Does Leptin Stimulate Nitric Oxide to Oppose the Effects of Sympathetic Activation?


Abstract—Leptin decreases appetite and increases sympathetic nerve activity and arterial pressure. Recent reports suggest that leptin may also have peripheral vasodilator actions that would tend to reduce arterial pressure. We tested the hypothesis that the direct vascular actions of leptin oppose sympathetically mediated vasoconstriction. We evaluated the effects of intravenous leptin (1 mg/kg over 3 hours) on arterial pressure and mesenteric, hindlimb, and renal blood flows in conscious rats. We then tested whether blockade of nitric oxide or the sympathetic nervous system would unmask a pressor or depressor effect of leptin, consistent with direct vascular actions. Acute intravenous administration of leptin alone did not change arterial pressure or regional blood flows. This was despite a significant increase in lumbar sympathetic nerve activity. Administration of the nitric oxide synthase inhibitor \( N^G \)-nitro-L-arginine methyl ester significantly increased arterial pressure and caused vasoconstriction. However, leptin did not have any significant effect on hemodynamics in the presence of \( N^G \)-nitro-L-arginine methyl ester despite continued sympathoactivation. \( \alpha \)-Adrenoceptor blockade with prazosin alone or combined with yohimbine significantly decreased arterial pressure and caused vasodilation. Again, leptin did not have any effect on arterial pressure or regional blood flow in the presence of sympathetic blockade. These data demonstrate that leptin does not have vasodilator actions in vivo at concentrations that are sufficient to increase sympathetic nerve activity. The absence of a pressor effect of leptin-induced sympathetic activation may merely reflect the brief duration of leptin administration. These data support the concept that the chronic hemodynamic actions of leptin are likely to be related to sympathetic activation. (Hypertension. 2001;38:1081-1086.)

Key Words: obesity ■ blood flow ■ nervous system, sympathetic renal ■ blood pressure

Leptin is a peptide hormone that is secreted by the adipocytes and acts in the hypothalamus to activate the sympathetic nervous system and decrease appetite.\(^1\)\(^-\)\(^5\) Leptin has been shown to increase sympathetic nerve activity (SNA).\(^4\)\(^,\)\(^5\) Surprisingly, despite elevated SNA, acute administration of leptin does not increase arterial pressure in anesthetized animals.\(^5\) This lack of effect on arterial pressure may reflect anesthesia, the short duration of studies, or an independent vasodilator effect. The latter would potentially oppose the vasoconstrictor effects of increased SNA. Indeed, there is evidence that leptin may have direct vascular effects. It has been found that long-form leptin receptors are present on endothelial cells.\(^6\) Lembo et al\(^7\) demonstrated that leptin causes endothelium-dependent vasodilation of rat aortic rings in vitro, which can be prevented by a NO synthase inhibitor. In addition, leptin has angiogenic activity both in vivo and in vitro.\(^6\) Furthermore, in anesthetized rats, leptin has been shown to decrease arterial pressure under conditions of sympathetic blockade and increase arterial pressure when NO synthase is blocked.\(^8\) Thus, endothelial NO generation may oppose leptin-induced sympathetic vasoconstriction.

We therefore tested the hypothesis that sympathetically mediated vasoconstrictor and pressor actions of leptin are masked by simultaneous stimulation of NO generation in conscious animals. We studied the effects of intravenous leptin on arterial pressure and regional blood flows with and without blockade of the sympathetic and NO systems in conscious rats.

Methods

Animals and Materials

Experiments were performed in 3-month-old male Sprague-Dawley and Wistar rats from Harlan Sprague-Dawley that weighed 300 to 325 g. All procedures were approved by the University of Iowa and Iowa City Veterans Affairs Animal Research Committees. Recombinant murine leptin was provided by Amgen Biologicals.

Procedures

Regional blood flows were measured by ultrasound Doppler probes (model ES, Iowa Doppler Products) as previously described.\(^9\) Under anesthesia (50 \( \mu \)g/kg IP Nembutal), Doppler probes were placed around the mesenteric (1.0-mm diameter), lower aortic (1.3-mm), and renal arteries (0.8-mm) and tied into place after a clear signal.
was obtained. The wires were tunneled subcutaneously and then exited out between the scapula. After the surgery was completed, animals were given 1500 U IM penicillin and allowed to recover 5 to 7 days. In some studies, SNA to the hindlimb was measured in conscious rats by placing an electrode on the lumbar sympathetic nerve, as previously described.5,10

**Study Design**

Rats were briefly anesthetized (40 mg/kg IP methohexitol sodium) for implantation of carotid artery and femoral vein catheters. After a complete recovery from anesthesia, each rat was placed in a Plexiglas restraining cage. Wires from the previously implanted Doppler probes were connected to a directional pulsed Doppler flowmeter, and the range was adjusted to achieve the maximum voltage. Arterial blood was taken before the administration of leptin and after 3 hours leptin or 0.9% NaCl infusion for assay of murine leptin concentrations.

**Effect of Leptin Alone on Hemodynamics**

Two groups of Doppler-instrumented Sprague-Dawley rats (n=34) received 0.9% NaCl alone for 30 minutes. One group (n=17) was then given a bolus of leptin (0.5 mg/kg) over 5 minutes, followed by an infusion of leptin (0.167 mg/kg per hour) for 3 hours (total leptin dose, 1 mg/kg), whereas the second group (n=17) was given only 0.9% NaCl for the same amount of period.

**Effect of Leptin Alone on SNA**

Rats that had previously had implantation of lumbar nerve electrodes were studied in the conscious state 3 hours after electrode implantation. One group (n=10) was given leptin as described above for 3 hours. A second group (n=8) was given 0.9% NaCl for the same time period.

**Effect of NO Blockade on Hemodynamic Response to Leptin**

Two groups of Doppler-instrumented Sprague-Dawley rats (n=38) received 0.9% NaCl alone for 30 minutes, followed by a 1-hour infusion of N^6-nitro-l-arginine methyl ester (L-NAME) (30 μg/kg per hour). One group (n=19) was given leptin as described above with continued infusion of L-NAME (30 μg/kg per hour) for 3 additional hours. The second group (n=19) received only continued infusion of L-NAME for the same 3-hour period.

**Effect of NO Blockade With Nitroprusside Clamp on Sympathetic and Blood Pressure Response to Leptin**

Two groups of Sprague-Dawley rats (n=19) that previously had implantation of lumbar nerve electrodes were studied in the conscious state 3 hours after recovery. Animals received 0.9% NaCl alone for 30 minutes, followed by L-NAME (30 μg/kg per hour). To block the pressor effects of L-NAME, sodium nitroprusside was infused (20 to 30 μg/kg per hour) to clamp the blood pressure at pre-L-NAME levels for 60 minutes. Thereafter, one group (n=10) was given leptin as described above with continued infusion of L-NAME and nitroprusside for 3 additional hours. The second group (n=9) received continued infusion of L-NAME and nitroprusside for 3 hours, without addition of leptin. The dose of nitroprusside was not changed after leptin was started.

**Effect of α₁-Adrenoceptor Blockade on Hemodynamic Response to Leptin**

Two groups of instrumented Sprague-Dawley rats (n=37) received 0.9% NaCl alone for 30 minutes, followed by a 200-μg/kg bolus of the α-adrenoceptor antagonist prazosin, and then 1-hour infusion of prazosin (300 μg/kg per hour). One group (n=15) was then given leptin as described above along with prazosin (300 μg/kg per hour) for 3 additional hours. The second group (n=22) received only continued prazosin infusion at the same dose for 3 hours. This dose of prazosin completely blocked the pressor effect of a 25-μg/kg IV bolus dose of phenylephrine (ΔMAP phenylephrine+prazosin: −142±7 mm Hg; P<0.05). Prazosin partially attenuated the pressor effect of a 0.1-μg/kg IV bolus dose of norepinephrine (ΔMAP norepinephrine alone: +30±5 mm Hg; ΔMAP norepinephrine+prazosin: +20±2 mm Hg; P<0.05).

**Effects of α₁- and α₂-Adrenoceptor Blockade on Hemodynamic Response to Leptin**

This protocol was identical to the prazosin-alone protocol except that 2 groups of 10 rats received the α₂-adrenoceptor antagonist yohimbine (bolus 5 mg/kg; maintenance: 1 mg/kg per hour) in addition to prazosin. This combination of prazosin and yohimbine completely blocked the pressor effect of a 0.1-μg/kg IV bolus dose of norepinephrine (ΔMAP norepinephrine alone: +30±5 mm Hg; ΔMAP norepinephrine+α-blockers: −6±4 mm Hg; P<0.05).

**Effects of NO Blockade on Leptin Responses in Anesthetized Animals**

To determine whether anesthesia or strain had altered the hemodynamic response to leptin, studies were performed in anesthetized Wistar rats with the same protocol as a previously published study.8 These animals did not have Doppler probes implanted. Catheters were placed into the femoral artery and vein. Animals were anesthetized with intravenous chloralose (50 mg/kg initially, then 25 mg/kg per hour). Two groups of Wistar rats (n=10) received a bolus dose of L-NAME (30 μg/kg). After 15 minutes, one group (n=5) was administered a bolus of leptin (1 mg/kg) over 5 minutes, whereas the other group (n=5) was given a bolus of 0.9% NaCl. Both groups were monitored for 90 minutes after the leptin or 0.9% NaCl injection.

**Data Analysis**

Signals from the Doppler flowmeter and arterial pressure transducer were digitized, displayed, and stored on an Apple Macintosh (8500/180), with the use of a MacLab (model 8/e) analog-to-digital converter (AD Instruments) with Chart v 3.6/s software. Aortic flow measurements were recorded in megahertz and corrected for background noise by subtracting postmortem measurements from measurements taken when alive. (To convert flow measurements to conductance, the corrected values were divided by the MAP at the time and expressed as MHz/mm Hg). Results are expressed as absolute mean±SEM values in tables and as percentage of baseline in figures. Baseline values were obtained by averaging 3 time points, separated by 5 minutes each, during 0.9% NaCl infusion (control period 1). Post-L-NAME and postprazosin values were obtained by averaging data collected between 50 to 60 minutes after L-NAME or prazosin infusion (control period 2) and 50 to 60 minutes (1 hour), 110 to 120 minutes (2 hours), and 170 to 180 minutes (3 hours) after leptin. SNA values were obtained by averaging data collected between 0 to 15 minutes and 345 to 360 minutes (6 hours). Statistical comparisons were made with the use of repeated-measures factorial ANOVA, with Scheffé’s test used for significance testing.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionahaj.org.

**Results**

**Effect of Leptin Alone on Hemodynamics**

There were no significant differences between the leptin-treated and non–leptin-treated animals in MAP or heart rate (Table 1 Online). Both groups had a slight decrease in blood pressure over time (saline, P=0.022; Figure 1); (leptin, P=0.191; Figure 1). Leptin did not significantly change mesenteric (P=0.833; Figure 2), hindlimb (P=0.581; Figure 3), or renal conductance (P=0.577; Figure 4). Plasma murine leptin concentrations were significantly higher than baseline in leptin-treated animals (baseline, 1.5±0.5 ng/mL; leptin, 227±40 ng/mL; P<0.001).
Effect of Leptin on SNA
In conscious Sprague-Dawley rats, intravenous leptin slowly increased, with the maximal effect observed 6 hours after starting the leptin infusion (baseline SNA, 88±13 sp/s; 6-hour SNA, 143±19 sp/s; P<0.01) (Figure 5). Saline infusion did not increase lumbar SNA (baseline SNA, 93±11 sp/s; 6-hour SNA, 66±8 sp/s; P=0.13).

Effect of NO Blockade on Hemodynamic Response to Leptin
L-NAME significantly increased arterial pressure (by 18±2 mm Hg; P<0.001) and decreased heart rate (by 41±7 bpm; P<0.001) and regional blood flows (Table 2 Online). However, administration of leptin after NO blockade by L-NAME did not change arterial pressure (P=0.231; Figure 1) or heart rate (P=0.511). Mesenteric conductance decreased slightly after leptin administration in L-NAME-treated animals, but there was no significant difference from animals receiving L-NAME alone (P=0.162; Figure 2). In addition, as compared with animals receiving L-NAME alone, there was no significant effect of leptin on hindlimb (P=0.703; Figure 3) or renal conductance (P=0.373; Figure 4) in the presence of L-NAME.

Figure 2. Mesenteric conductance (% of baseline) during infusion of 0.9% NaCl (○), leptin (●), L-NAME (●), leptin and L-NAME (●), prazosin (●), and leptin and prazosin (●). Control period 1 is during 0.9% NaCl infusion; control period 2 is after 1-hour infusion of L-NAME or prazosin. Values are mean±SEM.

Effect of NO Blockade With Nitroprusside Clamp on Sympathetic and Blood Pressure Response to Leptin
Infusion of nitroprusside prevented the pressor effect of L-NAME (Table 1). Arterial pressure and heart rate levels in both groups during L-NAME infusion were similar to the control period and remained unchanged for the whole experiment. Importantly, infusion of leptin over 180 minutes produced a significant increase in conscious lumbar SNA (P<0.01), without changing blood pressure (Figure 5 and Table 1). Saline infusion did not increase lumbar SNA or blood pressure.

Effect of α1-Adrenoceptor Blockade on Hemodynamic Response to Leptin
Prazosin significantly decreased arterial pressure (by 10±2 mm Hg; P<0.001; Figure 1) and increased heart rate (by 48±7 bpm; P<0.001) (Table 3 Online). Leptin had no effect on arterial pressure (P=0.565), heart rate (P=0.812), or on conductance in mesenteric (P=0.672; Figure 2), hindlimb (P=0.332; Figure 3), or renal beds (P=0.489; Figure 4).

**Figure 3.** Hindlimb conductance (% of baseline) during infusion of 0.9% NaCl (○), leptin (●), L-NAME (●), leptin and L-NAME (●), prazosin (●), and leptin and prazosin (●). Control period 1 is during 0.9% NaCl infusion; control period 2 is after 1-hour infusion of L-NAME or prazosin. Values are mean±SEM.
**Effects of α1- and α2-Adrenoceptor Blockade on Hemodynamic Response to Leptin**

The combination of α1- and α2-adrenoceptor blockade with prazosin and yohimbine significantly decreased arterial pressure (by 28±7 mm Hg; P<0.001), with no change in heart rate (Table 2). In addition, combined α-blockade produced a significant increase in hindlimb conductance (P<0.001), with no significant changes in mesenteric and renal conductance (Table 2). Infusion of leptin for 3 hours had no additional effects on arterial pressure (P=0.538) or heart rate (P=0.371) or on conductance through the mesenteric (P=0.625), hindlimb (P=0.499), and renal beds (P=0.422).

**Effects of NO Blockade on Leptin Responses in Anesthetized Animals**

In anesthetized Wistar rats, bolus administration of L-NAME increased arterial pressure from 117±4 to 162±4 mm Hg at 10 minutes, with arterial pressure falling to 135±3 mm Hg at 100 minutes. This arterial pressure response was not altered by administration of leptin at 15 minutes after L-NAME, with arterial pressure increasing from 130±4 to 163±3 mm Hg 10 minutes after L-NAME, then declining to 140±5 mm Hg at 100 minutes.

**Discussion**

Intravenous leptin alone clearly did not alter arterial pressure or regional blood flows in conscious animals over a 3-hour period, despite increased SNA. This might suggest activation of compensatory vasodilator mechanisms. However, blockade of NO synthase with L-NAME did not unmask a pressor effect of leptin, even when NO activity was clamped at basal levels. Similarly, leptin had no depressor effect after α-adrenoceptor blockade with prazosin alone or in combination with yohimbine. Our findings suggest that leptin does not have substantial direct or indirect vasodilator actions at concentrations sufficient to increase SNA in vivo. Incidentally, this study provides the first demonstration that leptin increases directly measured sympathetic nerve traffic in the conscious state, either alone or after NO synthesis inhibition.

Leptin has been shown to increase endothelial NO generation in isolated aortic rings. However, our study does not support an effect of leptin on NO activity in resistance vessels in vivo because leptin had no effect on hemodynamics even after blockade of NO generation. It is possible that the findings of previous in vitro studies represent a pharmacological dose of leptin or differences between types of vessel. We achieved leptin concentrations (>200 ng/mL) that were sufficient to cause substantial sympathoactivation and can increase arterial pressure when given long-term. Most human subjects with obesity have leptin concentrations <100 ng/mL, so it is unlikely that effects of leptin at higher concentrations will be physiologically or pathophysiologically relevant.

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**Table 2. Prazosin and Yohimbine Protocol: Hemodynamic Data Obtained During 0.9% NaCl Infusion, After 1-Hour Prazosin/Yohimbine Infusion, and in the Third Hour of Experimental Infusion of Prazosin/Yohimbine and Leptin (1 mg/kg) or Prazosin/Yohimbine Alone**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Prazosin/Yohimbine + Leptin</th>
<th>Prazosin/Yohimbine + Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>115±3</td>
<td>117±7</td>
</tr>
<tr>
<td>+ Prazosin/yohimbine</td>
<td>87±7*</td>
<td>89±6*</td>
</tr>
<tr>
<td>+ Vehicle/leptin</td>
<td>96±6*</td>
<td>99±7*</td>
</tr>
</tbody>
</table>

| HR, bpm                          | 456±40                      | 477±44                      |
| + Prazosin/yohimbine             | 443±23                      | 492±32                      |
| + Vehicle/leptin                 | 437±31                      | 478±36                      |

| Mesenteric conductance, mm/MHz   |                             |                             |
| + Prazosin/yohimbine             |                             |                             |
| + Vehicle/leptin                 |                             |                             |

| Hindlimb conductance, mm/MHz     |                             |                             |
| + Prazosin/yohimbine             |                             |                             |
| + Vehicle/leptin                 |                             |                             |

| Renal conductance, mm/MHz        |                             |                             |
| + Prazosin/yohimbine             |                             |                             |
| + Vehicle/leptin                 |                             |                             |

*P<0.05 vs saline.

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**Figure 4.** Renal conductance (% of baseline) during infusion of 0.9% NaCl (●), leptin (●), L-NAME (●), leptin and L-NAME (●), prazosin (●), and leptin and prazosin (●). Control period 1 is during 0.9% NaCl infusion; control period 2 is after 1-hour infusion of L-NAME or prazosin. Values are mean±SEM.

**Figure 5.** A, Examples of lumbar SNA before and after administration of leptin (1 mg/kg over 3 hours) in conscious Sprague-Dawley rat. B, Examples of lumbar SNA before and after administration of leptin (1 mg/kg over 3 hours) in conscious Sprague-Dawley rat during NO clamp with L-NAME and nitroprusside.
Frühbeck et al. found that under NO blockade, leptin caused a significant increase in arterial pressure in anesthetized rats. We were unable to demonstrate such an action in conscious rats. Because differences in anesthesia or strain may have caused these discrepancies, we performed studies in anesthetized Wistar rats by using the same published protocol and were unable to demonstrate a pressor effect of leptin after NO blockade. These contradictory findings may relate to other factors such as laboratory conditions or perhaps the leptin preparation. For example, manufacturing of recombinant leptin may be associated with contamination by endotoxin. Such inadvertent endotoxin contamination could potentially induce NO generation in vivo.

Why does leptin not decrease regional blood flows or increase arterial pressure, given that it causes sympathetic activation? The lack of change in hemodynamics with leptin under conditions of sympathetic or NO blockade suggests that leptin does not have opposing vasodilator actions. The lack of a pressor effect may therefore reflect nonvascular actions of the sympathetic nerves that were activated, such as thermogenesis or lipolysis. Alternatively, the degree and duration of sympathetic activation may have been insufficient to acutely increase arterial pressure. However, sustained increases in SNA of similar magnitude could potentially have substantial effects on cardiovascular function and structure.

If leptin does not have vasodilator actions, then the overall hemodynamic effect of chronic elevations in leptin probably will relate to its actions to increase SNA. Indeed, there is experimental evidence to support this concept. For example, Shek et al. have demonstrated that chronic systemic administration of leptin for 12 days significantly increased arterial pressure and heart rate in conscious rats. In addition, transgenic mice with hepatic overexpression of leptin have increased arterial pressure that can be normalized by sympathetic blockade. Furthermore, we have recently shown that chronic intracerebroventricular administration of nonsystemic doses of leptin slowly increases arterial pressure over 2 weeks at doses that are without hemodynamic effect when administered intravenously. Finally, obese ob/ob mice that are deficient in leptin have substantially lower arterial pressure than lean littermate control mice in the conscious state.

Together these studies suggest that chronic sympathetic activation by leptin may contribute to the physiological maintenance of arterial pressure.

There are several potential limitations to this study that need to be addressed. First, we did not study the effects of other endothelial mediators such as endothelium-derived hyperpolarizing factor. Our studies with L-NAME would suggest that there is no functionally consequential stimulation of NO by leptin. In addition, leptin did not cause vasodilation or a decrease in arterial pressure in the presence of complete α-adrenoceptor blockade with prazosin and yohimbine. If leptin did stimulate generation of other vasodilators, blockade of sympathetically mediated vasoconstriction should have unmasked a depressor effect, which was clearly not the case. This suggests that leptin does not stimulate generation of non-NO vasodilator substances. Second, although prazosin completely blocked the pressor effect of the α₁-adrenoceptor agonist phenylephrine, it clearly did not completely block the pressor response to the nonselective agonist norepinephrine. It is therefore possible that leptin continued to exert vasoconstrictor actions through α₁-adrenoceptors and that this masked any vasodilator actions. However, leptin did not have any depressor or vasodilator actions when both α₁- and α₂-adrenoceptors were completely blocked with prazosin and yohimbine. Third, it is possible that inhibition of NO synthase with L-NAME administration interfered with sympathoactivation or that the pressor action of L-NAME altered the hemodynamic response to leptin. However, when NO activity was clamped with a combination of L-NAME and nitroprusside, leptin-induced sympathoactivation persisted and leptin continued to have no effect on arterial pressure. Fourth, we used murine leptin in the rat, which may have different effects than rat leptin. However, we have shown that our preparation of murine leptin was biologically active in the conscious rat, acting to increase lumbar SNA. Fifth, it is possible that the sensitivity of the Doppler technique was insufficient to detect a small effect of leptin on regional blood flows. However, power calculations with the use of our Doppler signal variability revealed that our sample sizes were sufficient to detect a 12% change in regional blood flow, with 80% power at the 0.05 significance level.

In summary, the present study demonstrates that leptin has no resistance vessel vasodilator actions in conscious animals at concentrations that cause sympathoactivation. These results suggest that the putative vasodilator actions of leptin probably are confined to conduit vessels or occur at pharmacological rather than physiological concentrations. Our data support the concept that the main hemodynamic actions of leptin are likely to be related to chronic sympathetic activation.

Acknowledgments

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References

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