Leptin Suppresses Food Intake and Body Weight in Corticosterone-Replaced Adrenalectomized Rats1,2

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ABSTRACT  Intracerebroventricular (ICV) injections of leptin decrease food intake and body weight while increasing energy expenditure. Some of these effects are reportedly enhanced in bilaterally adrenalectomized (ADX) rats. The purpose of the present experiment was to establish the time course of the suppression in body weight and food intake after an ICV injection of leptin. We wanted to establish the effect of varying doses of corticosterone (CORT) on body weight and food intake suppression by using separate groups of ADX, ADX and corticosterone-treated and sham-operated Sprague-Dawley rats. All rats were implanted with cholesterol pellets that varied in CORT content. During the same surgical session, all rats were fitted with a cannula in the lateral ventricle. After recovering from surgery, each rat was administered a 5-μg ICV injection of leptin. ADX rats that were treated with CORT replacement lost more (P < 0.05) weight and took longer (P < 0.05) to return to baseline body weight than sham-operated controls. Leptin injection decreased food consumption to a greater extent (P < 0.05) in the ADX groups treated with CORT than in the sham-operated controls. Plasma insulin increased in a dose-dependent manner in the ADX rats as a function of CORT replacement. The higher of the two CORT replacement doses used in this experiment restored circulating CORT to levels observed in sham-operated controls. Contrary to earlier reports, physiological doses of CORT appear to enhance leptin-induced weight loss. J. Nutr. 133: 504–509, 2003.

KEY WORDS:  • adrenalectomy • corticosterone • leptin • food intake • body weight • hypothalamus

Leptin is a 167-amino-acid peptide (1) produced primarily by adipose tissue (2) and, to a lesser extent, by the stomach (3), the brain (4) and the placenta (5). It is thought to play a role in normal appetite regulation by providing feedback from the adipocytes to hypothalamic centers mediating hunger and satiety. Since first being described by Friedman et al. in 1994 (1), dysregulation of leptin or its receptors has been implicated in several models of obesity. Both systemic and intracranial injections of leptin result in the suppression of food intake and body weight as well as an increase in metabolic rate.

Spinedi and Gaillard (6) first reported that circulating leptin is under adrenal glucocorticoid control. Adrenalectomy (ADX)4 results in decreased circulating leptin concentrations, and corticosterone (CORT) replacement restores it to levels observed in sham-operated controls (SHAM). Leptin synthesis is increased in humans in response to chronic elevation of cortisol and visceral obesity (7) and in cultured rat adipocytes when treated with glucocorticoids (8,9). Circulating levels of leptin rise in rats (6,9) and humans (10) as a result of exogenous administration of glucocorticoid. However, ADX mice infused with leptin decrease body weight, suggesting that an intact hypothalamic-pituitary-adrenal axis is not required for leptin to affect energy balance (11,12).

Adrenal steroids play a role in most experimental models of obesity as well as in normal intake (13–19). Exogenous administration of adrenal steroids in the treatment of inflammation promotes increased hunger, intake and weight gain. Cushing’s disease, a syndrome characterized by hypercortisolism, causes profound truncal obesity. Recently, Zakrzewska et al. (20) proposed the hypothesis that glucocorticoids act as counterregulatory hormones of leptin in the control of intake and body weight. They have shown that ADX rats injected intracerebroventricularly (ICV) with a single bolus of leptin lost significantly more body weight than did SHAM controls. Further, dexamethasone (DEX)-treated ADX rats lost significantly less body weight than did untreated ADX rats.

The present study was performed to replicate and extend the findings of Zakrzewska et al. (20) by examining the effects of a single ICV leptin injection in ADX rats as well as in ADX rats treated with one of two replacement doses of CORT, the principal glucocorticoid in the rat. Meijer et al. (21) have shown that DEX is a poor substitute for depletion of the endogenous glucocorticoid. In particular the brain appears to be protected against moderate amounts of DEX, leading these authors to the conclusion that its use could be expected to
destabilize neuronal homeostasis and lead to hippocampal dysfunction.

Methods

Animals. Forty-eight male Sprague-Dawley rats (weight, 180–200 g) were purchased from Charles River Laboratory (Wilmington, MA). Rats were individually housed in plastic cages in a 23°C temperature, 45% humidity, light-controlled room. Lights were on a 12:12 light/dark cycle; with lights on at 0800 h. Rats had access to a nonpurified diet (Lab Diet 5010 ground Rodent Diet; PMI Nutrition International, Brentwood, MO) and tap water ad libitum for 12 d. Proximate composition of the diet was 25.8% protein, 17.2% fat, 59.8 g of carbohydrate and 5.3 g of crude fiber/100 g of diet and provided 12.72 KJ of metabolizable energy/g. The bottom of each cage was covered with bedding (Care Fresh; Absorption, Bellingham, WA), which was replaced every 3–4 d. Food intake and body weight were measured daily. Food spillage was collected and weighed.

Lateral ventricle cannulation. After the 12-d laboratory accommodation period, all rats were surgically fitted with an ICV cannula before ADX. On d 13–17, cannulation was performed using sterile surgical technique. Each rat was anesthetized by an intraperitoneal injection of sodium pentobarbital (65 mg/kg) and then placed in a stereotaxic instrument (Stoelting, Wood Dale, IL), with its skull fixed in a level orientation with the incisor bar 3.0 mm below ear level. A 1.5-cm dural incision was made so that bregma, midline and interaural lines at the top of the skull were exposed. A 1.0-mm hole was drilled 0.8 mm posterior to bregma and 1.6 mm lateral from midline. A 22-gauge stainless steel guide cannula was placed so that its tip was 3.5 mm below the interior surface of the skull. The placement of the cannula was fixed by placing four self-tapping anchor screws in the skull around the cannula and pouring a mixture of cranioplast powder and liquid (Plastics One, Roanoke, VA) around the cannula and on the top of the screws. The animal was removed from the stereotaxic instrument once the cranioplast cement dried. An obdurater (C313G; Plastics One) was placed in the cannula guide and screwed into place.

Adrenalectomies. Immediately after cannulation, rats underwent either bilateral ADX or SHAM using our laboratory’s established surgical procedures described by Trocki et al. (15). Rats were also implanted with a 250-mg pellet, which was placed subcutaneously in the interscapular region. Each pellet contained one of the following amounts of CORT: 0 mg CORT (0% CORT; ADX0; n = 14), 25 mg CORT (10% CORT; ADX10; n = 10) and 50 mg CORT (20% CORT; ADX20; n = 9). SHAM rats (n = 15) were implanted with 100% cholesterol pellet at the time of surgery. The midline dural incision was then closed with stainless steel wound clips, and Panalog triple antibiotic was applied to the site of the wound. Rats assigned to the SHAM group underwent the same procedures as rats assigned to the ADX group, with the exception that the adrenals were left intact. After ADX was complete, each rat was placed on its side lying on a surgical warming blanket until full motor control was reacquired. Rats were allowed 10–14 d for recovery before ICV injections. ADX rats were given access to 0.15 mol of NaCl/L ad libitum for the remainder of the study, whereas SHAM rats continued to have access to tap water throughout the experiment.

Injection of aCSF or leptin. Rats from each surgical group were then assigned to one of two treatment subgroups and received an injection of either artificial cerebrospinal fluid (aCSF) or leptin. On d 27 of the experiment rats assigned to the leptin subgroup received a 5-μL ICV injection of 5 μg of leptin in aCSF, and rats in the aCSF subgroup received 5 μL of aCSF (vehicle) at a rate of ~1 μL/s (treatment 1). The rats were returned to their cages after injection. Food intake and body weight were measured daily for 10 subsequent days.

On d 39 of the experiment (treatment 2), rats assigned to the leptin group in treatment 1 were assigned to the aCSF group in treatment 2. Rats assigned to the aCSF group in treatment 1 were assigned to the leptin group in treatment 2. Each rat received a 5-μL ICV injection of either 5 μg of leptin in aCSF or aCSF at a rate of ~1 μL/s.

Body weight and food intake. Body weight and food intake, measured to the nearest 0.1 g, was recorded daily between 1030 and 1200 h throughout the experiment. To reduce spillage of food, standard 120-mL baby food jars were glued to the bottoms of ceramic saucers (~15 cm in width) and used as food cups. One such spill-proof food cup was placed in each cage. On the day of injection, food intake and body weight were recorded before treatment.

Blood and tissue sampling. On d 59 of the study, all rats received an injection of sodium pentobarbital (65 mg/kg), and blood was collected via cardiac puncture into EDTA-treated vials (Sarstedt, Newton, NC). Rats were perfused with 0.15 mol of saline/L followed with a 4% paraformaldehyde solution to preserve the brains for cannula placement verification and further analysis. Brains were dissected and placed in a solution of 4% paraformaldehyde. Blood was centrifuged (Beckman Model TJ-6) at 1,225 × g for 20 min at 4°C. Plasma was stored at −20°C before analysis of leptin, insulin and CORT by radioimmunoassay. All procedures were performed in accordance with the guidelines and with the approval of the University of Maryland’s Animal Care and Use Committee.

Analysis of leptin, insulin and CORT. Plasma leptin and insulin were measured by radioimmunoassay using reagents obtained from Linco Research (St. Charles, MO). CORT was analyzed using a 125I RIA Kit (ICN Biomedicals, Costa Mesa, CA). The intra-assay coefficient of variation for the insulin assay was 3.3%; for leptin, 4.1%; and for CORT, 2.2%. The interassay CV for the insulin assay was 6.0%; for leptin, 7.1%; and for CORT, 4.5%.

Data analysis. In addition to the conventional daily intake and body weight measurements that are typically reported in an investigation of this type, several other measures of the effects of a single injection are presented here. ‘Baseline’ intake values for each rat were determined using a mean of 5 d of preinjection food intake as the intake for each individual rat. In addition to change in body weight, the number of days that elapsed since injection to achieve maximum weight loss was calculated for each rat. Leptin injections typically resulted in body weight loss over the next 24 h. We found that in some cases weight loss continued for >1 d. It was not unusual to record body weights of leptin-treated rats 48 h after injection that were lower than the body weight that was recorded 24 h after the injection. Consequently, we defined ‘Time to maximum weight loss’ as the number of consecutive days in which body weight was lower than it had been 24 h earlier. ‘Maximum weight loss’ was defined as the difference between each rat’s preinjection body weight and the lowest 24-h body weight recorded postinjection. Similarly, the number of days after injection that elapsed to achieve maximal food intake suppression was also calculated. It was not unusual to record 24-h intakes on the d 2 after leptin injection that were lower than those during the 24 h immediately after the injection. ‘Maximal food intake suppression’ was defined as the greatest difference in 24-h intake between the baseline intake and any 24-h postinjection intake measurement. ‘Time to maximum food suppression’ was defined as the number of days to achieve maximal food intake suppression.

‘Time to food intake recovery’ was defined as the number of days it took for each individual rat to return to its pretreatment food intake within a 95% confidence limit for the mean of preinjection levels. ‘Time to body weight recovery’ subsequent to an ICV injection was defined as the number of days it took for each rat to return to 100% of the pretreatment weight.

The 5 d after injection of either aCSF or leptin was analyzed by mixed model procedures (SAS Institute, Cary, NC). Leptin and aCSF treatments for each surgical group were pooled for analysis because there was no effect due to order of treatment. The treatment means for leptin and aCSF for each surgical group were analyzed for the following: days to recover to baseline for both body weight and food intake, maximum amount of weight lost and the day it occurred, maximum amount of food reduction and the day it occurred. In addition, plasma insulin, leptin and CORT on d 59 were measured. Data were analyzed using a multivariate analysis of variance. Multiple mean comparison tests were performed using the LSD procedure. All data are reported as means ± SEM. Residuals were analyzed for normality. Differences were considered significant at P < 0.05.

RESULTS

Body weight. There were no differences in body weight among any of the experimental groups before surgery or ICV
Injections. The rats weighed 322 ± 3.2 g before surgery and 356 ± 4.7 g before the first treatment with either leptin or aCSF.

Maximum weight loss. Maximum weight loss as a result of aCSF injection did not differ among surgical groups. Varying levels of CORT replacement in the ADX rat did not change the maximum amount of weight lost after aCSF injection (Fig. 1A). Leptin injection increased (P < 0.05) the maximum weight loss in the ADX10 and ADX20 groups compared with the SHAM controls (Fig. 1A). The difference between the ADX0 group and the controls was not statistically significant. The ADX10 and ADX20 groups lost more than twice the body weight that was lost by the SHAM controls. Leptin injection in the ADX20 group resulted in an increase (P < 0.05) in maximum weight loss that was more than two and a half times greater than the weight loss observed in response to aCSF (Fig. 1B).

Time to body weight recovery. The number of days for rats to return to their preinjection body weights did not differ among surgical groups as a result of an IV injection of aCSF. Varying levels of CORT replacement did not alter the number of days that it took ADX rats to achieve maximum weight loss compared with controls in response to aCSF injection (Table 1).

Food intake. Before the first injection treatment, food intake of the ADX10 group was lower (P < 0.05) than in the SHAM group. No other differences were significant (Fig. 2).

Maximal food intake suppression. No differences between groups in maximal food intake suppression were observed in response to aCSF injections. (Fig. 3). The suppressive effects of a single leptin injection were most apparent in the ADX20 group. Maximal food intake suppression was significantly greater in the ADX20 group compared with that observed in SHAM controls treated with leptin as well as the ADX20 group treated with aCSF. After leptin injection, the ADX20 group suppressed food intake by 50% compared with both SHAM controls and the ADX20 group treated with aCSF (Fig. 3).

Time to maximal food intake suppression. Day of maximal food intake suppression in response to either aCSF or leptin injection did not differ among surgical groups. Varying levels of CORT replacement had no effect on the number of days that it took ADX rats to return to their baseline food consumption compared with controls (Table 1).

Time to food intake recovery. The mean number of days for the rats to return to their preinjection food intake did not differ among surgical groups as a result of either an IV injection of aCSF or leptin. Varying levels of CORT replacement did not alter the number of days that it took ADX rats to return to their baseline food consumption compared with controls (Table 1).

<p>| TABLE 1 |
| Effects of intracerebroventricular injections in adult male Sprague-Dawley rats on the mean time to maximum weight loss, the mean time to maximum food reduction and number of days to recover food intake after a single 5-µL injection | d |</p>
<table>
<thead>
<tr>
<th>SHAM</th>
<th>ADX0</th>
<th>ADX10</th>
<th>ADX20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to maximum weight loss</td>
<td>1.6 ± 0.3</td>
<td>1.4 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>aCSF</td>
<td>1.3 ± 0.2</td>
<td>1.6 ± 0.3</td>
<td>1.8 ± 0.4</td>
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<tr>
<td>leptin</td>
<td>1.5 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Time to maximum food reduction</td>
<td>1.5 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>aCSF</td>
<td>1.5 ± 0.3</td>
<td>1.4 ± 0.2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>leptin</td>
<td>2.1 ± 0.3</td>
<td>3.0 ± 0.5</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>Time to recovery</td>
<td>2.4 ± 0.3</td>
<td>2.9 ± 0.7</td>
<td>3.6 ± 0.6</td>
</tr>
</tbody>
</table>

1 Values are mean ± SEM, n = 9–15.
2 SHAM, sham-operated; ADX0, adrenalectomized and replaced with 0% corticosterone pellet; ADX10, adrenalectomized and replaced with 10% corticosterone pellet; ADX20, adrenalectomized and replaced with 20% corticosterone pellet.
3 aCSF, artificial cerebrospinal fluid.
Hormones

Corticosterone. Plasma CORT levels were reduced in ADX0 and ADX10 groups \((P < 0.05)\) compared with SHAM controls. However, 20% CORT replacement restored circulating levels to those in the SHAM controls. The ADX20 group had circulating CORT levels that were higher \((P < 0.05)\) than levels observed in the ADX0 group (Fig. 4A).

Insulin. ADX and CORT replacement had no effect on mean circulating insulin on d 59. At that time the SHAM controls had \(1.4 \pm 0.25\) pmol of insulin/L in circulation. By comparison, plasma insulin in the ADX0 group was \(1.1 \pm 0.26\) pmol/L. Similarly plasma insulin in the ADX10 group was \(1.4 \pm 0.19\) pmol/L, and that in the ADX20 group was \(1.7 \pm 0.34\) pmol/L. Plasma insulin rose in a dose-dependent manner with CORT replacement \((r = 0.971, P < .01)\).

Leptin. Plasma leptin levels were reduced in the ADX0 group compared with the ADX20 group. CORT replacement in the ADX20 group increased leptin levels \((P < 0.05)\) compared with the ADX0 group. Leptin levels were reduced by 46% in the ADX0 group compared with the SHAM controls. ADX with 10% CORT replacement increased leptin levels 68% above those observed in ADX with no CORT replacement. ADX with 20% CORT increased leptin 132% above levels observed in the ADX0 group (Fig. 4B).

DISCUSSION

We found that after an ICV leptin injection, ADX rats that received either 10% or 20% CORT replacement took longer (more than twice the number of days) to return to preinjection body weight compared with SHAM controls or nonreplaced ADX rats. The ADX10 and ADX20 groups also lost more weight after an ICV injection of leptin than did the SHAM controls or the ADX0 group. These results suggested that leptin has a more pronounced effect on weight loss in ADX rats with CORT replacement than it does on ADX rats without CORT replacement. The weight loss that was observed in the ADX10 and ADX20 groups occurred within 1–2 d after injection, which was the same for all the groups. Although leptin injection resulted in significant and prolonged weight loss in the ADX10 and ADX20 groups, leptin injection in the ADX20 group resulted in a marked suppression of food intake. This suppression of intake was not observed in the ADX0 or ADX10 groups.

Results from this study were noteworthy for several reasons. Unlike previously published reports (20), we presented a detailed analysis of the time course for the suppression of intake in response to leptin injection on food intake and body weight.
We reported the maximum food and weight reduction in rats and reported the day that these reductions occur after an ICV injection of leptin. We measured baseline values to determine recovery and report that the ADX rats with CORT replacement took longer to recover from leptin injection than ADX rats without CORT (ADX0) replacement. In the absence of CORT, rats shift from glucose to fat mobilization for energy metabolism, during the early hours of the night (22). ADX rats lose adipose tissue while sparing lean body mass (16–19,23). We speculated that although the ADX0 group only nominally and nonsignificantly reduced body weight and food intake after a leptin bolus, the group did not differ from the SHAM controls. This may be due to less adipose tissue to rely on during fasting. Rats do not have internal mechanisms to metabolically correct for reduced adiposity and may maintain food intake to maintain body weight. ADX rats without CORT replacement have circulating leptin levels, which are reduced by 46% compared with SHAM controls. However, 20% CORT replacement increases circulating leptin levels, which are reduced by 46% compared with SHAM controls. These increased levels of leptin may indicate higher adipose stores and an increased reserve of adipose tissue to mobilize for energy. We speculated that is this increased adipose that allowed the ADX20 group to respond to the leptin injection in such a way as to decrease intake compared with SHAM controls. ADX reduces body fat in Sprague-Dawley rats (15), ob/ob mice (8,13) and lean and obese Zucker rats (16–19). In addition to reduced body fat, ADX rats have a decrease in the size of adipose fat cells (17,24). The ADX0 group may not have adequate fat to mobilize for energy, therefore, do not suppress food intake.

CORT replacement made the ADX rats more responsive to a single ICV leptin injection than either SHAM or ADX rats that were not treated with CORT. The ADX10 and ADX20 groups took more time to achieve maximum weight loss and to recover from the leptin injection. In this study it appeared that physiological doses of CORT enhanced the effect of leptin on body weight. It should be pointed out that adrenal status had no effect on the day of maximum food reduction or food intake recovery day. Although these results are unexpected, it should be noted that leptin regulates its own expression through a negative feedback loop, which may involve the interaction of glucocorticoids (9). CORT replacement in our study may have acted as stimulant of leptin production, which resulted in a reduction of food intake and body weight in the rats replaced with more CORT. The results from this study differed from what has been reported by Zakrzewska et al. (20), where ICV leptin injection results in significant and prolonged increase in food intake and body weight in ADX rats and DEX replacement was able to reverse this trend. It should be noted, however, that in our study, we replaced CORT in ADX20 (60.9 ± 20 μg/L) rats to levels in the SHAM controls (81.0 ± 19.1 μg/L) (Fig. 4A). In the Zakrzewska study, rats were injected daily with a supraphysiological dose of 1.0 mg of DEX/l. The authors presented no measurement of circulating glucocorticoids in their experiment. DEX is a synthetic glucocorticoid that has a higher affinity for the type II glucocorticoid receptor than CORT (25). High levels of DEX may not be either mimicking or amplifying the effects of CORT. Meijer et al. (21) argued that DEX is a poor substitute for endogenous adrenal glucocorticoids.

Circulating CORT levels in the ADX20 group were restored to those in the SHAM controls. Plasma insulin levels rose in a dose-dependent manner according to CORT replacement. Plasma leptin levels in the ADX20 group were higher than levels in the ADX0 group, which appears to be a function of the CORT replacement. These results are consistent with both in vitro and in vivo studies in which exogenous sources of steroids result in increases of leptin expression and secretion (9,26). Gosselin et al. (27) report that ADX results in circulating leptin levels that were lower than SHAM controls. In our study leptin levels rose in a dose-dependent manner as a function of CORT replacement.

Finally, one of the more unexpected outcomes of the present experiment was the observation that aCSF ICV injections result in transient but consistent decreases in both body weight and food intake. The effects of this control procedure were less than ideal. Nevertheless, their transient nature is consistent with those reported by others (L. Bellinger, Baylor College of Dentistry and V. Mendel, University of California, Davis, personal communication). One of the methods of dealing with this type of “control” injection data has been to express the effect of an injection as a percentage of either intake or weight loss that is observed in aCSF controls. Unfortunately, this statistical manipulation of the data does not clearly present the effect of the SHAM procedure. We elected to report the nontransformed data in both experimental and SHAM controls so as to clearly present the transient nature of the effect of the aCSF injection and at the same time contrast it to that of the continued suppressive effects of the leptin injection.

Results from this study are consistent with the suggestion that CORT and/or insulin is acting to increase the leptin levels circulating in ADX rats (20). It is possible that increased insulin or CORT may be increasing endogenous production of leptin and that having sufficient leptin in circulation as well as sufficient adipose reserves may permit the animal to decrease food intake and weight when injected ICV with leptin. More research is needed in this area to determine if leptin is suppressing food intake and body weight as a function of adrenal status.

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LITERATURE CITED


