Renal effects of leptin in normotensive, hypertensive, and obese rats

DANIEL VILLARREAL, GARRY REAMS, RONALD H. FREEMAN, AND AMIR TARABEN
Departments of Internal Medicine and Physiology, University of Missouri and Harry S. Truman Memorial Veterans' Hospital, Columbia, Missouri 65212

Villarreal, Daniel, Garry Reams, Ronald H. Freeman, and Amir Tarabenn. Renal effects of leptin in normotensive, hypertensive, and obese rats. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R2056–R2060, 1998.—The hemodynamic, hormonal, and renal excretory effects of intravenous bolus administration of synthetic murine leptin were examined in groups of anesthetized normotensive (Sprague-Dawley), hypertensive (spontaneously hypertensive), and both lean and obese Zucker rats. In the normotensive animals (n = 8) an intravenous bolus of 400 µg/kg of leptin produced a significant six- to sevenfold elevation in sodium excretion compared with controls (n = 8). The onset of natriuresis was delayed for ~30–45 min. Mean arterial pressure (MAP), creatinine clearance, plasma renin activity (PRA), and plasma aldosterone concentration (PAC) remained unchanged. In contrast, the hypertensive rats were refractory to the natriuretic effects of leptin when infused either with 400 (n = 8) or 1,600 (n = 8) µg/kg. Also in these animals MAP, creatinine clearance, PRA, and PAC were unmodified. Finally, whereas lean Zucker rats (n = 8) responded very similarly to the Sprague-Dawley animals, the natriuretic effect of the hormone was attenuated in the obese Zucker groups. At 400 µg/kg (n = 8) no natriuresis was elicited, but at 1,600 µg/kg (n = 8) a modest but significant two- to threefold increment in sodium excretion was observed in the obese rats. In both Zucker groups, MAP, creatinine clearance, PRA, and PAC were unchanged. Collectively, these results demonstrate a significant natriuretic effect of exogenous leptin in the normal rat and a blunted saluretic response in hypertension and obesity. It is suggested that leptin may be a potential salt-excretory factor in normal rats and may function pathophysiologically in obesity and hypertension.

The association between hypertension and obesity has been previously described, but the pathophysiological basis of obesity-induced hypertension remains unclear (11, 14). Mechanisms suggested to be involved include increased plasma volume and cardiac output, hyperinsulinemia and insulin resistance, enhanced sympathetic nervous system activity, and sodium retention with dysfunction of salt-regulating hormones (11, 14). Although the renal mechanisms that lead to obesity-related sodium retention have not been fully evaluated, these do not appear to be related to either renal vasoconstriction or decreased filtered sodium load. Obesity, however, is associated with an enhanced absorptive and fractional sodium reabsorption that may occur at distal nephron sites (12).

Leptin, the product of the ob/ob gene, is an adipose tissue-derived secreted protein that has been implicated primarily in the regulation of food intake as well as other metabolic parameters (19, 21). The expression of the ob gene and circulating leptin levels correlate with body fat content. It has been suggested that leptin is a sensing protein for adipose tissue, reducing food intake in a negative feedback manner. The leptin receptor is a member of the extended class I cytokine receptor family having at least six splice variants ob-R(a-f) (4, 19, 21). The ob-Ra variant has been postulated to transport leptin across the blood-brain barrier (5, 18, 30). Ob-Rc and ob-Rd have been implicated in the clearance of leptin from the circulation, and the ob-Res variant is a putative soluble receptor (5, 18, 30). The ob-Rb variant encodes a receptor with a long intracellular domain, which is essential for intracellular signal transduction (5, 18, 30); and finally, the recently recognized ob-Rf variant has as yet no identified function (5, 18, 30).

High tissue levels of leptin receptor gene expression occur in the lung, moderate levels in the kidney, and low levels in the heart, brain, spleen, liver, and muscle (6, 14, 30), as demonstrated by reverse transcription-polymerase chain reaction analysis and Southern blot analysis. Expression of the extracellular domain of the leptin receptor ob-R and the short splice variant ob-Ra has been shown in many peripheral tissues (32); however, the long splice variant ob-Rb was detectable only in the adrenal gland and kidneys (27). Within the kidney, in situ hybridization occurs over the inner zone of the medulla and the pyramid, appearing to be associated with collecting tubules and ducts (14).

Leptin circulates in humans in the bound form, and its levels are increased in obesity primarily in the form of free leptin. Interestingly, it has also been reported that circulating levels of leptin are elevated in patients with essential hypertension (1). More recently, it was demonstrated that exogenous leptin produced a natriuresis in anesthetized, normotensive rats when infused directly into the renal artery (16), suggesting a potential role of this hormone in the regulation of sodium volume balance. Moreover, chronic low-dose administration of leptin into conscious rats has been shown to produce modest but sustained increases in mean arterial pressure (MAP) and heart rate (28). Notwithstanding this initial information, the involvement, if any, of leptin for control of sodium excretion in obesity and hypertension remains undefined. Thus this study was designed to examine the acute hemodynamic and renal...
effects of synthetic leptin in rat models of normotension, hypertension, and obesity.

METHODS

Animal models. Sprague-Dawley male rats (SDR; Harlan, Indianapolis, IN), spontaneously hypertensive rats (SHR; Taconic Farms, Germantown, NY), and both lean and obese Zucker rats (Harlan), with body weights between 250 and 400 g were utilized for this study. All animals were housed in individual cages in a room on a 12:12-h light-dark cycle and maintained on a regular rat chow diet (Purina, St. Louis, MO) for at least 7 days before the study. Tap water was available ad libitum. All experiments were performed in the postabsorptive state at least 18 h after the last meal. All animal care provided during the conduction of these studies met institutional guidelines. On the day of the acute experiment, anesthesia was induced with Inactin (100 mg/kg ip; Lockwood and Associates, Sturtevant, WI). A tracheostomy was performed, and polyethylene catheters (PE-50) were inserted into the carotid artery and jugular vein. The urinary bladder was exteriorized and cannulated. The arterial catheter was connected to a Statham P23 Db strain gauge pressure transducer (Oxnard, CA) to a Hewlett-Packard 7714-041A recorder (St. Louis, MO) for continuous MAP monitoring. After completion of all surgical procedures, a 0.75-ml intravenous bolus of a creatinine-saline solution with a creatinine concentration of 370 mg/ml was administered and immediately followed by a sustained infusion at a rate of 25 µl/min (Sage Instruments, Boston, MA) for the duration of the experiment to measure creatinine clearance.

Experimental design. After 15 min of an equilibration period, an intravenous bolus of synthetic murine leptin (PeproTech, Rocky Hill, NJ) was infused over 30 s into the jugular vein. The SDR and the lean Zucker rats were infused with either 400 µg/kg of leptin (n = 8) or a saline vehicle (n = 8); the SHR and the obese Zucker rats were infused with either 400 µg/kg (n = 8) or 1,600 µg/kg (n = 8) of leptin or a saline vehicle (n = 8). After an additional 30-min equilibration interval, two 45-min experimental renal clearance periods (E1 and E2) were obtained. Arterial blood (1.0 ml) was obtained at the midpoint of each renal clearance period for measurements of plasma creatinine, sodium, and potassium. This blood volume loss was replaced intravenously with an equal volume of 6% albumin diluted in normal saline. Urine was collected for the entire period and assayed for creatinine, sodium, and potassium. Finally, after completion of the second renal clearance period, arterial blood (3.0 ml) was obtained for measurement of plasma renin activity (PRA) and plasma aldosterone concentration (PAC).

Assays. Creatinine was assayed by autoanalyzer. Sodium and potassium were determined by flame photometry. PRA and PAC were determined by radioimmunoassay as previously described (31).

Statistical analysis. Group data were expressed as means ± SE. Between appropriate groups, data were analyzed using ANOVA with a two-factor mixed design and least-significant difference as post hoc test (2). A difference was considered statistically significant at P < 0.05.

RESULTS

The systemic hemodynamic and renal excretory responses to leptin in all groups of rats are shown in Figs. 1–3 and Table 1. MAP remained unchanged (P > 0.05) throughout the experiment in the vehicle control and leptin-treated animals of the SDR, SHR, and Zucker groups, although in all of the series a slight, nonsignificant (P > 0.05) reduction in MAP was observed in E2 compared with E1 (Fig. 1).

In SDR, urinary sodium excretion was increased sevenfold after leptin infusion compared with control animals infused with the vehicle (Fig. 2). Creatinine clearance (Table 1) remained unchanged in both the control and leptin groups of SDR throughout the study, whereas fractional sodium excretion was two- to fourfold higher in the leptin group compared with control rats (P < 0.05), indicating a predominant tubular effect of leptin for the promotion of natriuresis (Fig. 2). Similar, although less marked, responses in urinary flow rate and urinary potassium excretion were observed in the leptin-treated SDR compared with the control group (Table 1).

Like the SDR, no systemic hemodynamic changes were observed in the SHR with leptin administration compared with the control group even when the dose of the hormone was quadrupled to 1,600 µg/kg (Fig. 1). Similarly, leptin had no effect on creatinine clearance in the hypertensive rats at either dose (Table 1). Interestingly, however, in this spontaneously hypertensive model, leptin produced no significant increases in uri-

Table 1. Renal effects of leptin in rats

<table>
<thead>
<tr>
<th></th>
<th>Creatinine Clearance, ml/min</th>
<th>Urinary Potassium Excretion, neq/min</th>
<th>Urinary Flow Rate, µl/min</th>
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<tbody>
<tr>
<td></td>
<td>E1</td>
<td>E2</td>
<td>E1</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.91 ± 0.33</td>
<td>2.51 ± 0.33</td>
<td>400 ± 86</td>
</tr>
<tr>
<td>Leptin (400 µg/kg)</td>
<td>2.33 ± 0.32</td>
<td>2.68 ± 0.24</td>
<td>805 ± 146†</td>
</tr>
<tr>
<td>Spontaneously hypertensive</td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>1.67 ± 0.18</td>
<td>1.72 ± 0.12</td>
<td>561 ± 128</td>
</tr>
<tr>
<td>Leptin (400 µg/kg)</td>
<td>1.99 ± 0.12</td>
<td>2.27 ± 0.06</td>
<td>177 ± 32</td>
</tr>
<tr>
<td>Leptin (1,600 µg/kg)</td>
<td>1.93 ± 0.21</td>
<td>2.22 ± 0.11</td>
<td>347 ± 115</td>
</tr>
<tr>
<td>Lean Zucker</td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>1.51 ± 0.14</td>
<td>1.73 ± 0.16</td>
<td>895 ± 182</td>
</tr>
<tr>
<td>Leptin (400 µg/kg)</td>
<td>1.51 ± 0.39</td>
<td>1.99 ± 0.39</td>
<td>1,413 ± 364</td>
</tr>
<tr>
<td>Obese Zucker</td>
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</tr>
<tr>
<td>Control</td>
<td>2.53 ± 0.36</td>
<td>2.69 ± 0.42</td>
<td>483 ± 117</td>
</tr>
<tr>
<td>Leptin (400 µg/kg)</td>
<td>2.86 ± 0.10</td>
<td>3.30 ± 0.15</td>
<td>641 ± 251</td>
</tr>
<tr>
<td>Leptin (1,600 µg/kg)</td>
<td>2.42 ± 0.15</td>
<td>2.11 ± 0.13</td>
<td>1,076 ± 322†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 in each group. *P < 0.05 vs. equilibration period (E1). †P < 0.05 vs. control of corresponding experimental period.
nary sodium, potassium, or volume excretion at both the 400- and 1,600-µg/kg infusion doses compared with vehicle control SHR (Fig. 2, Table 1). This observation in SHR is in contrast to the marked natriuretic response to leptin administration in the SDR model. No alterations in MAP occurred in either the lean or obese Zucker rat with leptin administration compared with their respective control groups (Fig. 1). The natriuretic, diuretic, creatinine clearance, and fractional sodium excretion responses with leptin in the lean Zucker rats were comparable to the normotensive SDR (Fig. 3 and Table 1), although a kaliuresis was not observed (Table 1). Like SHR and in contrast to lean animals, the obese Zucker rats infused with leptin at the 400 µg/kg dose did not respond with enhanced electrolyte or fluid-volume excretion rates compared with the control group. However, when leptin administration was increased to 1,600 µg/kg, a modest but significant increase in natriuresis and kaliuresis in the obese Zucker rat did occur (Fig. 3). Lastly, with either low- or high-dose leptin, both creatinine clearance and urinary flow rate remained unchanged (P > 0.05) compared with control animals.

The data for PRA and PAC in all of the series of SDR, SHR, and Zucker rats is presented in Table 2. In the three strains of rats, the plasma levels of the two hormones remained unchanged from their respective controls (P > 0.05) with the administration of leptin, indicating little if any effect on this renal-adrenal hormonal axis.

DISCUSSION

Leptin is a circulating polypeptide protein produced by an adipocyte-specific gene (4, 19, 21). It regulates energy balance by binding to receptors in the hypothalamus, leading to alterations in food intake, temperature, and energy expenditure (3, 4, 19, 21). Adipose tissue leptin mRNA and serum leptin levels directly correlate with the amount of body fat, and considerable information indicates that leptin is a signaling factor for...
regulation of body weight (15, 20, 25). Leptin has also been shown to increase norepinephrine turnover in thermogenic, brown adipose tissue, suggesting that increased sympathetic activity may in part modulate its action (4). Additionally, leptin-induced elevations in sympathetic activity have been demonstrated in nonthermogenic tissue, specifically the kidney (13). Relevant to the regulation of body fluid volume and pressures it is pertinent to point out that enhanced efferent renal sympathetic nerve activity (ERSNA) has been demonstrated to exert antinatriuresis by a variety of direct and indirect mechanisms (9) and that elevated circulating levels of leptin have been measured in patients with essential hypertension (1). However, considering the association of obesity with sodium retention and hypertension, there is minimal information on the renal effects of leptin in these pathophysiological situations.

Natriuresis and diuresis have been reported with increasing intrarenal infusion doses of leptin in normotensive, anesthetized rats (16). In this previous study (16), intrarenal administration of leptin had no effect on arterial pressure, heart rate, renal blood flow, glomerular filtration rate, or potassium excretion. The renal excretory effects of intrarenal infusion of human leptin were not immediate, requiring some time to be fully expressed. The reason for this phenomenon is unclear; however, Jackson and Li (16) have suggested that the delayed time course observed in renal excretory function with human leptin is consistent with a signal transduction pathway that involves alteration in gene transcription. The threefold increase in sodium excretion was confined to the infused kidney, suggesting a local effect (16). In the present study a natriuresis and diuresis was likewise demonstrated in the anesthetized, normotensive SDR when murine leptin was administered systemically; moreover, the full expression of natriuresis and diuresis was delayed, confirming the previous findings reported during intrarenal infusion of human leptin (16). Furthermore, in both of these studies, the observed increased fractional excretion of sodium suggests a tubular mechanism for leptin-induced natriuresis in normotensive rats.

To our knowledge the current study represents the first investigation of the renal effects of leptin in hypertension. Unlike the observations in the normotensive SDR model, systemic infusion of leptin failed to produce a natriuresis or diuresis in SHR. Even when the dose of leptin was fourfold increased from 400 to 1,600 µg/kg, again no changes were observed in renal excretory function or creatinine clearance. These infusion doses of leptin were clearly of pharmacological magnitude and presumably elevated plasma levels markedly. The reasons for the impaired natriuretic response to leptin observed in the SHR are unclear. However, a preliminary study (10) from this laboratory in this experimental model has indicated that acute renal denervation restored at least in part the natriuretic response to leptin. This initial observation suggests that the elevated level of ERSNA characteristic of the SHR (8) and/or potential leptin-induced increases in ERSNA (13) may have contributed to attenuate the renal tubular actions of leptin in this hypertensive rat model. Moreover, a preliminary study in an experimental rat model of hypertension and obesity, the Koletsky strain, has demonstrated that leptin administered to the lean Koletsky rat exhibited renal sympathetic activation, which was not apparent in its obese counter-part (7). Of interest, however, leptin-induced natriuresis was not observed in either the lean or obese Koletsky rat (7).

Similar to hypertension, minimal information is available on the renal effects of leptin in animal models of obesity. The Zucker rat is an autosomal-recessive model of obesity characterized by proteinuria and glomerulosclerosis, usually apparent after 14 wk of age (28, 29). Insulin resistance, hyperinsulinemia, and hypertension in response to high dietary intake of sodium are also characteristics of this genetic model of obesity (23). Recently, a specific missense mutation was found in the extracellular domain of all leptin receptor isoforms in the Zucker rat (29). This mutation was found only in the obese Zucker rat but not in either its lean littermate or SDR (29).

In the current study, both natriuresis and diuresis were demonstrated in the anesthetized, lean Zucker rat after leptin infusion. These responses in the lean Zucker rat were quantitatively and qualitatively similar to the response obtained on the SDR. The increase in fractional excretion of sodium, without significant changes in either creatinine clearance or the renin–aldosterone axis, again suggests a tubular mechanism for leptin-induced natriuresis in this rat model. In the obese Zucker rat, an attenuated natriuretic and diuretic effect was observed. Because a deficit in the leptin receptor has been demonstrated and hypothalamic leptin resistance is postulated in this genetic animal model of obesity (29), it is possible that a similar renal mechanism may explain the attenuated natriuretic and diuretic response to leptin obtained in the current study. Indeed, four times more leptin was required in the obese Zucker rat to achieve similar renal excretory responses as observed in its lean littermate. Because leptin may not cause sympathoactivation in the obese

<table>
<thead>
<tr>
<th>Plasma renin activity, ng·ml⁻¹·h⁻¹</th>
<th>Control (400 µg/kg)</th>
<th>Leptin (1,600 µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprague-Dawley</td>
<td>3.5 ± 0.5</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>Spontaneously hypertensive</td>
<td>8.7 ± 1.2</td>
<td>6.9 ± 0.6</td>
</tr>
<tr>
<td>Lean Zucker</td>
<td>6.5 ± 0.4</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td>Obese Zucker</td>
<td>5.7 ± 0.4</td>
<td>5.8 ± 0.5</td>
</tr>
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</table>

| Plasma aldosterone, ng/ dl          | Sprague-Dawley      | 14.8 ± 0.7           | 14.6 ± 0.6           |
|                                     | Spontaneously hypertensive | 13.1 ± 1.0           | 10.6 ± 0.6           | 13.2 ± 1.1 |
|                                     | Lean Zucker          | 10.4 ± 0.4           | 11.1 ± 0.8           |
|                                     | Obese Zucker         | 13.0 ± 0.9           | 14.8 ± 0.6           | 13.0 ± 1.3 |

Values are means ± SE; n = 8 in each group. For each leptin-infused group, values were not different (P > 0.05) compared with respective control.
Zucker rat (22), it is possible that unidentified mechanisms other than leptin-induced enhanced ERSNA contributed to the attenuated natriuretic actions of the hormone.

In summary, the composite results of the current investigation suggest that the acute infusion of synthetic leptin produces renal enhancement of sodium excretion in the normal rat via an action at the tubular level. The exact mechanism(s) for leptin-induced natriuresis and the nature of the blunted natriuretic response in genetic hypertension and obesity require further investigation. Whereas leptin clearly is an important circulating signal for body weight homeostasis, it now appears to be a potential salt-excreting factor and may function pathophysiologically as a common link to obesity and hypertension.

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Address for reprint requests: D. Villarreal, 1E65 Health Sciences Center, One Hospital Drive, Columbia, MO 65212.

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