The Weight-Reducing Effect of an Intracerebroventricular Bolus Injection of Leptin in Genetically Obese *fa/fa* Rats

Reduced Sensitivity Compared With Lean Animals

Isabelle Cusin, Françoise Rohner-Jeanrenaud, Alain Stricker-Krongrad, and Bernard Jeanrenaud

The effect of different doses of leptin, given as an intracerebroventricular (ICV) bolus, on body weight gain and food intake was investigated during refeeding, following a 24-h fast in lean (*FA/fa*) rats. It was observed that ICV leptin resulted in a dose-dependent decrease in body weight gain, compared with vehicle injection, a difference that persisted for at least 6 days. This was associated with a transient reduction in food intake over the first 2 days after leptin injection. More importantly, the effect of leptin was also observed in genetically obese *fa/fa* rats but at the expense of two to ten times higher leptin concentrations, indicating the presence of decreased leptin sensitivity. Furthermore, ICV leptin injections were able to decrease neuropeptide Y (NPY) levels in the arcuate and paraventricular hypothalamic nuclei in both lean and genetically obese *fa/fa* rats, although a higher leptin dose was again needed in the obese group. These observations provide further evidence for the implication of NPY and leptin in a regulatory loop controlling body homeostasis. This loop is functional in lean and genetically obese *fa/fa* rats, provided that leptin levels in the central nervous system are high enough in the obese group, in particular. Since human obesity is frequently associated with elevated circulating leptin levels, a state of decreased leptin sensitivity (i.e., leptin resistance), similar to that described here in *fa/fa* rats, could possibly occur in human syndromes as well. *Diabetes* 1446–1450, 1996

Leptin, the product of the *ob* gene (1), is a satiety factor secreted by white adipose tissue that, when injected to mice, reduces body weight (2–4) and increases energy expenditure (2). It exerts these effects by acting on an isoform or isoforms of its receptor (5,6) that are located, in particular, in the hypothalamus, a brain region known to control body homeostasis. Mutation of the *ob* gene is responsible for the obesity syndrome of the *ob/ob* mice (1), while a mutation of the *ob* receptor, leading to a deficient intracellular domain of one isoform of the receptor, is responsible for the syndrome of the *db/db* mice (5,6). It has been reported that the *fa* gene is the same as the *db* and *ob* receptor genes (7). Since the obesity-diabetes syndrome of the *fa/fa* rat is less severe than that of the *db/db* mice, we hypothesized that the *fa* mutation could lead to an alteration of the *ob* receptor activation by leptin, without completely preventing leptin action. This hypothesis is in keeping with the very recent discovery that the *fa* mutation affects the extracellular domain of the *ob* receptor and, thus, possibly results in decreased sensitivity to leptin (8).

Therefore, the aim of the present study was to test the effect of an intracerebroventricular (ICV) bolus injection of leptin on body weight and food intake in lean and genetically obese *fa/fa* rats. Furthermore, as recent studies (9–11) have suggested that a hypothalamic neuropeptide, neuropeptide Y (NPY), and leptin are part of a regulatory loop controlling body homeostasis, the hypothalamic NPY levels were measured in ICV vehicle- and leptin-injected lean and obese *fa/fa* rats.

RESEARCH DESIGN AND METHODS

Adult male lean (*FA/fa*) and genetically obese (*fa/fa*) rats were used throughout the study. At 12 weeks of age, all animals were equipped with a cannula that was placed in the right lateral cerebral ventricle. After 2 weeks of recovery, rats were fasted for 24 h (7:00 P.M.–7:00 P.M.), then refed and studied for an additional 5 days. Leptin at different doses or its vehicle (Tris 0.1 mol/l, pH 8.0) was injected as a bolus over 2 min in a constant volume of 5 µl 3–4 h before the end of the 24-h fast (i.e., 3:00–4:00 P.M.). Leptin was expressed in *Escherichia coli* and purified as previously described (12). Body weight and food intake were measured daily starting 2 days before and up to 5 days after the 24-h fast. The same protocol was used for the measurement of hypothalamic NPY levels, except that the animals were not refed after the 24-h fast and were killed 6 hours after ICV leptin or vehicle injection. Brains were quickly removed and frozen on dry ice. Serial sections were cut and discrete hypothalamic nuclei were microdissected, as previously described (13). Bilateral tissue samples were placed in a solution of HCl, aprotinin, and EDTA. NPY was extracted and measured with a specific radioimmunoassay. NPY antibodies were produced in rabbits and did not cross-react with hPYY, pPYY, PP, NPY(1-13), NPY(13-36), and NPY(22-36). Porcine NPY (Bachem, Bubendorf, Switzerland) was used as standard. Antiserum and standard or sample were preincubated for 24 h at 4°C. Then, ^{125}I-labeled NPY (Amersham, Amersham, UK) was added and incubated for 16 h.

From the Laboratory of Metabolic Research (I.C., F.R.-J., B.J.), Faculty of Medicine, University of Geneva, Geneva; and Ciba-Geigy (A.S.-K.), Pharmaceutical Division, Research Department, Basle, Switzerland.

Address correspondence and reprint requests to Dr. I. Cusin, Laboratory of Metabolic Research, Faculty of Medicine, University of Geneva, 64 avenue de la Roseraie, 1211 Geneva 4, Switzerland.

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ARC, arcuate; ICV, intracerebroventricular; NPY, neuropeptide Y; PVN, paraventricular.

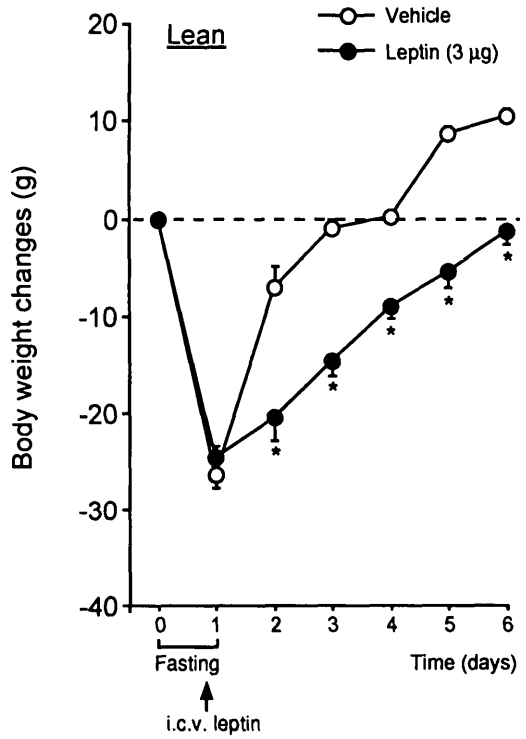

FIG. 1. Changes in body weight in ICV leptin (3 µg) and vehicle-injected lean (*FA/fa*) rats during refeeding after a 24-h fast. Data are means ± SE of 5–7 animals per group. *P < 0.001.

The separation of bound and free peptide was performed with a sheep Fc-specific anti-rabbit IgG. In these conditions, maximal zero binding was 53%, and nonspecific binding was <6%. The decrease of 50% of the bound activity was obtained for 0.07 ng/ml. Intra-assay variation was 1.3% for 2 ng/ml.

Two-tailed unpaired Student's t test was used for all parameters except for the dynamic changes of body weight as a function of time where one-way analysis of variance with repeated measures, followed by multiple Bonferroni comparisons, was used.

RESULTS

Figure 1 shows the effect of a single ICV injection of leptin (3 µg) or its vehicle on body weight changes of 24-h fasted lean (*FA/fa*) rats that were refed and followed for 5 days. Such protocol was used to optimize the effects of leptin, since leptin is presumed to have its satiety effect by decreasing hypothalamic NPY levels (9–11) and since fasting is known to increase hypothalamic NPY concentrations (14). It can be seen that the 24-h fast produced a profound decrease in body weight in both experimental groups. Vehicle-injected controls regained a normal body weight within 2 days of refeeding. In marked contrast, leptin injection in lean rats resulted in a profound reduction of body weight gain after fasting, a reduction that lasted up to the 6th day of the experiment. This effect of leptin on body weight was related to a transient inhibition of food intake over 2 days that was not present thereafter (Table 1). A dose-response curve of the effect of ICV leptin injection on body weight gain was carried out in lean rats. For each dose of leptin tested, the dynamic changes of body weight were similar to those described in Fig. 1 (data not shown). The dynamic changes observed were then calculated as integrated areas of body weight changes over a period of 6 days in leptin-injected compared with vehicle-injected rats, the latter group being referred to as –100%. The results thus obtained are shown in Fig. 2. It can be seen that 0.15 and 0.3 µg ICV leptin resulted in decreases in body weight gain after fasting (–189 ± 55 and –182 ± 45%, respectively, compared with vehicle-injected rats, –100 ± 10%), although these decreases failed to reach statistical significance. ICV leptin doses from 1.5 to 36 µg were effective in significantly decreasing body weight gain after fasting compared with vehicle-injected rats, with maximal responses being reached at 18 and 36 µg of leptin. Table 1 shows the effect of the various doses of ICV leptin on food intake expressed as a percentage of that of vehicle-injected rats. Upon refeeding (day 1–2), ICV leptin produced an inhibitory effect that was maximal at a dose of 3 µg. In contrast, on the next day (day 2–3), the maximal inhibitory effect of leptin was reached with the highest leptin dose (36 µg). It should be noted that the inhibitory effect of ICV leptin injection on food intake never lasted more than 2 days after leptin injection (i.e., day 1–3).

That ICV leptin injection was also efficient in decreasing body weight gain during the 24-h fast, then refed genetically obese *fa/fa* rats is shown in Fig. 3. Indeed, ICV leptin injection markedly reduced body weight gain in the obese group, with dynamic changes that were similar to those obtained in the leptin-injected lean group (compare Figs. 1 and 3). The dose-response curves carried out with leptin or vehicle-injection in the obese group suggested, as depicted by Fig. 4, that about ten times higher leptin doses were needed in obese *fa/fa* rats to produce the same effects as those observed in lean

TABLE 1
Food intake in ICV leptin-injected lean (FA/*fa*) and obese (*fa/fa*) rats during refeeding after 24-h fasting expressed as percent of respective controls

	Dose of leptin (µg/rat)					
	0.15	0.3	1.5	3.0	18	36
Lean						
Days 1–2	82.9 ± 6.5	81.6 ± 6.0*	80.9 ± 2.0*	66.2 ± 4.3*	71.4 ± 5.5*	80.8 ± 11.2
Days 2–3	98.6 ± 6.1	90.1 ± 5.9	88.0 ± 2.0*	84.4 ± 3.1*	76.6 ± 4.0*	67.3 ± 8.4*
Obese						
Days 1–2	–	–	–	71.4 ± 12.8	68.6 ± 5.2*	76.3 ± 6.2*
Days 2–3	–	–	–	98.8 ± 9.2	93.9 ± 2.7	85.7 ± 4.7*

Day 1–2: first day of refeeding after fasting. Values are means ± SE of 5–7 rats/group. *P at least < 0.05 versus respective vehicle injection (100%).

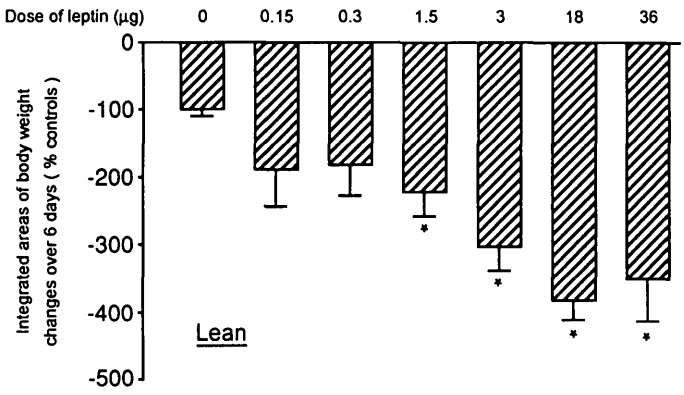

FIG. 2. Integrated areas of body weight changes over 6 days in ICV leptin-injected lean (*FA/fa*) rats during refeeding after a 24-h fast, expressed as percentage of vehicle-injected rats. Data are means ± SE of 5–7 animals per group. *$P < 0.025$ (at least) vs. vehicle injection.

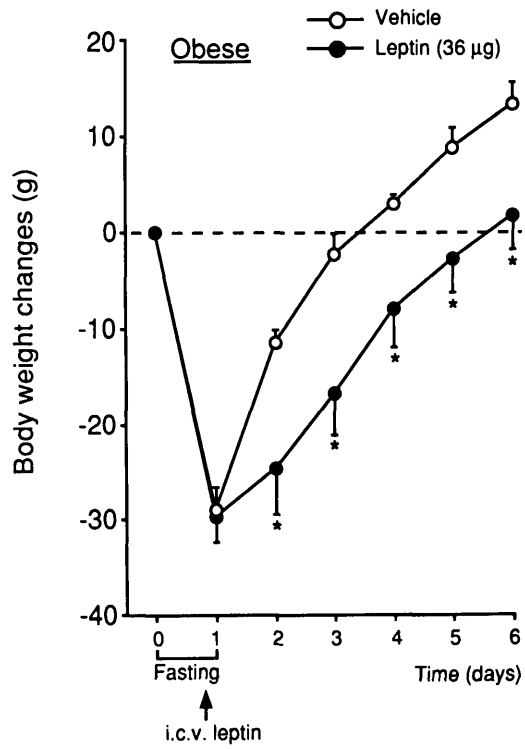

FIG. 3. Changes in body weight in ICV leptin (36 µg) and vehicle-injected genetically obese *fa/fa* rats during refeeding after a 24-h fast. Data are means ± SE of 5–7 animals per group. *$P < 0.05$.

animals. However, such a conservative statement should be modulated by the observation that the body weight lowering effect of the lowest leptin concentration used in obese rats (i.e., 3 µg) was similar to that obtained with 1.5 µg leptin in the lean group. This indicated that, at those concentrations, there may be only a twofold difference in leptin sensitivity between lean and obese animals. The inhibition of food intake by ICV leptin injection in obese *fa/fa* rats followed the same pattern as in lean rats, at the expense of higher leptin doses (Table 1). To rule out the nonspecific effects of the leptin preparation at high doses, 18 µg of a biologically inert fragment of leptin (a C-terminus leptin fragment of about 90 amino acids, obtained by treatment of leptin with cyanogen bromide) were ICV injected and found not to alter body weight nor food intake in lean rats (data not shown).

Figure 5 shows that ICV leptin injection resulted in decreases in NPY content in the arcuate (ARC) and the paraventricular (PVN) hypothalamic nuclei in lean rats. Similar results were obtained in genetically obese *fa/fa* rats, when a tenfold higher leptin dose was injected. It is noteworthy to mention that ARC and PVN NPY levels were similar in lean and obese vehicle-injected rats. This is in keeping with the observations that NPY levels increase in lean rats after a 24-h fast, while it fails to do so in obese animals that already have elevated hypothalamic NPY content (15).

DISCUSSION

This study demonstrates that an ICV bolus injection of leptin decreases body weight gain and food intake in rats. It also evaluates the impact of a single ICV leptin injection on body weight gain and food intake over a 6-day experimental period. Such an acute leptin injection results in a marked dose-dependent reduction in body weight gain in lean rats, a reduction that persists over at least 6 days. It should be noted that similar effects of leptin on body weight gain and food intake are observed in ad libitum fed rats (data not shown). These leptin-induced reductions of body weight gain are partly attributable to the transient satiety effect of leptin. In addition, the observation that ICV leptin injection prevents the catch-up growth of both fasted and fed rats suggests that leptin is able to prevent the compensatory mechanisms that are responsible for regaining normal body weight after fasting or food restriction, such as, for instance, an increase in glucose carbon incorporation into fatty acids, an increase in lipoprotein lipase activity, or a decrease in energy dissipation (16–18). This is in keeping with the indirect measurements, suggesting that leptin is able to increase energy expenditure in *ob/ob* mice (2,19).

Another prominent finding of the present study is that a bolus of leptin, given in an ICV injection, is active in genetically obese *fa/fa* rats. The effects of ICV leptin on body weight gain and food intake of obese *fa/fa* rats are qualitatively similar to those obtained in lean animals but at the expense of about two to ten times higher doses of leptin (see RESULTS). Finally, the present study demonstrates the ability of leptin to decrease NPY levels in its sites of synthesis (ARC nucleus) and release (PVN nucleus) in lean rats. This provides additional evidence for the implication of NPY and leptin in a regulatory loop controlling body homeostasis (9–11), although additional mechanisms may be involved, as shown in transgenic mice lacking NPY that were normally responsive to leptin (20,21). Of interest is the observation that similar results can be achieved in genetically obese *fa/fa* rats when, relative to lean animals, a two to ten times higher leptin concentration is injected. All these observations indicate the presence in the obese group of a state of reduced sensitivity to leptin. The *fa* mutation just described (8) appears to alter the extracellular domain of all *ob* receptor isoforms. This alteration could still allow leptin to bind to its receptor (8), while it could decrease

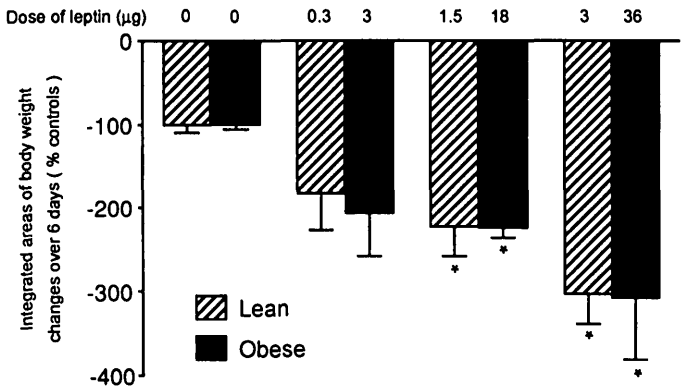

FIG. 4. Integrated areas of body weight changes over 6 days in ICV leptin-injected lean (*FA/fa*) and genetically obese (*fa/fa*) rats during refeeding after a 24-h fast, expressed as percentage of respective controls. Data are means ± SE of 5–7 animals per group. *$P < 0.025$ (at least) vs. respective vehicle injection.

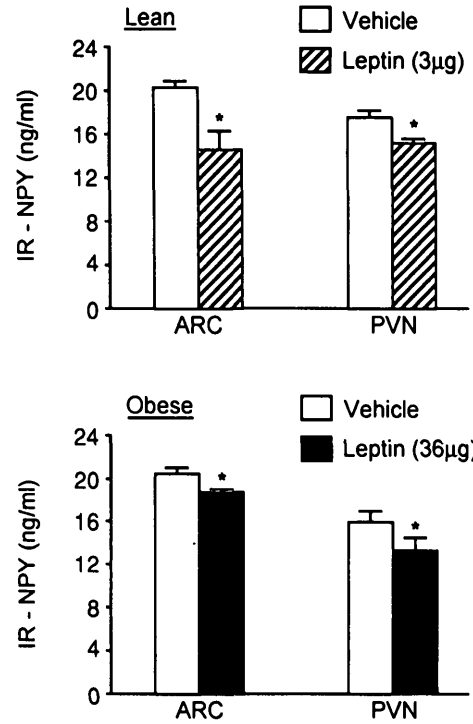

FIG. 5. Changes in NPY content in ARC and PVN hypothalamic nuclei in 27-h fasted lean (*FA/fa*) and genetically obese (*fa/fa*) rats injected either with vehicle or leptin. Data are means ± SE of 5–7 animals per group. *$P < 0.05$ (at least) vs. respective vehicle injection.

the receptor affinity for leptin. When given in an ICV injection, leptin acts directly at its hypothalamic receptor, while circulating leptin needs to be transported through the blood-brain barrier, possibly by binding to the receptor isoform located in the choroid plexus (6). Given that the *fa* mutation affects all *ob* receptor isoforms (8) and based on our results, the sensitivity of all receptors would be rightward-shifted. Thus, in obese *fa/fa* rats whose circulating leptin levels are already increased (22), a further increase of leptinemia achieved by exogenous leptin administration could be beneficial, not only for restoring a normal body weight homeostasis, but also for ameliorating some hormonal profiles and reproductive functions as has been shown in fasted lean C57BL mice (23). Leptin analogs, aimed at a better interaction with the choroid plexus receptors at the level of the blood-brain barrier, the hypothalamic receptors, or both, could also ameliorate the abnormalities of the obese group. This could prove to be similar for several human obesity syndromes in which elevated plasma leptin levels have also been reported (22,24,25). Thus, the genetically obese *fa/fa* rat with its hormonal and metabolic disorders is a good model for the better understanding of human obesity associated with leptin resistance. The present finding of a decreased leptin sensitivity in obese *fa/fa* rats that can be overcome by exogenous ICV leptin administration potentially opens new possibilities for the treatment of leptin-resistant obesities.

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