Chronic cardiovascular and renal actions of leptin during hyperinsulinemia

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Submitted 12 August 2002; accepted in final form 30 December 2002

Kuo, Jay J., O. Barrett Jones, and John E. Hall. Chronic cardiovascular and renal actions of leptin during hyperinsulinemia. Am J Physiol Regul Integr Comp Physiol 284: R1037–R1042, 2003.—Hyperinsulinemia and hyperleptinemia occur concurrently in obese subjects, and both have been suggested to mediate increased blood pressure associated with excess weight gain. The goal of this study was to determine whether chronic hyperleptinemia exacerbates the effects of insulin on arterial pressure and renal function. Group I and II rats were infused with insulin (1.5 mU·kg⁻¹·min⁻¹) for 21 days while maintaining euglycemia. After 7 days of insulin infusion, group II rats received leptin (1.0 μg·kg⁻¹·min⁻¹) for 7 days, concomitant with insulin. Insulin plus glucose infusion reduced food intake to 55 ± 7% of control, while leptin + insulin lowered food intake further to 22 ± 4% of the initial control. Insulin initially raised mean arterial pressure (MAP) by 12 ± 1 mmHg; then MAP declined to 5–8 mmHg above control during continued hyperinsulinemia. Leptin + insulin infusion increased MAP by 7 ± 2 mmHg above the level observed in rats infused with insulin alone. Insulin raised heart rate (HR) by 17 ± 5 beats/min, whereas leptin + insulin increased HR by 34 ± 5 beats/min. Thus leptin appears to increase the effects of insulin to suppress appetite and to raise arterial pressure and HR.

Blood pressure; heart rate; food intake; obesity; hypertension; kidney

OBESITY IS ASSOCIATED with metabolic disturbances that cause marked increases in plasma insulin and leptin concentrations. Increased insulin secretion occurs as a compensatory response to decreased insulin sensitivity (i.e., insulin resistance) of peripheral tissues, and leptin is secreted from adipocytes in proportion to the amount of body fat (2). Although these two peptides are widely recognized as important regulators of energy balance through their actions on peripheral tissues and the central nervous system (CNS), they have been also suggested to have important sympathetic, renal, and cardiovascular actions and to mediate obesity-associated hypertension (12, 22). In support of this concept, previous studies have shown that chronic hyperleptinemia, produced by leptin infusions in rats or by ectopic oversecretion of leptin in transgenic mice, caused modest hypertension and tachycardia despite marked reductions in food intake that would usually tend to lower blood pressure and heart rate (HR) (7, 21, 28). Chronic hyperinsulinemia, comparable to that found in obesity, also caused tachycardia and small increases in arterial pressure in rats (5, 6), although insulin-induced hypertension was not observed in dogs (11).

The hypertensive effects of both leptin and insulin have been suggested to be mediated via sympathetic activation (8, 15, 25), and both peptides act on receptors in the same regions of the hypothalamus (2). Although leptin and insulin both reduce appetite, recent studies suggest that these peptides may combine subadditively in acute regulation of food intake. That is, the combined effects of leptin and insulin to reduce food intake for the first few hours of coadministration was less than predicted from their individual effects (1). On the other hand, after 24 h the combined effects appeared to be additive (1). Whether the chronic effects of insulin and leptin, acting over a period of several days, to decrease food intake and to raise blood pressure act through redundant mechanisms and are subadditive, additive, or synergistic is still unknown. Because obesity is associated with concurrent increases in plasma leptin and insulin, it is possible that the combined effects of these peptides could play a significant role in mediating hypertension associated with excess weight gain. However, the importance of the combined effects hyperleptinemia and hyperinsulinemia in raising blood pressure have, to our knowledge, not been previously reported. Therefore, the primary goal of this study was to determine whether leptin exacerbates the chronic cardiovascular and renal actions of insulin in nonobese rats. We also examined the interactions of leptin and insulin in chronic regulation of food intake.

MATERIAL AND METHODS

Animal surgery. All experiments and procedures for these studies conformed 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), weighing 300–350 g, were anesthetized with 50 mg/kg of pentobarbital sodium (Nembutal), and atropine sulfate (0.37 mg/kg) was administered to

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prevent excessive airway secretions. Chronic arterial and venous catheters were implanted by methods that have been previously described (7, 21, 28).

After several days of recovery from surgery, the rats were housed in individual metabolic cages in a quiet, air-conditioned room with a 12:12-h light-dark cycle and an ambient temperature of 22°C. 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 femoral venous catheter was connected, via the swivel, to a syringe pump for continuous infusions, and the arterial catheter was filled with heparin (1,000 USP U/ml) and connected to a pressure transducer (Maxxim). Arterial pressure signals were sent to an analog-to-digital converter and analyzed by computer with customized software. The analog signal was sampled at 500 samples/s for 4 s every minute, 24 h/day, throughout the experimental protocol.

All rats received food and water ad libitum throughout the study. Total sodium intake was maintained constant at ~3.1 meq/day by a continuous infusion of 40 ml/day of 0.45% saline combined with a sodium-deficient rat chow (0.006 mmol sodium/g food, Teklad). All solutions were infused via a sterile Millipore filter (22 μm), and the saline infusion was started immediately after placement of rats in their metabolic cages. An acclimation period of 4–7 days was allowed before control measurements were begun.

Experimental protocols. Rats were divided into two groups. Group 1 (insulin, n = 9) rats were infused with saline vehicle during a 5-day control period, followed by a 21-day intravenous infusion of insulin (porcine insulin, Eli Lily) at a rate of 1.5 mU·kg⁻¹·min⁻¹ while maintaining euglycemia with infusion of a 50% sterile glucose solution (19.8 mg glucose·kg⁻¹·min⁻¹ iv). Body weight of the rats averaged 350 ± 3 g at the beginning of the experiments, and this weight was used to calculate the rates of glucose and insulin infusions. A 5-day vehicle infusion recovery period followed the termination of the 21-day insulin and glucose administration.

Group 2 rats (insulin + leptin, n = 9) were infused with saline vehicle during the 5-day control period followed by 7 days of insulin infusion (1.5 mU·kg⁻¹·min⁻¹) and glucose (19.8 mg glucose·kg⁻¹·min⁻¹). Murine leptin (Amgen) was then infused intravenously at a rate of 1.0 μg·kg⁻¹·min⁻¹ for 7 days in combination with the insulin and glucose infusion. An average body weight of 350 ± 4 g was used to determine the amount of insulin, glucose, and leptin administrations. After termination of leptin infusion, insulin and glucose were infused for an additional 7 days followed by a 5-day vehicle infusion recovery period.

Statistical methods. Data are expressed as means ± SE. Data were analyzed using two-factor ANOVA with repeated measures. The Tukey-Kramer test was used for all comparisons between the insulin and insulin + leptin groups. A one-way ANOVA and Dunnett’s test were used for multiple comparisons within each group. Statistical significance was accepted at P < 0.05.
Hemodynamic and renal actions of leptin and insulin. Insulin infusion raised mean arterial pressure (MAP) by an average of $12 \pm 1 \text{ mmHg}$ after 4 days of infusion; thereafter, MAP slowly declined before reaching a plateau of approximately $5-8 \text{ mmHg}$ above control levels during the second and third weeks of insulin infusion (Fig. 2); during the second and third weeks of insulin infusion, MAP was increased by an average of $7 \pm 2$ and $7 \pm 2 \text{ mmHg}$, respectively, compared with the initial control level. HR increased by 15–20 beats/min during the first 2 wk of insulin infusion and remained elevated by 15 ± 2 beats/min after 3 wk of insulin infusion (Fig. 2). The final 3 days of leptin infusion during insulin infusion further increased MAP to $11 \pm 2 \text{ mmHg}$ above the initial control level and to $7 \pm 2 \text{ mmHg}$ above the level observed during insulin infusion alone (Fig. 2). Leptin infusion also increased HR by 17 ± 5 beats/min above the level measured in rats infused with insulin alone. After termination of leptin infusion while continuing insulin infusion, MAP and HR remained slightly elevated and did not return to the control levels observed before leptin infusion until after 5–6 days (Fig. 2).

GFR remained stable during 21 days of insulin infusion (Table 1). Leptin administration during insulin infusion also had no significant effect on the GFR. Urine volume and sodium excretion were not significantly altered by insulin or leptin + insulin infusions. However, urinary potassium excretion was markedly reduced from a control of $3.1 \pm 0.2 \text{ to } 1.7 \pm 0.1 \text{ mmol/day in rats infused with insulin alone. In leptin + insulin-infused rats, urinary potassium excretion decreased further to } 1.1 \pm 0.1 \text{ mmol/day, paralleling the greater decrease in food intake in this group.}

**DISCUSSION**

Leptin and insulin are both widely recognized as important endocrine regulators of food intake and metabolism. Multiple studies suggest that these peptides may also have significant sympathetic, cardiovascular, and renal actions that could contribute to obesity-associated hypertension (12, 22). Most of the evidence supporting this concept is derived from observational studies showing a correlation between insulin, leptin, and blood pressure, or from acute studies showing that infusion of these peptides causes sympathetic activation and renal effects that, if sustained, could lead to hypertension.

A few studies have shown that chronic leptin or insulin infusions can raise blood pressure in rodents (5, 28). In contrast, chronic insulin infusions did not ele-
vate arterial pressure in dogs and in some cases actually reduced blood pressure (11). The increases in blood pressure observed during either leptin or insulin infusions in rats were modest, suggesting that neither hyperinsulinemia nor hyperleptinemia alone can fully explain obesity-induced hypertension. However, leptin and insulin activate similar pathways in the hypothalamus, and it is possible that these peptides could interact to raise blood pressure to a greater extent than either peptide alone.

The present study was therefore designed to determine whether hyperleptinemia exacerbates the chronic cardiovascular, renal, and metabolic actions of insulin. The primary new finding of this study is that chronic insulin infusion modestly raised arterial pressure for 21 days, and rats administered leptin during hyperinsulinemia had further increases in arterial pressure compared with those receiving insulin alone. Moreover, these increases in arterial pressure occurred despite decreases in food intake that may have attenuated some of the hypertensive effects of these peptides in our experiments.

**Arterial pressure and HR responses to leptin during hyperinsulinemia.** We have previously shown in nonobese rats that leptin infusion for 5–7 days, at the same rate used in the present study (1.0 μg·kg⁻¹·min⁻¹), raises plasma leptin concentration to ~94 ng/ml, increases HR by ~20 beats/min, and causes a slow rise in arterial pressure of 7–10 mmHg (28). These effects appear to be mediated by activation of the sympathetic nervous system because they are completely abolished by adrenergic blockade (7). Although the mechanisms by which leptin causes sympathetic activation are poorly understood, acute studies suggest that the proopiomelanocortin (POMC) pathway may play a key role. Leptin stimulates POMC expression in the arcuate nucleus, resulting in increased production of α-melanocyte stimulating hormone, an endogenous ligand for the melanocortin-3 and 4 receptor (MC3/4-R) (2, 29). Moreover, blockade of the MC3/4-R prevents leptin-induced sympathoexcitation in the kidneys but not in brown adipose tissue (14). Thus the POMC pathway may mediate, at least in part, leptin’s renal sympathoexcitatory effects. Whether the POMC pathway mediates the chronic effects of leptin on blood pressure, however, remains to be determined.

Hyperinsulinemia also causes modest increases in arterial pressure and HR in nonobese rats (5, 6), although insulin infusions in dogs caused vasoconstriction and decreased arterial pressure (11). We have previously reported in rats that insulin infusion for 5–7 days raised arterial pressure by about 7–10 mmHg (5, 6), comparable to the results obtained in the present study. The chronic blood pressure effects of insulin in rats are attenuated or abolished by inhibition of thromboxane (20) or angiotensin-converting enzyme inhibition (4), but not by adrenergic blockade (18). These observations suggest that insulin’s hypertensive actions in rats may be mediated primarily by interactions of ANG II and thromboxane rather than sympathetic activation.

In the present study, the hypertensive effects of insulin were modest and diminished slightly during the second and third week of insulin infusion. The mechanisms responsible for the diminished response are unclear. It is possible that the rats became resistant to the blood pressure actions of insulin during the prolonged hyperinsulinemia. Alternatively, the decreased food intake associated with prolonged hyperinsulinemia and glucose infusion may have contributed to the diminished blood pressure response because fasting is known to decrease blood pressure and to stimulate other hormones such as ghrelin that may decrease sympathetic activity (26).

Leptin administration during hyperinsulinemia caused further decreases in food intake, but despite the very low intake of food, arterial pressure remained elevated. In the absence of decreased food intake, as occurs in obese subjects in which food intake is actually increased, the interactions of leptin and insulin to raise blood pressure may be even more pronounced than observed in the present study.

We previously reported that blood pressure and HR returned to control values, or even below control, 2–3 days after cessation of chronic leptin infusion (7, 21, 28). In the present study, blood pressure remained elevated for 5–6 days after stopping leptin infusion during hyperinsulinemia. This suggests that an interaction between insulin and leptin resulted in a sustained increase in arterial pressure after leptin administration was stopped. It is possible that leptin may have prevented the development of resistance to insulin’s blood pressure effects. Also, hyperinsulinemia during euglycemia increases free leptin levels (23), and insulin stimulates leptin transport through the blood-brain barrier in rodents (17). Thus it is possible that CNS leptin levels remained elevated in hyperinsulinemic rats for several days after termination of leptin infusion, resulting in a sustained increase in blood pressure and HR. However, these possibilities must be considered speculative in the absence of measurements of CNS or plasma levels of leptin.

Previous studies have shown that leptin, in supra-physiological concentrations, markedly increases renal sympathetic activity (8, 15). However, potential interactions of leptin and insulin in causing chronic increases in blood pressure have, to our knowledge, not been previously reported. Dunbar and Lu (9) found that intracerebroventricular administration of insulin prevented acute leptin-induced lumbar sympathetic activation, suggesting that insulin and leptin may interact subadditively to stimulate sympathetic activity or that insulin may actually block the sympathoexcitatory effects of leptin. Whether this applies to leptin’s chronic effects on renal sympathetic activity or blood pressure is unclear. Nevertheless, the results of the present study are consistent with the hypothesis that hyperleptinemia and hyperinsulinemia, acting together, could contribute to elevations in blood pressure, at least in rodents. However, the importance of these peptides in raising blood pressure in other species, including humans, is still unclear. In addition, the
role of leptin and insulin in raising blood pressure in obesity is complicated by the possibility that obesity may induce “resistance” to the hypothalamic effects of leptin and insulin that cause sympathetic activation. Previous studies have clearly documented the development of obesity-induced resistance to the effects of leptin and insulin on various metabolic actions and food intake (10, 12, 24). However, it is not yet clear whether obesity attenuates the chronic effects of these peptides to stimulate sympathetic activity and raise blood pressure.

Renal responses to leptin during hyperinsulinemia. Acute injections or infusions of large doses of leptin have been reported to cause natriuresis and diuresis (16). In the present study and in previous studies (7, 21, 28), we found no significant changes in sodium excretion or urine volume during chronic infusion of leptin at rates that raised plasma concentrations to levels comparable to those found in obesity. We also found no major changes in sodium balance during chronic hyperinsulinemia, nor did insulin infusion significantly alter the effects of leptin on sodium balance. Insulin infusion reduced urinary potassium excretion, but this was likely due to decreased potassium intake secondary to reduced food intake. When hyperleptinemia was combined with hyperinsulinemia, potassium excretion decreased even further, paralleling the additive effects of these peptides to reduce food intake.

The observation that neither insulin nor leptin infusion, alone or in combination, caused marked sodium retention does not necessarily imply that these peptides have no significant chronic effect on renal function. The fact that hyperinsulinemia and hyperleptinemia did not raise sodium excretion despite increased arterial pressure suggests that leptin and insulin shifted renal pressure natriuresis to higher blood pressures. In the absence of altered pressure natriuresis, a rise in blood pressure would raise sodium and water excretion (13). The shift of pressure natriuresis did not appear to be caused by decreased GFR, because GFR was not significantly altered during hyperinsulinemia or hyperleptinemia. This suggests that these peptides may shift pressure natriuresis mainly by increasing tubular reabsorption, although the precise mechanisms by which renal function is altered during combined hyperinsulinemia and hyperleptinemia remain unclear.

Hormonal and metabolic effects of leptin during hyperinsulinemia. Both leptin and insulin have been suggested to suppress appetite and to function as adiposity signals for long-term weight control (2). Recent evidence suggests that these peptides may share intracellular and neuronal signaling pathways. Both insulin and leptin stimulate POMC and inhibit neuropeptide Y expression in the hypothalamus (1, 2). Also, phosphatidylinositol-3-OH kinase, an enzyme that mediates intracellular insulin signaling, appears to play a major role in mediating leptin-induced appetite suppression (27). These observations support the concept that leptin and insulin share common pathways in mediating appetite suppression. An important observation in the present study is that hyperinsulinemia and hyperleptinemia both caused marked reductions in food intake, although some of the effects of insulin may have been mediated by the glucose that was infused to maintain euglycemia. The effect of both peptides on food intake was pronounced and together caused an even greater decrease in appetite than observed when either was infused alone. However, the design of our experiments does not permit us to determine whether these effects are strictly additive.

In the present study, neither hyperinsulinemia alone nor combined with hyperleptinemia caused significant changes in PRA. However, we previously demonstrated that angiotensin-converting enzyme inhibition completely abolished the rise in blood pressure associated with insulin infusion, suggesting that ANG II contributes to insulin-induced hypertension in rats (4). One possible explanation for these observations is that chronic hyperinsulinemia may enhance ANG II sensitivity despite normal PRA (19). Another possibility is that ANG II may modulate the blood pressure responsiveness to insulin by interacting with other mechanisms such as thromboxane (20).

Perspectives

Although obesity is associated with multiple metabolic changes, two key hormonal abnormalities include hyperinsulinemia and hyperleptinemia, both of which have been suggested to mediate obesity-associated hypertension. The results of the present study indicate that hyperinsulinemia and hyperleptinemia together raise blood pressure in rats to a greater extent than observed with either peptide alone. However, the importance of interactions between leptin and insulin in regulating blood pressure in other species, especially in humans, has not been examined and remains an important area for further investigation. It seems likely that obesity-induced hypertension results from multiple mechanisms that increase sympathetic activity, including hyperleptinemia, and other factors such as activation of the renin-angiotensin system and structural and functional changes in the kidney that impair its ability to excrete sodium and water.

We thank H. Zhang for radioimmunossay of plasma renin activity and insulin concentration in these studies. Murine leptin for these studies was kindly provided by Amgen (One Thousand Oaks, CA). This research was supported by National Heart, Lung, and Blood Institute Grant P01-HL-51971.

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