Central Leptin Acutely Reverses Diet-Induced Hepatic Insulin Resistance

Alessandro Pocai, Kimyata Morgan, Christoph Buettner, Roger Gutierrez-Juarez, Silvana Obici, and Luciano Rossetti

Voluntary overfeeding rapidly induces resistance to the effects of systemic insulin and leptin on liver glucose metabolism. To examine whether central administration of recombinant leptin can restore leptin and insulin action on liver glucose fluxes, we infused leptin in the third cerebral ventricle of conscious overfed rats during pancreatic-insulin clamp studies. The effect of leptin on the phosphorylation of the signal transducer and activator of transcription-3 in the arcuate nuclei of the hypothalamus was similar in animals fed a regular diet or a high-fat diet for 3 days. The infusion of leptin in the third cerebral ventricle markedly inhibited glucose production in rats fed a high-fat diet mainly by decreasing glycogenolysis. The inhibition of glycogenolysis was sufficient to normalize glucose production and was accompanied by leptin-induced decreases in the hepatic expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. Thus central administration of leptin rescues the hepatic insulin resistance induced by short-term hyperphagia. Diabetes 54:3182–3189, 2005

Insulin and leptin resistance ensued within 3 days of voluntary overfeeding in a rodent strain (Sprague-Dawley rats) susceptible to age- and diet-dependent weight gain (21). In particular, short-term overfeeding induced a severe defect in the ability of a systemic infusion of leptin (50 μg) to modulate hepatic glucose fluxes (21). Because we have shown that 1.5 μg leptin i.c.v. entirely reproduced the effect of 50 μg leptin infused systemically on hepatic glucose fluxes in standard diet rats (19), our aim is to elucidate whether the central administration of leptin rescues the action of insulin on liver carbohydrate metabolism.

RESEARCH DESIGN AND METHODS

Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were studied. Rats were housed in individual cages and subjected to a standard light-dark cycle (lights on at 0600). Fourteen days before the in vivo study, rats were equipped with indwelling catheters placed in the third cerebral ventricle by stereotaxic surgery (19,22). After full recovery (~10 days), catheters were placed in the right internal jugular vein and left carotid artery (19,22). Rats were then randomly divided into two groups and fed either a standard diet or a highly palatable high-fat diet for 3 days. The standard chow (catalog no. 5001; Purina Mills) provided 59% calories from carbohydrates, 20% from protein, and 21% from fat. The high-fat diet chow (catalog no. 5005)
Table 1
General characteristics of the experimental groups

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Standard-diet rats</th>
<th>Leptin</th>
<th>Overfed rats</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Vehicle</td>
<td>6</td>
<td>Vehicle</td>
</tr>
<tr>
<td>Basal</td>
<td>7</td>
<td>77 ± 3</td>
<td>81 ± 4</td>
<td>156 ± 4*</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td>295 ± 7</td>
<td>299 ± 4</td>
<td>315 ± 3</td>
<td>307 ± 5</td>
</tr>
<tr>
<td>ΔBody wt (g)</td>
<td>3 ± 4</td>
<td>8 ± 4</td>
<td>24 ± 6</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>7.8 ± 0.7</td>
<td>7.4 ± 0.2</td>
<td>8.0 ± 0.3</td>
<td>7.7 ± 0.2</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>1.1 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>2.2 ± 0.4</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.7 ± 0.1*</td>
<td>1.9 ± 0.3*</td>
</tr>
<tr>
<td>Free fatty acids (mmol/l)</td>
<td>0.8 ± 0.0</td>
<td>1.1 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.1</td>
</tr>
</tbody>
</table>

Clamp
| Glucose (mmol/l) | 7.6 ± 0.3 | 7.8 ± 0.2 | 7.2 ± 0.4 | 8.2 ± 0.5 |
| Insulin (ng/ml)  | 3.2 ± 0.2 | 2.5 ± 0.3 | 2.96 ± 0.1 | 3.0 ± 0.1 |
| Leptin (ng/ml)   | 1.7 ± 0.1 | 2.2 ± 0.4 | 2.0 ± 0.3 | 2.2 ± 0.4 |
| Glucagon (pg/ml) | 35.1 ± 1.4 | 34.2 ± 2.9 | 28.4 ± 1.7 | 32.3 ± 2.4 |
| Free fatty acids (mmol/l) | 0.8 ± 0.1  | 0.7 ± 0.1 | 1.0 ± 0.2 | 0.8 ± 0.1 |

Biochemical parameters represent the average ± SE of at least five basal measurements in each rat. Food intake represents the average ± SE of the last 3 days preceding the study. *P < 0.01 vs. vehicle or regular diet.

RESULTS

Central leptin administration restores insulin sensitivity in overfed rats. To test the impact of short-term voluntary overfeeding on the central action of leptin on glucose homeostasis and insulin sensitivity, male Sprague-Dawley rats were randomized to receive highly palatable high-fat diet chow or the standard chow (Table 1). After 3 days on the assigned diet, overfed rats had moderately increased plasma insulin and leptin concentrations (Table 1) but failed to adapt to the enhanced caloric content of the diet and markedly increased their daily caloric intake (148 vs. 79 kcal/day). The acute metabolic response to central leptin was examined by combining the infusion of leptin (1.5 μg/6 h) within the third cerebral ventricle (intracerebroventricular) with a pancreatic-insulin (3 mU·kg⁻¹·min⁻¹) clamp procedure designed to generate a physiological increase (approximately threefold; Table 1) in the plasma insulin concentration (Fig. 1B).

During pancreatic-insulin clamp studies, plasma glucose and insulin concentrations were similar in all groups (Table 1). In standard-diet rats, leptin did not affect the...
rate of glucose infusion (~15 mg · kg\(^{-1} \cdot \text{min}^{-1}\)) required to prevent changes in the plasma glucose levels (Fig. 1C). As expected, overfed rats developed insulin resistance so that markedly less glucose (9.5 ± 1.3 mg · kg\(^{-1} \cdot \text{min}^{-1}\); \(P < 0.05\)) had to be infused during the clamp procedure (Fig. 1C). Surprisingly, the acute intracerebroventricular infusion of leptin markedly increased the rate of glucose infusion in overfed animals to levels similar to those observed in standard-diet rats (14.4 ± 2.1 mg · kg\(^{-1} \cdot \text{min}^{-1}\)) (Fig. 1C).

Central leptin administration normalizes hepatic insulin action in overfed rats. To examine the mechanism(s) by which leptin restored whole-body insulin sensitivity in the overfed group, we assessed glucose kinetics by tracer dilution methodology. The basal glucose production (13.1 ± 0.4 vs. 14.2 ± 0.5 mg · kg\(^{-1} \cdot \text{min}^{-1}\) vehicle vs. leptin in standard diet rats and 13.3 ± 1.1 vs. 11.5 ± 0.6 mg · kg\(^{-1} \cdot \text{min}^{-1}\) vehicle vs. leptin in overfed rats) and the rate of glucose uptake during the clamp (Fig. 1D) were not significantly affected by intracerebroventricu-
ular leptin nor by the short-term exposure to a high-fat diet. As previously reported (19), central leptin did not elicit changes in glucose production during the clamp in standard-diet rats (Fig. 1E). Physiological increases in the plasma insulin concentration failed to suppress glucose production in overfed rats (11.5 ± 1.2 vs. 5.2 ± 0.4 mg · kg⁻¹ · min⁻¹, standard diet vs. overfed, *P < 0.05) (Fig. 1E), confirming the rapid onset of severe hepatic insulin resistance in this model (21). Strikingly, the acute intracerebroventricular infusion of leptin restored hepatic insulin action to normal levels in the overfed group (11.5 ± 1.2 vs. 3.8 ± 0.7 mg · kg⁻¹ · min⁻¹, leptin vs. vehicle, *P < 0.05) (Fig. 1E). The action of insulin on glucose production can also be expressed as percent suppression from basal levels (Fig. 1F). Physiological hyperinsulinemia inhibited glucose production by ~65% in standard-diet rats in the presence of either intracerebroventricular vehicle or leptin. After short-term overfeeding, the inhibitory effect of insulin on glucose production was reduced to 15 ± 10% in overfed rats receiving intracerebroventricular vehicle, but it was rapidly increased to 67 ± 4.9% by intracerebroventricular leptin administration (Fig. 1F). Thus, central leptin completely reversed the hepatic insulin resistance induced by short-term overfeeding.

Mechanisms by which central leptin inhibits glucose production in overfed animals. The net output of glucose by the liver (glucose production) is the result of the dephosphorylation of glucose-6-phosphate derived via gluconeogenesis and glycogenolysis. However, part of the glucose entering the liver is phosphorylated by glucokinase and then dephosphorylated by G6Pase. This futile cycle between glucokinase and G6Pase is named glucose cycling, and it accounts for the difference between the total flux through G6Pase and glucose production. To further delineate the mechanism by which central leptin rapidly modulates liver glucose homeostasis, we estimated the in vivo flux through G6Pase (G6Pase flux) and the relative contribution of glucose cycling to G6Pase flux. Table 2 displays the [³H]UDP-glucose and the [³H]glucose specific activities used to calculate the contribution of plasma glucose (percent direct) to the hepatic glucose-6-phosphate pool. As shown in Fig. 2, leptin administration did not alter the rates G6Pase flux (Fig. 2A) or glucose cycling (Fig. 2B) in the standard-diet group. In the overfed rats, the increased glucose production during the clamp was mirrored by increases G6Pase flux (Fig. 2A) and glucose cycling (Fig. 2B), suggesting that the in vivo activity of G6Pase is markedly elevated in the liver of overfed rats. Central infusion of leptin dramatically decreased G6Pase flux (from 14.3 ± 2.1 to 5.3 ± 1.0 mg · kg⁻¹ · min⁻¹, *P < 0.05) and glucose cycling (from 3.8 ± 0.8 to 1.3 ± 0.3 mg · kg⁻¹ · min⁻¹; *P < 0.05) in overfed rats. Importantly, intracerebroventricular leptin induced hepatic G6Pase expression in standard-diet rats, but it decreased liver G6Pase mRNA in overfed rats (Fig. 2C). We next examined the effect of intracerebroventricular leptin

Data are means ± SE. Specific activities of UDP-glucose (UDP-Glc) and PEP were used to calculate the contribution of PEP gluconeogenesis (indirect pathway) and plasma glucose (direct pathway) to the hepatic UDP-glucose pool after [U-¹⁴C]lactate and [³H]-glucose infusions. V, vehicle; L, leptin; SC, standard chow; OF, overfed rats. *P < 0.05 vs. vehicle or controls.

**TABLE 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>[³H]Glc (dpm/nmol)</th>
<th>[³H]UDP-Glc (dpm/nmol) % Direct</th>
<th>[¹⁴C]PEP (dpm/nmol)</th>
<th>[¹⁴C]UDP-Glc (dpm/nmol) % Indirect</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC-V</td>
<td>27.4 ± 0.8</td>
<td>7.1 ± 0.5</td>
<td>21.9 ± 1.9</td>
<td>12.9 ± 2.5</td>
</tr>
<tr>
<td>SC-L</td>
<td>38.7 ± 2.9</td>
<td>3.9 ± 0.7</td>
<td>22.5 ± 1.9</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>OF-V</td>
<td>29.7 ± 3.6</td>
<td>6.5 ± 1.2</td>
<td>24.6 ± 2.5</td>
<td>5.2 ± 0.7</td>
</tr>
<tr>
<td>OF-L</td>
<td>32.4 ± 5.4</td>
<td>5.8 ± 1.0</td>
<td>26.3 ± 6.2</td>
<td>7.7 ± 0.9</td>
</tr>
</tbody>
</table>

FIG. 2. Central leptin normalizes G6Pase flux, glucose cycling, and liver G6Pase mRNA in overfed rats. In the standard-chow (SC) group, intracerebroventricular leptin () did not alter G6Pase flux (A) and glucose cycling (B); however, G6Pase mRNA (C) was significantly increased by leptin. Short-term hyperphagia led to a significant increase in G6Pase flux (A), glucose cycling (B), and G6Pase mRNA (C), and intracerebroventricular leptin entirely restored their levels to those observed in standard-diet rats. OF, overfed rats. *P < 0.05 vs. standard diet, P < 0.05 vs. vehicle control.
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**FIG. 3.** Effect of intracerebroventricular leptin on gluconeogenesis, glycogenolysis, and liver PEPCK mRNA in overfed rats. Central leptin administration markedly decreased glycogenolysis (A) in both standard-chow (SC) and overfed (OF) rats. Conversely, central leptin stimulated gluconeogenesis (B) in standard-diet but not in overfed rats. C: Furthermore, intracerebroventricular leptin markedly increased the hepatic expression of PEPCK in standard-diet rats. Short-term hyperphagia (OF) per se increased the hepatic expression of PEPCK (C), but the central administration of leptin significantly inhibited PEPCK mRNA expression (C). *P < 0.05 vs. standard-diet, \( P < 0.05 \) vs. vehicle control.

UDP-glucose pool in standard-diet animals. This contribution was markedly increased to 65% after intracerebroventricular leptin administration in standard-diet rats. Conversely, central leptin infusion failed to stimulate the indirect pathway in overfed animals (21% in high-fat diet–vehicle to 24% in high-fat diet–leptin). Based on these data, we calculated the relative contribution of gluconeogenesis and glycogenolysis to glucose production. Central leptin stimulates gluconeogenesis (Fig. 3B) but did not alter glucose production due to a compensatory decrease in glycogenolysis (Fig. 3A) (4,19) in standard-diet rats. Conversely, central leptin markedly suppressed glycogenolysis (Fig. 3A) and failed to stimulate the rate of gluconeogenesis (Fig. 3B) in overfed rats. To provide a reference for the current studies, we also assessed the effect of physiological increases in circulating insulin and leptin in standard-diet and overfed rats after systemic infusion (intravenous) of leptin (30 μg·kg\(^{-1}\)·h\(^{-1}\) for 6 h, total dose of 50 μg). As previously reported, intravenous leptin failed to enhance the inhibition of glucose production by insulin in standard-diet rats (4,19), as well as in overfed rats (21). In addition, intravenous leptin stimulated gluconeogenesis but did not alter glucose production because of a compensatory decrease in glycogenolysis in standard-diet rats (4). However, the same dose of intravenous leptin did not affect the rate of gluconeogenesis (5.8 ± 3.5 vs. 4.9 ± 0.3 mg·kg\(^{-1}\)·min\(^{-1}\)) and glycogenolysis (5.6 ± 3.7 vs. 4.9 ± 2.5) in overfed rats.

We next assessed the liver abundance of PEPCK mRNA after central infusion of vehicle or leptin. In standard-diet rats, central leptin increased PEPCK expression (4) (Fig. 3C). By contrast, central leptin treatment resulted in a marked suppression of PEPCK expression (Fig. 3C) in overfed rats. Thus, central leptin in overfed rats decreased glucose production mainly via inhibition of glycogenolysis and via decreased expression of PEPCK and G6Pase coupled with a lack of stimulation of gluconeogenesis.

**Intracerebroventricular leptin normally activates hypothalamic STAT3 in overfed rats.** Because the early step of leptin signal transduction involves phosphorylation and activation of STAT3, we also examined the effect of intracerebroventricular leptin on hypothalamic STAT3 phosphorylation in standard-diet and overfed animals (Fig. 4). Thirty minutes after the intracerebroventricular injection of leptin (2.5 μg) or vehicle, rats were killed, and mediobasal wedges of the hypothalamus were dissected and analyzed by Western blot. Total STAT3 protein and β-actin (not shown) levels were similar among all groups. Intracerebroventricular leptin induced a marked increase in STAT3 tyrosine phosphorylation in position 705 (Tyr705) (25) to similar extent in standard-diet and overfed rats. The baseline levels of Stat3-705 were not elevated in overfed rats. Furthermore, the transcriptional activity of Stat3 can be further enhanced by a leptin-independent serine phosphorylation at position 727. Interestingly,
serine phosphorylation of Stat3 (Ser727) was neither affected by leptin, nor by short-term overfeeding at this site.

DISCUSSION
Leptin resistance has been demonstrated in numerous animal models of voluntary overfeeding (14–16,21,26). It has been suggested that a "nutrient counter-regulatory" system is likely to fail during a sustained exposure to nutrient excess in susceptible animals and individuals (27–29). This dynamic regulation of leptin sensitivity in response to changes in nutrient availability could be viewed as a key component of the "thrifty" genotype/phenotype that appears to contribute to the recent rise in the incidence of type 2 diabetes and obesity in developed...
and developing countries (30). Most studies have focused primarily on the impairment in the acute anorectic action of leptin that occurs in diet-induced (14–16) and genetic (31,32) rodent models. However, there is growing evidence that leptin also plays an important role in the regulation of glucose homeostasis and insulin action (4,18–20). It is therefore of great importance to delineate the impact of short-term nutritional manipulations on the pleiotropic actions of leptin on glucose metabolism and insulin action. In a model of short-term (3 days) voluntary hyperphagia, we have previously shown a rapid collapse of "nutrient counter-regulation" as manifested by the rapid onset of resistance to the behavioral and/or metabolic actions of leptin, insulin, and fatty acids (21,33).

Human and animal studies suggest that impaired transport of leptin across the BBB contributes to leptin resistance in the obese state. Several reports from genetic models of obesity (26,34), mice with diet-induced obesity (15,16), but also human studies (35) report a defect in the transport of leptin into the CNS that may partly account for their diminished sensitivity to leptin. In addition to defective leptin transport across the BBB, alterations in leptin receptor expression and in cellular signaling (14,15) have also been observed within the hypothalamus and in the arcuate nucleus in particular. Leptin exerts several of its central actions on energy homeostasis by engaging the melanocortin pathway (22,36). In this regard, Clegg and colleagues (36,37) have recently shown that consumption of a high-fat diet decreases the activation of the melanocortin pathway by a synthetic agonist. Thus, in terms of the effects of leptin on energy metabolism, long-term (>2 weeks) exposure to a high-fat diet appears to induce multiple defects at the levels of BBB transport, receptor expression, STAT3 activation, and downstream effector pathways such as the melanocortin pathway.

The acute effects of leptin on glucose homeostasis are quite complex. As it concerns the rapid regulation of hepatic glucose fluxes, we have recently identified melanocortin-dependent and melanocortin-independent effects of either systemic or central leptin (4). Leptin robustly inhibits hepatic glycogenolysis in conscious rats via a melanocortin-independent mechanism. However, the activation of the central melanocortin pathway by leptin also leads to stimulation of gluconeogenesis and of the hepatic expression of PEPCK and G6Pase. Of interest, leptin markedly suppressed glucose production in lean rats when the activation of the central melanocortin pathway was selectively blocked (4).

The present investigation sought to test whether the leptin resistance observed after systemic administration of leptin in overfed rats (21) could be overcome by its central administration. Surprisingly, we show that the acute central administration of leptin restores hepatic insulin sensitivity in overfed rats. Because this effect is not elicited by the systemic infusion of leptin (21), the present study suggests that a defect in the transport of leptin across the BBB is an early event in the development of leptin resistance in this model. Furthermore, the striking effects of central leptin on liver glucose fluxes in overfed rats closely resemble the melano-cortin-independent effects of leptin in a lean animal (4). Thus, we propose that an additional site of leptin resistance in this model is within the signaling of leptin through the melanocortin pathway. Because the latter process requires activation of STAT3 by leptin (38,39), we also investigated whether short-term overfeeding alters the stimulatory effect of leptin on the hypothalamic phosphorylation of STAT3. It should be noted that more prolonged exposure to high-fat diets had been shown to result in an impairment in leptin signaling to STAT3, particularly in the arcuate nucleus (14,15,40). However, in the present study, central leptin similarly enhanced the tyrosine phosphorylation of STAT3 (Tyr705) in the arcuate nucleus of standard-diet and overfed rats. We also examined whether high-fat diet or leptin altered the serine phosphorylation of STAT3 (Ser727). Phosphorylation at this site has been shown to enhance STAT3 DNA-binding activity (41), but also to negatively regulate its tyrosine phosphorylation and activation (42,43). Because we could not demonstrate effects of either leptin or high-fat diet on STAT3 serine phosphorylation, it is not likely that alterations in this signaling system play a causative role in the loss of leptin action on gluconeogenesis observed in this model. The downstream signaling steps mediating the changes in leptin action on the lever in overfed rats remain to be delineated.

In conclusion, we propose that two distinct alterations in the metabolic actions of leptin are rapidly induced by overfeeding in Sprague-Dawley rats. The first is a defect in the transfer of circulating leptin to its sites of action in the hypothalamus, and the second is a selective impairment in central leptin signaling toward hepatic gluconeogenesis likely via the melanocortin pathway (Fig. 5). Perhaps most important, the selective alterations in central leptin signaling that are induced by overfeeding in this model appear to unleash a powerful effect of central leptin on hepatic glucose production. The latter effect is sufficient to completely reverse the severe hepatic insulin resistance induced by high-fat feeding. Taken together, these results advance the notion that increasing the central availability of leptin may be a novel strategy to the treatment of diet-induced hepatic insulin resistance.

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REFERENCES


