF. The blood sample was replaced with an equal volume of saline.

Analitical Methods

PRA was measured by radioimmunoassay using 125I-angiotensin I from New England Nuclear and antibody from Axel Plasma insulin, aldosterone, and corticosterone concentrations were measured by radioimmunoassay (Diagnostic Products) Plasma leptin concentration was measured by enzyme immunoassay (Amgen Inc.) Plasma protein concentration was measured by refractometry. Urine sodium concentration was determined by using ion-sensitive electrodes (Nova). Blood glucose was measured by using an AccuCheck III blood glucose monitor (Boehringer Mannheim Corpora-

Figure 1. Effect of chronic bilateral carotid artery or intravenous infusion of leptin at 0.1 µg/kg/min (5 days) and 1.0 µg/kg/min (7 days) on daily food intake in conscious normal SD rats (n=5 per group). *P<.05 compared to control.
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**Figure 2.** Effect of chronic bilateral carotid artery or intravenous infusion of leptin at 0.1 µg/kg/min (5 days) and 1.0 µg/kg/min (7 days) on mean arterial pressure in conscious normal SD rats (n=5 per group). *P<.05 compared to control.

decreased rapidly to levels below control levels after leptin infusion was stopped in both carotid artery and IV infusion groups.

Chronic carotid artery infusion of leptin at 0.1 µg/kg/min did not change heart rate significantly, but at the 1 µg/kg/min dose, HR increased from 323±4 beats/mm to 355±5 beats/mm by day 7 of the infusion (Fig 3). Similar to the effect of carotid artery infusion, chronic IV infusion of leptin did not affect HR at the low dose, but heart rate increased significantly at the high dose, from 305±9 beats/mm during the control period to 322±10 beats/mm by day 7 of the leptin infusion period (Fig 3). HR decreased rapidly in both carotid artery and IV infusion groups after leptin infusion was stopped and was significantly below control (302±5 beats/mm and 284±5 beats/mm, respectively) by day 3 of the recovery period.

**Effects of Leptin Infusion on Renal Function**

Although there was a tendency for urine volume to increase during leptin infusion at 1 µg/kg/min in both carotid artery and IV infusion groups, the increase was not statistically significant (Fig 4). There was also no significant change in urinary sodium excretion in either group during leptin infusion at 0.1 or 1 µg/kg/min (Fig 5). GFR, RPF, and RVR did not change during

**Figure 3.** Effect of chronic bilateral carotid artery or intravenous infusion of leptin at 0.1 µg/kg/min (6 days) and 1.0 µg/kg/min (7 days) on heart rate in conscious normal SD rats (n=5 per group). *P<.05 compared to control.

**Figure 4.** Effect of chronic bilateral carotid artery or intravenous infusion of leptin at 0.1 µg/kg/min (5 days) and 1.0 µg/kg/min (7 days) on urine volume in conscious normal SD rats (n=5 per group). *P<.05 compared to control.

**Figure 5.** Effect of chronic bilateral carotid artery or intravenous infusion of leptin at 0.1 µg/kg/min (6 days) and 1.0 µg/kg/min (7 days) on urinary sodium excretion in conscious normal SD rats (n=5 per group). *P<.05 compared to control.
Leptin-Induced Hypertension

Figure 6. Effect of chronic bilateral carotid artery or intravenous infusion of leptin at 0.1 μg/kg/min (5 days) and 1.0 μg/kg/min (7 days) on glomerular filtration rate (GFR), renal plasma flow (RPF), and renal vascular resistance (RVR) in conscious normal SD rats (n=5 per group). Data are expressed as percent control. *P < 0.05 compared to control.

Table 1. Effects of Chronic Bilateral Carotid Artery Infusion of Leptin at 0.1 and 1.0 μg/kg/min on Plasma Leptin, Fasting Insulin (Ins), Fasting Glucose (Glu), Plasma Renin Activity (PRA), Plasma Aldosterone (Aldo), and Plasma Corticosterone (Cort)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leptin (ng/mL)</th>
<th>Ins (μU/mL)</th>
<th>Glu (mg/dL)</th>
<th>PRA (ng Al/mL/h)</th>
<th>Aldo (ng/dL)</th>
<th>Cort (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.2 ± 0.4</td>
<td>41.2 ± 10.5</td>
<td>104.4 ± 2.9</td>
<td>31 ± 0.4</td>
<td>40.7 ± 10.8</td>
<td>131.3 ± 43.1</td>
</tr>
<tr>
<td>Leptin (0.1 μg/kg/min)</td>
<td>1.4 ± 0.5</td>
<td>40.2 ± 6.7</td>
<td>106.2 ± 2.2</td>
<td>36 ± 0.5</td>
<td>35.5 ± 43.8</td>
<td>94.5 ± 12.6</td>
</tr>
<tr>
<td>Leptin (10 μg/kg/min)</td>
<td>91.0 ± 4.8*</td>
<td>7.2 ± 1.3*</td>
<td>93 ± 4.4*</td>
<td>37 ± 0.3</td>
<td>25.1 ± 49.0</td>
<td>87.3 ± 28.1</td>
</tr>
<tr>
<td>Recovery</td>
<td>0.8 ± 0.2</td>
<td>42.2 ± 10.4</td>
<td>109.2 ± 3.5</td>
<td>3.2 ± 0.6</td>
<td>31.4 ± 5.9</td>
<td>105.8 ± 33.6</td>
</tr>
</tbody>
</table>

Values are mean ± SE, n=5 rats

*P < 0.05 compared with control, leptin (0.1 μg/kg/min), and recovery.

Discussion

The most significant finding of this study is that chronic leptin infusion, at rates that increased plasma leptin concentrations to levels comparable to those found in obese humans, markedly reduced food intake and elevated arterial pressure and heart rate in normal rats. The tendency for arterial pressure to increase only in the carotid artery infusion group at the low dose is consistent with the possibility that the pressor effect of leptin may have a CNS component. However, the higher infusion rate produced similar increases in both plasma levels of leptin and arterial pressure.

Humoral and Metabolic Actions of Leptin

Food intake did not change during carotid artery or IV leptin infusion at 0.1 μg/kg/min but was decreased significantly at 1 μg/kg/min in both groups. These observations are consistent with previous studies that chronic injection of leptin in ob/ob mice, animals with deficient leptin production, significantly reduces food intake. The mechanism for leptin's effect to reduce food intake has been suggested to be suppression of hypothalamic NPY mRNA expression and NPY release, although most previous studies have used very high concentrations of leptin to demonstrate these effects. In the present study, increased circulating leptin concentration, to levels comparable to those found in obese humans, markedly reduced food intake. These observations provide further support for the concept that leptin is an important physiological regulator of energy balance.

Increased circulating leptin also markedly decreased plasma insulin concentration and modestly reduced blood glucose. Although the mechanisms responsible for leptin's effects on plasma insulin and glucose levels are not clear, a recent study by Kieffer et al. indicates that there are leptin receptors located on the pancreatic β-cells, although the function of these receptors...
is not known. Leptin may also decrease insulin release by stimulating \( \alpha \)-adrenergic receptors in the pancreas via its effect on sympathetic activity. However, the finding that leptin decreased plasma glucose suggests additional effects besides a simple inhibition of insulin secretion. Another possibility is that leptin increased glucose utilization or improved insulin sensitivity in peripheral tissues, which enhanced glucose disposal in skeletal muscle and fat cells and suppressed glucose output by the liver. Thus, the decrease in plasma insulin could be a compensatory response to a fall in plasma glucose.

Additional studies are needed, however, to determine how leptin affects glucose control and insulin secretion.

Leptin, at the higher dose, did not alter plasma renin activity, but corticosterone and aldosterone tended to decrease, although not significantly. The reduced plasma aldosterone levels may be related to reduced potassium intake associated with reduced food consumption.

**Arterial Pressure and Heart Rate Responses to Leptin**

Previous studies have reported that arterial pressure and heart rate were not changed by acute leptin infusion. One possible explanation is that arterial pressure and heart rate were measured under anesthesia in those studies, whereas our studies were conducted in conscious rats. In addition, leptin was administered acutely in previous studies, either by a single bolus injection or by short-term infusion. Our results are consistent with those findings, because the increases in arterial pressure and heart rate were not apparent until 3 to 4 days after the leptin infusion was started. This was true even for the low infusion rate, which appeared to have a small centrally mediated effect on arterial pressure, since there was no significant change in systemic plasma leptin concentration and IV leptin infusion at this rate caused no changes in heart rate and arterial pressure. Thus, both the arterial pressure and heart rate responses to physiological increases in circulating leptin levels appear to be slow in onset.

The mechanisms by which increased circulating levels of leptin elevate arterial pressure and heart rate chronically are not entirely clear. One possible explanation is activation of the sympathetic nervous system. The finding that animals with deficient leptin production (ob/ob mice) or defective leptin receptors (db/db mice) also have decreased metabolic activity and hyperthermia is consistent with the concept that leptin interacts with hypothalamic receptors to reduce food intake and activate the SNS. Furthermore, leptin has been shown to increase norepinephrine turnover in interscapular brown adipose tissue, and a recent study by Haynes et al. reported that acute IV infusion of leptin in rats increased sympathetic nerve activity in the adrenals, brown adipose tissue, and the kidneys.

Our finding that there was a tendency for arterial pressure to increase with low-dose carotid artery infusion suggests a possible CNS mechanism, but our studies cannot determine the precise role of CNS compared to systemic actions of leptin in chronic blood pressure regulation. Further studies are needed to determine the role of the CNS and peripheral effects of leptin in long-term blood pressure regulation.

Heart rate also increased significantly with chronic carotid artery and IV leptin infusion at 1 \( \mu g/kg/min \). Although leptin receptor mRNA has been shown to be expressed in the heart, the physiological role of this receptor remains to be determined. Leptin could increase heart rate by increasing cardiac sympathetic activity or by withdrawal of parasympathetic tone. A recent study in our laboratory, for example, demonstrated that the increased heart rate associated with 5 weeks of a high-fat diet in dogs was due mainly to decreased cholinergic activity. However, additional studies are required to determine the contribution of sympathetic and parasympathetic mechanisms in mediating leptin's effects on heart rate.

**Renal Effects of Leptin**

Previous acute studies have shown that infusion of leptin at high doses caused naturessis and diuresis. For example, Jackson and Le reported that intravenous leptin injection at 30 \( \mu g/min \) caused a twofold increase in urine volume and sodium excretion in normal rats. Reams et al. also reported that bolus IV injection of leptin at 400 \( \mu g/kg \) significantly increased urine volume and sodium excretion in normotensive Sprague-Dawley rats, although naturessis was not observed in spontaneously hypertensive rats.

In the present study, we found no significant changes in sodium excretion or urine volume during chronic infusion of leptin at a rate that produced physiological increases in plasma leptin concentration. However, the absence of significant changes in sodium excretion in our study does not necessarily imply that leptin had no effect on renal function. In fact, IV leptin infusion significantly decreased RPF and increased RVR. Moreover, the observation that leptin did not increase sodium excretion, despite raising arterial pressure, provides evidence that leptin, infused IV or via the carotid arteries, shifted the renal-pressure naturessis relationship to higher levels than previously observed.

**TABLE 2. Effects of Chronic Intravenous Infusion of Leptin at 0.1 and 1.0 \( \mu g/kg/min \) on Plasma Leptin, Fasting Insulin (Ins), Fasting Glucose (Glu), Plasma Renin Activity (PRA), Plasma Aldosterone (Aldo), and Plasma Corticosterone (Cort)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leptin (ng/mL)</th>
<th>Ins (( \mu U/mL ))</th>
<th>Glu (mg/dL)</th>
<th>PRA (ng AL/mL/h)</th>
<th>Aldo (ng/dL)</th>
<th>Cort (ng/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.9 ± 0.1</td>
<td>52.6 ± 10.2</td>
<td>113.8 ± 3.5</td>
<td>3.8 ± 0.5</td>
<td>45.8 ± 6.5</td>
<td>137.6 ± 20.2</td>
</tr>
<tr>
<td>Leptin (0.1 ( \mu g/kg/min ))</td>
<td>2.3 ± 0.7</td>
<td>39.1 ± 10.9</td>
<td>113.4 ± 2.4</td>
<td>3.6 ± 0.6</td>
<td>36.9 ± 5.9</td>
<td>118.0 ± 35.8</td>
</tr>
<tr>
<td>Leptin (10 ( \mu g/kg/min ))</td>
<td>94.0 ± 9.0*</td>
<td>103.4 ± 2.6*</td>
<td>34.0 ± 4.0</td>
<td>23.8 ± 7.7</td>
<td>106.6 ± 38.7</td>
<td></td>
</tr>
<tr>
<td>Recovery</td>
<td>0.7 ± 0.1</td>
<td>53.8 ± 15.8</td>
<td>114.0 ± 3.3</td>
<td>4.0 ± 0.3</td>
<td>35.4 ± 10.4</td>
<td>116.2 ± 26.8</td>
</tr>
</tbody>
</table>

*P<0.05 compared with control, leptin (0.1 \( \mu g/kg/min \)), and recovery.

Values are mean ± SE, n=5 rats.
blood pressures. In the absence of an impaired pressure
natmresls, increased arterial pressure would tend to increase
renal sodium and water excretion lg2" Whether this effect of
leptm to shift pressure natnresls IS due to direct renal action or
other effects, such as sympathetic stlmulatlon, is still unclear
In summary, our studies demonstrated that leptm, at phys-
ological concentration, markedly decreased food intake and
plasma msuhn, supportmg the concept that leptm plays an
important role m regulating energy balance Our studies also
indicate that increases in circulating leptm concentration, to
levels similar to those found in obesity, significantly elevate
arteral pressure and heart rate, findings suggesting a possible
role for leptm in obesity hypertension Further studies are
needed, however, to determine the precise role of the CNS
and peripheral effects of leptm in long-term blood pressure
regulation in obesity

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murme leptm
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