A comparison of the natriuretic/diuretic effects of rat vs. human leptin in the rat

EDWIN K. JACKSON AND WILLIAM A. HERZER
Center for Clinical Pharmacology, Departments of Pharmacology and Medicine, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15213-2582

Jackson, Edwin K., and William A. Herzer. A comparison of the natriuretic/diuretic effects of rat vs. human leptin in the rat. Am. J. Physiol. 277 (Renal Physiol. 46): F761–F765, 1999.—Intrarenal artery infusions of low-dose human, but not mouse, leptin cause diuresis/natriuresis in rats [E. K. Jackson and P. Li. Am. J. Physiol. 272 (Renal Physiol. 41): F333–F338, 1997]. The lack of effect of mouse leptin in the rat could be due to slight differences in the primary structure of mouse vs. rat leptin. To test this hypothesis, we infused single doses of rat (0.1, 0.3, 1, or 3 µg/min) or human (3 µg/min) leptin into the renal artery of rats for 140 min while continuously measuring blood pressure and the renal excretion rate of urine and electrolytes. Intrarenal infusions of rat leptin did not alter any measured parameter. Human leptin caused a delayed diuresis/natriuresis (P < 0.0006 and P < 0.0049, respectively) that required ~2 h to achieve a maximum effect and that was not accompanied by changes in blood pressure or potassium excretion. We conclude that low-dose human, but not low-dose rodent, leptin has direct diuretic/natriuretic activity. Our results can be explained from an evolutionary perspective, since obesity-induced hypertension would be a much greater selective force in hominids compared with rodents.

sodium excretion; obesity; hypertension; kidneys

THE ABILITY TO STORE ENERGY as fat is an important survival mechanism that allows organisms to endure times of deprivation by storing calories during times of plenty. However, for humans at least, this evolutionary solution to the erratic availability of dietary energy carries the risk of cardiovascular disease secondary to obesity-induced hypertension and diabetes.

In this regard, in a previous publication, we developed the hypothesis that leptin may serve to protect humans from the adverse effects of storing calories as fat (5). Leptin is a protein released from white adipose tissue that circulates to the brain and appropriately modifies food-seeking behavior and bodily energy expenditures so as to maintain a relatively stable body weight (1, 3, 8, 15). However, leptin also increases insulin sensitivity (12), and our recent work suggests that human leptin increases renal sodium excretion (5), a major determinant of the long-term levels of arterial blood pressure (2). Thus it is possible that leptin not only functions to limit adipose stores but also serves to protect the cardiovascular system from the well-known risks of adipose tissue, i.e., insulin resistance and hypertension (4, 9, 10).

Although evolution of a mechanism to protect against obesity-induced salt retention and hypertension would be beneficial for humans, it is difficult to imagine that the same would be true for rodents. Because rodents are low on the food chain, in the natural environment predation or starvation is the fate of most rodents, and death due to obesity-induced cardiovascular disease would be an extraordinarily rare event. Therefore, whereas mechanisms to protect against obesity-induced hypertension might evolve in humans, it seems only remotely possible that such mechanisms would evolve in rodents.

Consistent with this analysis is our recent finding that intrarenal infusions of low doses of human, but not mouse, leptin induce a diuretic/natriuretic response (5). However, these studies were conducted in rats, and it is possible that our negative findings with mouse leptin were due to the possibility that rat kidneys do not respond to mouse leptin but do respond to rat leptin. At the time we conducted our initial studies, rat leptin was unavailable. However, we recently obtained recombinant rat leptin from Amgen (Thousand Oaks, CA) and so were able to test and compare the effects of rat and human leptin in the rat kidney. Our results indicate that low doses of rat leptin do not have effects on renal excretory function and confirm that human leptin does.

METHODS

Forty-seven male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 240 ± 4 g (mean ± SE) were used in the experiments. The animals were housed at the University of Pittsburgh Animal Facility and fed Prolab RMH 3000 (PMI Feeds, St. Louis, MO) containing 0.26% sodium and 0.82% potassium and were fasted overnight before the experiment. Institutional guidelines for animal welfare were followed.

Rats were anesthetized with Inactin (100 mg/kg ip) and placed on a Deltaphase Isothermal Pad (Braintree Scientific, Braintree, MA). Body temperature was monitored with a digital rectal probe thermometer (Physitemp Instruments, Clifton, NJ) and maintained at 37°C by adjusting a heat lamp above the animal. After cannulation of the trachea to maintain airway patency, a polyethylene (PE-50) catheter was inserted into the left jugular vein, and an infusion of 0.9% saline was initiated at 50 µl/min and maintained for the duration of the experiment. A left carotid artery catheter (PE-50) was inserted and was connected to a digital blood pressure analyzer (Micro-Med, Louisville, KY) for continuous measurement of mean arterial blood pressure (MABP) and heart rate. The digital blood pressure analyzer was set to time-average MABP and heart rate at 20-min intervals. The left ureter was cannulated with a PE-10 catheter for continual collection of urine, a 32-gauge needle connected to a PE-10 catheter was carefully inserted into the renal artery, and an intrarenal artery infusion (50 µl/min) of vehicle for...
either rat leptin or human leptin was initiated (see below for details regarding composition of vehicle).

After an 80-min stabilization period, eight consecutive 20-min urine collection periods were performed. During the first 20-min urine collection, only vehicle for rat leptin or vehicle for human leptin was infused into the renal artery. In the rat leptin study, during the remaining seven 20-min urine collection periods, rats were randomized to receive an intrarenal artery infusion of either 1) vehicle for rat leptin, 2) 0.1 µg/min rat leptin, 3) 0.3 µg/min rat leptin, 4) 1 µg/min rat leptin, or 5) 3 µg/min rat leptin. In the human leptin study, during the remaining seven 20-min urine collection periods, rats were randomized to receive an intrarenal artery infusion of either vehicle for human leptin or 3 µg/min human leptin. Each rat received only one treatment.

Recombinant rat and human leptin were kindly supplied by Amgen. In addition, human leptin placebo (i.e., the vehicle in which the human leptin was shipped to us) was also provided by Amgen. These research materials were shipped packaged in dry ice by overnight delivery to our laboratory in Pittsburgh and were immediately stored at −70°C upon receipt. The rat leptin was shipped to us dissolved in Dulbecco’s phosphate-buffered saline without CaCl₂ or MgCl₂ (catalog no. 14190–144; Life Technologies, Grand Island, NY). Therefore, we obtained the same buffer, used this as our vehicle, and diluted in this buffer rat leptin to the final concentration for infusion. The human leptin was shipped to us dissolved in a proprietary buffer manufactured by Amgen. The composition of this buffer is unknown to us. Human leptin was diluted to final concentration for infusion in the same Dulbecco’s phosphate-buffered saline as used for the rat leptin experiments. To ensure that no vehicle effects confounded the human leptin experiments, the same volume of human leptin placebo was added to Dulbecco’s phosphate-buffered saline, and this solution was used as the vehicle for the human leptin study.

To determine the effects of treatments on urine volume and sodium and potassium excretion, the values for these parameters during the first 20-min urine collection period were divided into the values for these parameters at each of the subsequent periods. These ratios were then compared using a two-factor analysis of variance with repeated measures [1 factor was experimental period (7 levels), and the other factor was treatment (5 levels for the rat leptin study and 2 levels for the human leptin study)]. Statistical analysis was performed with the NCSS software package (version 6.0; NCSS, Kaysville, UT). All data are presented as means ± SE.

RESULTS

In the rat leptin study, during the first 20-min urine collection period, urine volumes (175 ± 10, 238 ± 51, 206 ± 27, 130 ± 12, and 183 ± 28 µl/20 min), sodium excretion rates (2.40 ± 0.18, 3.22 ± 0.68, 2.72 ± 0.32, 1.63 ± 0.24, and 2.46 ± 0.47 µeq/min), and potassium excretion rates (1.31 ± 0.31, 1.05 ± 0.19, 1.09 ± 0.09, 0.95 ± 0.07, and 0.88 ± 0.12 µeq/min) were similar in rats that were subsequently treated during the remainder of the experiment with either 0 (vehicle), 0.1, 0.3, 1, or 3 µg/min of rat leptin, respectively. As shown in Fig. 1, rat leptin did not significantly affect urine volume, sodium excretion, or potassium excretion at any dose tested.

In the human leptin study, during the first 20-min urine collection period, urine volumes (189 ± 26 and 191 ± 29 µl/20 min), sodium excretion rates (2.33 ± 0.24, 1.31 ± 0.12 µeq/min), and potassium excretion rates (0.95 ± 0.13 and 0.96 ± 0.12 µeq/min) were similar in rats that were subsequently treated during the remainder of the experiment with either 0 (vehicle) or 3 µg/min of human leptin, respectively. As shown in Fig. 2, human leptin caused a highly significant increase in urine volume (P = 0.0006; group × period interaction term in 2-factor analysis of variance) and sodium excretion rate (P = 0.0049; group × period interaction term in 2-factor analysis of variance) but did not significantly affect potassium excretion. The diuretic and natriuretic effects of human leptin developed gradually and were not statistically significant (Fisher’s least significant difference multiple comparison test) until ~80 min into the intrarenal artery infusion of human leptin.

It was important to exclude any perturbation of renal excretory function by human leptin placebo. Therefore, we compared the first urine collection period for all 28 rats in the rat leptin study with all 19 animals in the
Mean arterial blood pressures and heart rates in rat leptin study. In this regard, the only difference between the two groups was the absence or presence of human leptin placebo. There was no detectable effect of human leptin placebo. During the first 20-min collection period, urine volumes (186 ± 15 and 190 ± 19 µl/20 min), sodium excretion rates (2.48 ± 0.20 and 2.18 ± 0.31 µeq/min), and potassium excretion rates (1.05 ± 0.08 and 0.95 ± 0.09 µeq/min) were not significantly different (unpaired Student’s t-test) between rats not treated with human leptin placebo and rats treated with human leptin placebo, respectively. Also, as shown in Tables 1 and 2, arterial blood pressure and heart rate were similar in all groups of animals and were stable for the duration of the experiments.

**DISCUSSION**

In a previous study (5), we reported that in the rat, low-dose intrarenal infusions of human leptin approximately doubled sodium excretion and urine volume without affecting arterial blood pressure, renal blood flow, or glomerular filtration. Surprisingly, similar infusions of mouse leptin had no effect on either sodium excretion rate or urine volume.

Why is human leptin more effective as a diuretic/ natriuretic hormone compared with mouse leptin? There are several possibilities. First, even though mouse and rat leptins are 96% identical, differing only in six amino acids (7), it is possible that mouse leptin happens to have low diuretic/natriuretic activity in the rat, whereas rat leptin may exert a strong effect in this regard. At the time of our initial study, rat leptin was unavailable, and so we could not test this hypothesis. In the present study, we addressed this possibility by infusing directly into the renal artery low doses of recombinant rat leptin. Importantly, low-dose intrarenal infusions of rat leptin did not show any tendency whatsoever to increase urine volume or sodium excretion, indicating that, in general, rodent leptins, whether mouse or rat, have low diuretic/natriuretic potency.

However, neither our original findings nor the present findings should be taken to mean that rodent leptin is completely inactive as a diuretic/natriuretic agent. It is clear that large intravenous bolus doses (400–500 µg/kg) of mouse leptin do indeed increase

**Table 1. Mean arterial blood pressures and heart rates in rat leptin study**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>20–0 min</th>
<th>0–20 min</th>
<th>20–40 min</th>
<th>40–60 min</th>
<th>60–80 min</th>
<th>80–100 min</th>
<th>100–120 min</th>
<th>120–140 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean arterial blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>5</td>
<td>103 ± 5</td>
<td>102 ± 5</td>
<td>102 ± 6</td>
<td>102 ± 6</td>
<td>104 ± 6</td>
<td>103 ± 6</td>
<td>102 ± 5</td>
<td>102 ± 5</td>
</tr>
<tr>
<td>Rat leptin, 0.1 µg/min</td>
<td>6</td>
<td>114 ± 4</td>
<td>114 ± 4</td>
<td>113 ± 4</td>
<td>113 ± 5</td>
<td>111 ± 6</td>
<td>112 ± 6</td>
<td>114 ± 5</td>
<td>114 ± 5</td>
</tr>
<tr>
<td>Rat leptin, 0.3 µg/min</td>
<td>5</td>
<td>113 ± 5</td>
<td>113 ± 6</td>
<td>112 ± 6</td>
<td>111 ± 5</td>
<td>112 ± 5</td>
<td>113 ± 6</td>
<td>115 ± 5</td>
<td>117 ± 4</td>
</tr>
<tr>
<td>Rat leptin, 1 µg/min</td>
<td>6</td>
<td>106 ± 2</td>
<td>105 ± 2</td>
<td>104 ± 3</td>
<td>104 ± 3</td>
<td>102 ± 1</td>
<td>101 ± 2</td>
<td>102 ± 3</td>
<td>103 ± 3</td>
</tr>
<tr>
<td>Rat leptin, 3 µg/min</td>
<td>6</td>
<td>109 ± 2</td>
<td>108 ± 2</td>
<td>108 ± 2</td>
<td>109 ± 3</td>
<td>109 ± 3</td>
<td>111 ± 3</td>
<td>110 ± 2</td>
<td>112 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heart rate, beats/ min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>5</td>
<td>379 ± 21</td>
<td>376 ± 21</td>
<td>374 ± 19</td>
<td>373 ± 20</td>
<td>380 ± 22</td>
<td>383 ± 22</td>
<td>387 ± 22</td>
<td>389 ± 22</td>
</tr>
<tr>
<td>Rat leptin, 0.1 µg/min</td>
<td>6</td>
<td>404 ± 16</td>
<td>406 ± 15</td>
<td>404 ± 18</td>
<td>402 ± 17</td>
<td>403 ± 20</td>
<td>406 ± 21</td>
<td>409 ± 20</td>
<td>410 ± 19</td>
</tr>
<tr>
<td>Rat leptin, 0.3 µg/min</td>
<td>5</td>
<td>358 ± 8</td>
<td>362 ± 9</td>
<td>363 ± 11</td>
<td>363 ± 11</td>
<td>376 ± 14</td>
<td>377 ± 14</td>
<td>380 ± 13</td>
<td>384 ± 12</td>
</tr>
<tr>
<td>Rat leptin, 1 µg/min</td>
<td>6</td>
<td>398 ± 16</td>
<td>395 ± 17</td>
<td>394 ± 19</td>
<td>397 ± 19</td>
<td>399 ± 19</td>
<td>400 ± 18</td>
<td>403 ± 19</td>
<td>403 ± 21</td>
</tr>
<tr>
<td>Rat leptin, 3 µg/min</td>
<td>6</td>
<td>380 ± 17</td>
<td>374 ± 15</td>
<td>375 ± 14</td>
<td>380 ± 15</td>
<td>385 ± 13</td>
<td>390 ± 12</td>
<td>389 ± 11</td>
<td>392 ± 10</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of experiments.
believe that the response to human leptin was not due to a contaminant. First, Amgen produced, purified, and analyzed all three leptins, using similar technologies. If a biologically active contaminant were present in human leptin, then most likely it would also have been present in mouse and rat leptin. Since mouse and rat leptin were inactive, it is unlikely that a biologically active contaminant was present in human leptin. Second, two different batches of human leptin were employed (one in our previous study and one in the present study), and precisely the same results were obtained. It is extremely unlikely that a contaminant would be present in exactly the same proportions in two batches of human leptin made and purified by different people at different times. However, it is virtually impossible to logically exclude the possibility that human leptin contains trace amounts of a highly potent diuretic/natriuretic substance.

A fifth possibility, and the one we favor, is that human leptin is intrinsically more diuretic/natriuretic compared with rodent leptin. Inasmuch as human and mouse leptin differ by 28 amino acids (22 amino acids in the active mature protein) (7, 15), it is possible that the contrasting effects of rodent and human leptin on renal excretory function are due to differences in their primary amino acid structure. As eluded to in the introduction, an evolutionary argument supports this concept. In the natural world, cardiovascular disease would be a rather impotent threat to mice and rats compared with predation and starvation. Moreover, an obese rodent would be an easy target for predation and therefore would not live long enough for obesity-induced hypertension to inflict life-threatening sequelae. Thus there would be little, if any, selective pressures to evolve a form of leptin that enhances renal excretory function in rodents. In contrast, obesity-induced hypertension and cardiovascular disease could be life-threatening to primates who live for decades with excess body fat. In this regard, the ability of human leptin to enhance sodium and water excretion may be an adaptation to protect primates against obesity-induced hypertension. The hypothesis that the leptin-renal connection may differ in rodents vs. humans is underscored by the observations that rodent models with obesity secondary to leptin deficiency or leptin resistance actually had low, rather than high, arterial blood pressure (see Ref. 6 for review), whereas in three humans with obesity secondary to leptin deficiency due to a missense mutation, the average diastolic blood pressure was 97 ± 8 mmHg (mean ± SE, n = 3) (Ref. 13) with two of
the three individuals clearly in the hypertensive range. Thus it is conceivable that leptin deficiency/resistance may have qualitatively opposite cardiorenal consequences in rodents vs. humans.

What is the significance of the present findings? The significance of this study is twofold. First, our results confirm that human leptin is diuretic/natriuretic and suggest that human leptin may play a physiological role to protect humans from obesity-induced hypertension and cardiovascular disease. In this regard, our findings underscore the need to conduct carefully controlled studies in a clinical research center examining the effects of human leptin on sodium excretion in humans. Second, our results suggest that rodent models may not be appropriate for the study of obesity-induced hypertension in humans.

This work was supported in part by Amgen (Thousand Oaks, CA).

Address for reprint requests and other correspondence: E. K. Jackson, Center for Clinical Pharmacology, Univ. of Pittsburgh Medical Center, 623 Scaife Hall, 200 Lothrop St., Pittsburgh, PA 15213-2582 (E-mail: edj1@pitt.edu).

Received 12 February 1999; accepted in final form 30 June 1999.

REFERENCES