Glucocorticoids as Counterregulatory Hormones of Leptin

Toward an Understanding of Leptin Resistance

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The product of the *ob* gene, leptin, is a hormone secreted by adipose tissue that acts in the hypothalamus to regulate the size of the body fat depot. Its central administration has been shown to decrease food intake and body weight, while favoring energy dissipation. As glucocorticoids are known to play a permissive role in the establishment and maintenance of obesity syndromes in rodents, it was hypothesized that they do so by restraining the effect of leptin. Leptin injected intracerebroventricularly as a bolus of 3 μg in normal rats induced modest reductions in body weight and food intake. In marked contrast, the same dose of leptin had very potent and long-lasting effects in decreasing both body weight and food intake when administered to adrenalectomized rats. Further, glucocorticoid supplementation of adrenalectomized rats dose-dependently inhibited these potent effects of leptin. These data suggest that glucocorticoids play a key inhibitory role in the action of leptin. Under normal conditions, this inhibitory influence of glucocorticoids may prevent lasting hypophagia. In obesity with degrees of hypercorticism, it may contribute to "leptin resistance," whose etiology is still little understood. ***Diabetes*** **46:717–719, 1997**

Previous experiments have shown that the pathology of rodents with obesity of genetic (1–4), dietary (5), or hypothalamic (6–8) origin can be normalized by adrenalectomy and restored by glucocorticoid replacement (1,7,8). More recently and specifically, it was observed that neuropeptide Y (NPY)-induced obesity-like defects were prevented by adrenalectomy (9). As leptin and NPY have, in normal rats, opposite effects on body weight and food intake (10–19) and as the presence of glucocorticoids favors intracerebroventricular (ICV) NPY-elicited effects (9), it was hypothesized that glucocorticoids could have an inhibitory effect on the action of leptin. This could partly explain why the effect of central or peripheral leptin in decreasing food intake or body weight gain, although definitively present, is relatively weak in amplitude in normal rodents (11–13). Thus, the purpose of this study was to compare the effects of ICV leptin administration on body weight and food intake in normal and adrenalectomized rats.

RESEARCH DESIGN AND METHODS

Intact adult sham-operated female Sprague-Dawley rats (body weight, 285 ± 8 g; $n = 5$) and bilaterally adrenalectomized ones (body weight, 275 ± 5 g; $n = 8$) purchased from IFFA CREDO (L'Arbresle, France) were used. They were fed ad libitum a standard laboratory diet (Provimi-Lacta, Cossonay, Switzerland) and had free access to tap water or tap water supplemented with 9 g/l NaCl for adrenalectomized rats. At twelve weeks of age all animals were equipped with a cannula placed in the right lateral cerebral ventricle (20). Adequate placement of the cannulae was tested with the drinking response to an ICV injection of angiotensin (25 ng in 5 μl phosphate-buffered saline; Novabiochem, Laüfelfingen, Switzerland) (21). The corticosteronemia of adrenalectomized rats was below the limit of detection of the radioimmunoassay (22). After a 10-day recovery period, fed intact or adrenalectomized rats were intracerebroventricularly injected with a bolus of leptin (3 μg per rat) given within 2 min in 3 μl. Body weight and food intake were measured during the following 5 days at 9:00 A.M. and 6:00 P.M., respectively. Leptin was expressed in *Escherichia coli* and purified as previously described (23). When replacement therapy was applied to adrenalectomized rats, either 0.2 or 1.0 mg dexamethasone was administered subcutaneously once per day for 3 days before and 5 days after ICV injections of vehicle or leptin. Differences in body weight changes and food intake were analyzed by one-way analysis of variance with repeated measures followed by multiple Bonferroni comparisons, with $P < 0.05$ being accepted as statistically significant.

RESULTS

Compared with vehicle-injected control rats, an ICV bolus injection of leptin (3 μg) given to intact normal rats produced a decrease in body weight gain that failed, however, to reach statistical significance (Fig. 1, left panel). Quite different was the effect of the same dose of leptin similarly administered to adrenalectomized rats. Indeed, compared with adrenalectomized animals receiving ICV vehicle, adrenalectomized rats injected with leptin were characterized by a profound body weight loss (33 g at nadir), which lasted during the whole experimental period (Fig. 1, right panel). To substantiate that the pronounced effect of ICV leptin in adrenalectomized rats was related to the lack of glucocorticoids, glucocorticoid supplementation of adrenalectomized rats was carried out using dexamethasone, 0.2 mg or 1 mg administered subcutaneously (see METHODS). During this treatment, the effect of 3 μg leptin on body weight changes was measured and is depicted in Fig. 2. It may be seen that dexamethasone substitution of adrenalectomized rats reduced the effect of leptin on body weight changes in a dose-dependent manner. With the highest dose of dexamethasone, the effect of leptin, although present, was no longer statistically different from that of ICV vehicle injection. To further document the dynamic changes depicted by Fig. 2, integrated areas of body weight changes over the 5-day experimental period were calculated. These data are

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ICV, intracerebroventricular; NPY, neuropeptide Y.

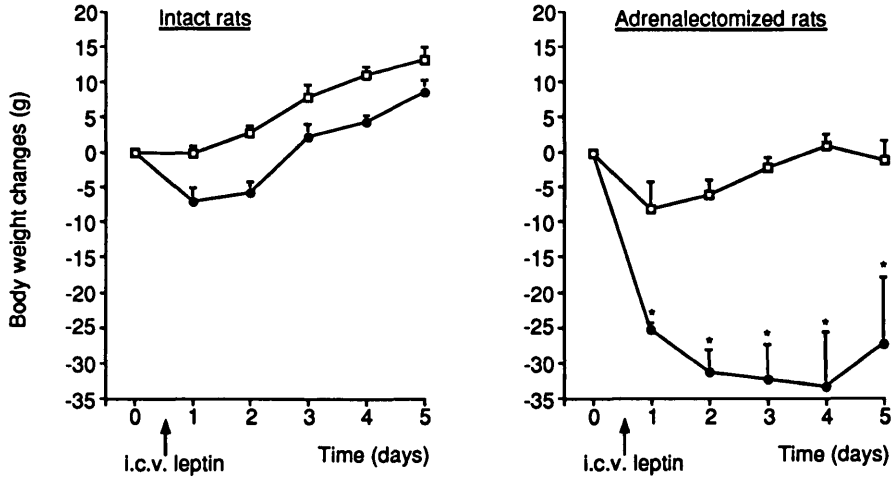

FIG. 1. Effect of a single ICV bolus injection of leptin (3 µg per rat in 3 µl) given at 3:00 P.M. (indicated with an arrow) on body weight changes measured during 5 consecutive days in intact and adrenalectomized rats, compared with respective controls intracerebroventricularly injected with vehicle (0.1 mol/l Tris, pH 8.1). Body weights were measured daily at 9:00 A.M. and are represented as changes from initial values (for further experimental details, see METHODS). Data are means ± SE of 5–8 animals per group. Statistical analysis was performed using one-way analysis of variance with repeated measures followed by multiple Bonferroni comparisons. *P < 0.05 vs. respective vehicle-injected controls. □, vehicle; ●, leptin.

shown by Fig. 3. Relative to adrenalectomized vehicle-injected controls, the loss of body weight was clearly highest in the adrenalectomized rats receiving ICV leptin only. Dexamethasone dose-dependently inhibited the marked effect of leptin on body weight loss.

Food intake was also measured. In intact rats, it was not significantly decreased by ICV injected leptin at the dose of 3 µg per rat (data not shown). In contrast, and as illustrated by Fig. 4, in adrenalectomized rats, this ICV leptin dose caused a marked reduction in food intake on days 1–3 after injection. This decrease became progressively less prominent with time, and there was no significant difference in food intake between leptin- and vehicle-injected rats from day 4 onward. Note that the food intake of adrenalectomized rats substituted with 1 mg dexamethasone per day failed to be decreased by ICV leptin (Fig. 4).

DISCUSSION

Recent data from this laboratory have shown that the effect of ICV leptin injection on body weight loss was dose-dependent in normal rats (24). The present study demonstrates that the presence of glucocorticoids restrains such ICV effects of leptin. This conclusion is based on the observation that an ICV dose of leptin (3 µg per rat), which barely affects body weight and food intake in normal rats, became powerful and long-lasting in doing so when it was intracerebroventricularly injected in adrenalectomized rats. Moreover, the supplementation of adrenalectomized rats with glucocorticoids (dexamethasone) inhibited the marked leptin response in a dose-dependent manner.

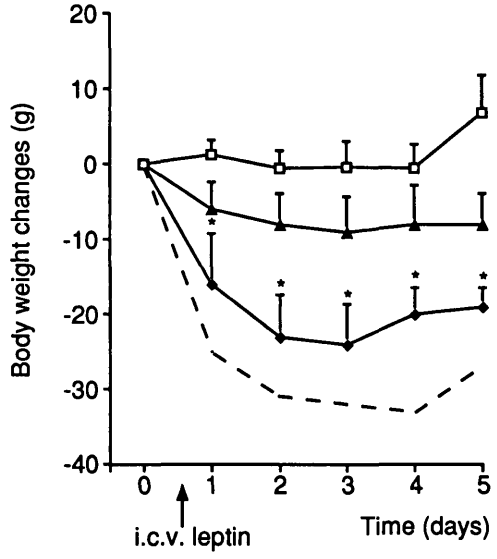

FIG. 2. Effect of a single ICV bolus injection of leptin (3 µg per rat in 3 µl) given at 3:00 P.M. (indicated with an arrow) on body weight changes measured during 5 consecutive days in adrenalectomized rats receiving leptin alone (dotted line; data from Fig. 1) or ICV leptin with subcutaneous dexamethasone substitution (0.2 or 1.0 mg/day), compared with adrenalectomized rats intracerebroventricularly injected with vehicle (0.1 mol/l Tris, pH 8.1) and supplemented with dexamethasone (1.0 mg/day). Rats were as described in legend to Fig. 1. Data are means ± SE of 5–8 animals per group. Statistical analysis was performed using one-way analysis of variance with repeated measures followed by multiple Bonferroni comparisons. *P < 0.05 vs. vehicle plus dexamethasone (1.0 mg). □, vehicle plus dexamethasone (1.0 mg); ▲, leptin plus dexamethasone (1.0 mg); ◆, leptin plus dexamethasone (0.2 mg); – –, leptin alone.

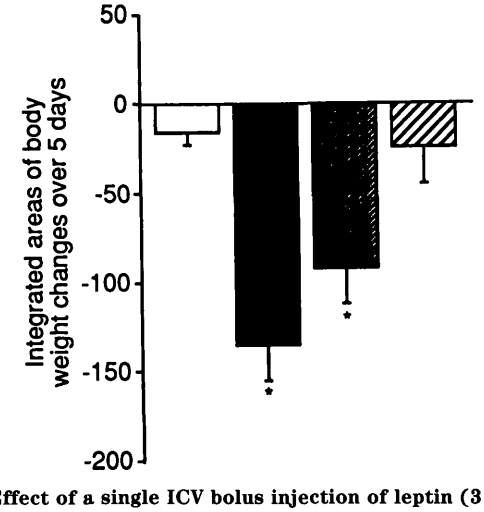

FIG. 3. Effect of a single ICV bolus injection of leptin (3 µg per rat in 3 µl) on integrated areas of body weight changes over 5 days. Integrated areas shown are calculated from the dynamics of body weight changes depicted in Figs. 1 and 2. Adrenalectomized rats received ICV vehicle (0.1 mol/l Tris, pH 8.1) or ICV leptin without or with supplementation with dexamethasone. Data are means ± SE of 5–8 animals per group. Integrated areas of vehicle-injected adrenalectomized rats substituted with 1.0 mg dexamethasone (data not shown) were not significant versus those of vehicle-injected adrenalectomized rats without dexamethasone substitution. Statistical analysis was performed using one-way analysis of variance followed by multiple Bonferroni comparisons. *P < 0.05 vs. vehicle-injected controls. □, vehicle alone; ■, leptin alone; ■, leptin plus dexamethasone (0.2 mg); ▨, leptin plus dexamethasone (1.0 mg).

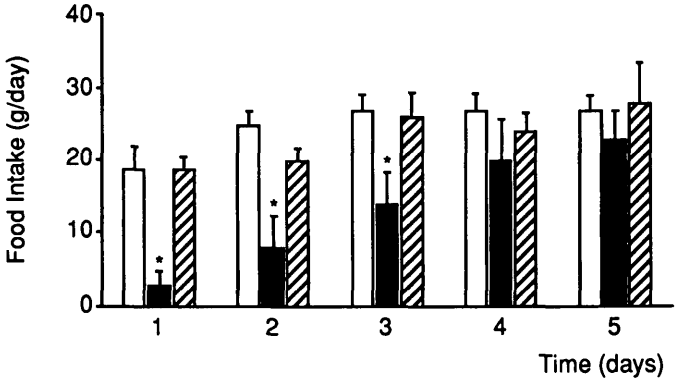

FIG. 4. Effect of a single ICV bolus injection of leptin (3 µg per rat in 3 µl) given at 3:00 P.M. on day 0 on daily food intake of adrenalectomized rats intracerebroventricularly injected with leptin, compared with that of adrenalectomized rats intracerebroventricularly injected with vehicle (0.1 mol/l Tris, pH 8.1) and with that of adrenalectomized rats intracerebroventricularly injected with leptin and supplemented with dexamethasone (1 mg/day). Food intake was measured daily at 6:00 P.M. Adrenalectomized animals were tested as indicated in Fig. 1. Data are means ± SE of 5–8 animals per group. Food intake of vehicle-injected adrenalectomized rats substituted with 1.0 mg dexamethasone was not significant versus that of vehicle-injected adrenalectomized rats without dexamethasone supplementation (data not shown). Statistical analysis was performed using one-way analysis of variance with repeated measures followed by multiple Bonferroni comparisons. *$P < 0.05$ vs. vehicle-injected controls. □, vehicle alone; ■, leptin alone; ▨, leptin plus dexamethasone (1.0 mg).

This study shows that, contrary to what occurs for NPY whose central effects require the presence of glucocorticoids (9,25), leptin effects (i.e., hypophagia and decreased body weight) are maximal in the absence of these hormones. Glucocorticoids thus appear to be a key modulator of body weight and food intake: they limit central leptin-induced effects, while favoring those of NPY. The modulatory role of glucocorticoids on the effects of leptin could be perturbed in adrenal deficiency (e.g., Addison's disease), which is characterized by marked anorexia. Such a modulatory role of glucocorticoids could also be altered in obesity, possibly contributing to the state of "leptin resistance" (26–29). The precise mode of action of glucocorticoids in the dual, yet opposite, effects on leptin and NPY remains to be established at the molecular level.

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