

Chronic Central Leptin Infusion Enhances Insulin-Stimulated Glucose Metabolism and Favors the Expression of Uncoupling Proteins

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Continuous (4 days) intracerebroventricular leptin infusion (12 µg/day) was performed in lean rats, and its hormonometabolic effects were determined. Intracerebroventricular leptin administration did not result in leakage of the hormone into the peripheral circulation. Thus, its effects were elicited by its presence within the central nervous system. Intracerebroventricular leptin infusion produced marked decreases in food intake and body weight gain relative to vehicle-infused fed ad libitum rats. Because decreases in food intake alter hormonometabolic homeostasis, additional control rats pair-fed to the amount of food consumed by leptin-infused ones were included in the study. Intracerebroventricular leptin-infused and vehicle-infused pair-fed rats were characterized, relative to vehicle-infused ad libitum-fed animals, by decreases in body weight and insulinemia and by increases in insulin-stimulated overall glucose utilization and muscle and brown adipose tissue glucose utilization index. Brown adipose tissue uncoupling protein (UCP)1, UCP2, and UCP3 mRNA levels were markedly decreased in pair-fed animals relative to those of fed ad libitum control animals, as were liver and white adipose tissue UCP2 and muscle UCP3 mRNA levels. In marked contrast, intracerebroventricular leptin administration was accompanied by the maintenance of high UCP1, UCP2, and UCP3 expression in all these tissues. Thus, despite analogies between leptin's effects and those of pair-feeding with regard to glucose handling, their respective underlying mechanisms differ. While leptin maintains or favors energy-dissipating mechanisms (UCP1, UCP2, and UCP3), the latter are markedly depressed in pair-fed rats. This effect of leptin may prevent subsequent excessive storage processes, thereby maintaining normal body homeostasis. *Diabetes* 47:1014-1019, 1998

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BAT, brown adipose tissue; HGP, hepatic glucose production; R_d , rate of glucose disappearance; RG, red gastrocnemius; RQ, red quadriceps; UCP, uncoupling protein; WAT, white adipose tissue; WG, white gastrocnemius; WQ, white quadriceps.

Leptin is a hormone produced by adipose tissue (1) that, after its release into the blood, acts within the hypothalamic area where specific long-form leptin receptors Ob-Rb are located (2,3). After binding to the receptors, leptin ultimately decreases food intake and presumably increases energy dissipation (4-7). Besides these effects, leptin has been reported to have direct ones on peripheral tissues. In isolated white adipocytes, it has been shown to decrease insulin-induced glucose uptake and lipogenic activity, while increasing lipolysis (8). More generally, leptin has been shown, in vivo and in vitro, to markedly deplete fat content of both adipocytes and non-adipocytes (i.e., muscles and liver) (9). In contrast, leptin was found to have insulin-like effects on glucose uptake and glycogen synthesis in myotubes, without affecting the insulin action per se (10). Leptin was also reported not to have any effect on basal or insulin-stimulated glycogen synthesis in isolated soleus muscle of wild-type mice, while it inhibited these processes in muscles of *ob/ob* mice (11).

Because of the observation that the main action of leptin is to decrease food intake and presumably increase energy expenditure by acting within the hypothalamus, the purpose of the present experiments was to determine whether hormonometabolic changes as well as alterations in the expression of different uncoupling proteins (UCPs) could be elicited by the presence of leptin within the central nervous system. To do this, leptin was continuously infused intracerebroventricularly for 4 days, and insulin-stimulated glucose metabolism, as well as the expression of UCP1, UCP2, and UCP3 in different tissues, was measured. It was hypothesized that leptin could influence carbohydrate metabolism as well as the expression of not only UCP1 in brown adipose tissue (BAT) (12) but also UCP2 (13) and UCP3 (14). Indeed, all such proteins, via their properties of generating heat, may be implicated in the regulation of body temperature and body weight. Furthermore, the presence of UCP2 in tissues other than the rodent BAT (e.g., in white adipose tissue [WAT], lung, kidney, and liver) (13) and the presence of UCP3 in BAT as well as in skeletal muscles, including human muscles (14), make UCP2 and UCP3 potential candidates for regulating body weight in large mammals, which usually lack BAT UCP1.

RESEARCH DESIGN AND METHODS

Animals. Heterozygote lean (*fa/fa*) male rats of the Zucker strain from our breeding colony, weighing about 220 g, were housed in individual cages under conditions of controlled temperature (23°C) and illumination (7:00 A.M.-7:00 P.M.).

Although these animals are heterozygous for a mutation of the leptin receptor gene (*fa*), they represent an adequate model of lean rats. Indeed, it has recently been demonstrated that the normal leptin receptor functions as a dominant negative toward the mutated *fa* receptor (15). They were allowed ad libitum access to water and standard laboratory food (Provimi Lacta, Cossonay, Switzerland) unless otherwise stated. Food intake and body weight were measured daily.

Chronic intracerebroventricular infusion of leptin. Rats were anesthetized with intramuscular ketamin/xylazine used at 45 mg/kg and 9 mg/kg, respectively (Parke-Davis and Bayer, Leverkusen, Switzerland), and equipped with a cannula positioned in the right lateral ventricle. After 1 week of recovery, osmotic minipumps (model 2001; Alza, Palo Alto, CA) delivering 12 μ g of leptin (recombinant mouse leptin provided by Novartis, Basel, Switzerland) per day for 4 days or its vehicle (Tris 0.1 mol/l, pH 9) were connected to the intracerebroventricular infusion cannula via a polyethylene catheter under ether anesthesia (16). Three groups of rats were investigated: 1) rats infused with leptin; 2) control rats infused with the vehicle and allowed to eat ad libitum; and 3) control rats infused with the vehicle but pair-fed to the amount of food consumed by leptin-infused animals. The pair-feeding regimen was performed as follows: average daily food intake for the leptin-treated group was calculated; one-third of this amount of food was given in the morning (8:00 A.M.), while the remaining two-thirds were given before the extinction of the light (6:00 P.M.), based on a preliminary study of food consumption during the day and the night. To ensure that leptin was present only within the cerebral ventricles, plasma leptin levels were measured after intracerebroventricular application.

Measurement of in vivo glucose utilization during euglycemic-hyperinsulinemic clamps. After 4 days of intracerebroventricular leptin infusion, 5-h fasted rats were anesthetized with sodium pentobarbital (55 mg/kg i.p.), and euglycemic-hyperinsulinemic clamps were performed as previously described (17). Mean steady-state values of insulinemia and glycemia during the clamps were, respectively, $3,480 \pm 135$ pmol/l and 6.6 ± 0.1 mmol/l. These insulinemia values enabled study of the half-maximal stimulation of glucose utilization by insulin (17). The in vivo insulin-stimulated glucose utilization index by individual tissues was measured during euglycemic-hyperinsulinemic clamps associated with the labeled 2-deoxy-D-glucose technique (2-deoxy-D-[1-³H]glucose; Amersham, Aylesbury, U.K.), as previously described and validated (18,19). Different fiber-type (red or white) muscles, such as white quadriceps (WQ), red gastrocnemius (RG), and white gastrocnemius (WG), and epididymal WAT and BAT were tested. Hepatic glucose production (HGP) and total glucose metabolism (rate of glucose disappearance [R_d]) were measured in basal and insulin-stimulated states during the clamps by infusing D-[U-¹⁴C]glucose (50 μ Ci/rat; Amersham), as previously described (17).

Analytical procedures relative to clamp studies. 2-Deoxy-D-[1-³H]glucose and D-[U-¹⁴C]glucose-specific activities were determined in deproteinized blood samples as described elsewhere (20). 2-Deoxy-D-[1-³H]glucose-6-phosphate, determined in tissues as described previously, allowed us to calculate the in vivo glucose utilization index by individual tissues and was expressed in nanograms per minute per milligram tissue (20). For the measurement of in vivo insulin-stimulated overall glucose turnover rate, supernatant of deproteinized blood was submitted to an ion-exchange resin (AG2X8; Bio-Rad, Richmond, CA) to avoid concomitant lactate measurement.

Plasma hormones and metabolites measurements. Plasma glucose was determined by the glucose oxidase method (Beckman Glucose Analyzer II; Fullerton, CA). Plasma insulin and corticosterone levels were measured by radioimmunoassays (21). Plasma leptin levels were determined using a commercial kit for rat leptin having 100% cross-reactivity with mouse leptin (Linco Research, St Louis, MO).

Northern blot. In additional experiments, the three groups of rats mentioned above (see **Animals**) were continuously infused with vehicle or leptin for six days, and at the end of the respective infusions, tissues were removed and total RNA was extracted (22) from WAT and BAT, as well as from liver and muscle (RO). Aliquots of 10 μ g were size-fractionated on 1.5% agarose gels, and blots were hybridized (Quikhyb; Stratagene) to random primed labeled cDNAs for UCP1 (12), UCP2 (13), UCP3 (GenBank Accession U92069), and β -actin (Clontech Laboratory) (23). Autoradiographs (X-OMAT-AR film; Kodak, Rochester, NY) were quantified by densitometry with Image Quant Software. Abundance of UCP1, UCP2, and UCP3 mRNA relative to that of β -actin was expressed as a percentage of corresponding fed ad libitum vehicle-infused control animals. Only signals obtained on the same Northern blot were compared.

Statistical analysis. For daily measurements of changes in body weight and plasma insulin concentrations, statistical analyses were made using one-way analysis of variance for repeated measures followed by multiple Bonferroni comparisons. For all other analyses, two-tailed Student's *t* test for unpaired data were used. Values of *P* < 0.05 were accepted as being statistically significant.

RESULTS

Comparisons were made among three intracerebroventricularly infused groups of rats: 1) vehicle-infused control rats who had free access to laboratory food; 2) leptin-infused rats; and 3) vehicle-infused rats pair-fed to the amount of food consumed by the leptin-infused group.

Impact of intracerebroventricular leptin infusion on body weight and food intake. Leptin-infused animals had, from experimental day 2, a significant decrease in food intake that remained $39.5 \pm 3.4\%$ lower than that of ad libitum-fed control rats throughout the study. By definition, vehicle-infused pair-fed rats had the same food intake as that of leptin-infused animals. The changes in body weight relative to the respective initial body weight for the three groups of rats are illustrated in Fig. 1. As can be seen, control rats gained weight during the 4-day experimental period, while both the leptin-infused rats and their respective pair-fed control rats lost weight during the same time. Body weight loss was identical in leptin-infused and in pair-fed control rats compared with ad libitum-fed control rats.

Impact of intracerebroventricular leptin infusion on hormonal changes and glucose metabolism. As can be seen in Table 1, plasma leptin levels were not significantly different in leptin-infused rats and pair-fed control rats, but they were significantly lower than those of ad libitum-fed control rats. This indicated that the leakage of intracerebroventricular leptin into the circulating blood was nil and that the plasma leptin levels were correlated with body weight. Corticosteronemia and glycemia were identical in the three groups of rats (Table 1).

Throughout the study, basal insulinemia was and remained within normal values in the ad libitum-fed control group. In contrast, leptin-infused rats, as well as pair-fed control rats, had low plasma insulin levels that, at the end of the experiment, were less than half those of the ad libitum-fed animals (Table 1).

Glucose handling was assessed during euglycemic-hyperinsulinemic clamps (Table 1). Basal R_d was the same in the three groups of rats. More importantly, and relative to ad libitum-fed control rats, both the leptin-infused rats and the respective pair-fed control rats had significantly higher rates of insulin-stimulated R_d during the clamps, indicating the presence of an increased overall insulin sensitivity in these two groups. Additional data on glucose handling, as assessed during euglycemic-hyperinsulinemic clamps, showed that the HGP was the same and was normally suppressed by insulin in the three groups of rats (data not shown).

Glucose uptake by individual tissues (referred to as glucose utilization index) was measured using euglycemic-hyperinsulinemic clamps associated with the labeled 2-deoxyglucose technique. For various muscles (including white and red type muscles), it was observed, as illustrated in Fig. 2, that compared with values of insulin-stimulated glucose utilization index obtained in ad libitum-fed control rats, those of either leptin-infused rats or their respective pair-fed control rats were significantly higher. An analogous pattern was observed in BAT, the glucose utilization index of which was higher in leptin-infused or pair-fed rats than in the ad libitum-fed control rats (Fig. 3). The situation was different in WAT. Indeed, as can be seen in Fig. 3, the insulin-stimulated glucose utilization index was lower in WAT of leptin-infused animals than in that of pair-fed control rats.

Impact of intracerebroventricular leptin infusion on the expression of UCP1, UCP2, and UCP3. Comparisons among vehicle-infused fed ad libitum, leptin-infused, and

vehicle-infused pair-fed rats also bore on the status of the UCP mRNA levels in different tissues. In separate experiments, it was observed that in BAT of control animals, the mRNA levels of UCP1 and UCP3 were similar, whereas those of UCP2 were about twofold lower (data not shown). As can be seen in Fig. 4, the pair-feeding regimen resulted, relative to the fed ad libitum situation, in very marked decreases in UCP1, UCP2, and UCP3 mRNA levels in BAT. In contrast, intracerebroventricular leptin infusion prevented the occurrence of such decreases in BAT UCP1 and UCP2 expression and resulted in a doubling of that in UCP3. As depicted in Fig. 5, UCP2 mRNA levels measured in the liver and WAT were also markedly decreased in vehicle-infused pair-fed animals. Such a decrease in liver UCP2 expression was prevented by intracerebroventricular leptin infusion. Additionally and relative to UCP2 expression in WAT, leptin had a stimulatory effect on UCP2 expression in this tissue. As shown in Fig. 6, muscle UCP3 mRNA levels were markedly decreased by the pair-feeding regimen, a decrease that was prevented by intracerebroventricular leptin infusion and replaced, when compared with values observed in ad libitum-fed control rats, by an increased expression, although the latter failed to reach statistical significance.

DISCUSSION

In vivo metabolic studies pertaining to the effects of leptin are still relatively scarce. Acute intravenous leptin administration to normal rats was shown to be followed by an increased sensitivity of glucose utilization to insulin, as assessed by euglycemic-hyperinsulinemic clamps (24). Also, acute (5-h) intravenous or intracerebroventricular leptin administration to normal mice has been shown to increase basal (i.e., not insulin-stimulated) R_d and to stimulate basal glucose utilization index (as assessed by the labeled 2-deoxyglucose technique) of both BAT and muscles. It was further suggested that the effects of leptin on glucose utilization were mediated by the central nervous system (25).

The aim of the present study was to investigate the effects of a continuous intracerebroventricular infusion of leptin for 4 days on body weight homeostasis, basal hormonal output, glucose handling, and the expression of UCP1, UCP2, and UCP3 in different tissues (12–14).

This study has been carried out in lean rats of the Zucker nondiabetic strain, heterozygous (FA/fa) for a mutation of the leptin receptor gene (fa), which might have interfered with the results. However, these animals seem to represent an adequate model of lean rats, since it has recently been demonstrated that the normal leptin receptor functions as a dominant

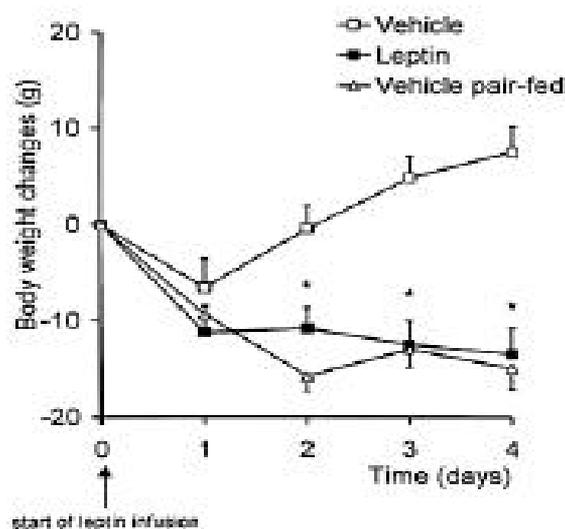


FIG. 1. Body weight changes over initial respective body weights of intracerebroventricular vehicle-infused control rats fed ad libitum, leptin-infused (12 μ g/day) rats, and vehicle-infused rats pair-fed to the amount of food consumed by the leptin-infused group. Continuous vehicle or leptin infusion over 4 days. Data are means \pm SE of 5–6 animals per group. * P < 0.05 for the two test groups vs. fed ad libitum control rats.

negative toward the mutated fa receptor (15). This does not seem to be the case in heterozygous lean rats (FA/fa) of the Zucker diabetic strain (ZDF), which display an impaired fatty acid-induced proinsulin mRNA response when compared with homozygous lean animals (FA/FA) (26).

Intracerebroventricular leptin infusion was not accompanied by any detectable increase in plasma leptin levels, indicating that, for the dosage used (12 μ g per rat per day), there was no leakage of the hormone into the circulating blood. Thus, the hormonometabolic changes observed were due to genuine action of leptin within the central nervous system, presumably within the hypothalamic area containing the known long-form (Ob-Rb) receptor isoform (2,3).

Central leptin infusion was shown to markedly decrease food intake, body weight, and basal insulinemia. These three parameters were similarly decreased by a pair-feeding regimen whereby vehicle-infused control rats were pair-fed to the same amount of food consumed by the leptin-infused group. The similar decreases in basal plasma insulin levels in leptin-infused rats and vehicle-infused pair-fed control rats are likely related to decreased substrate availability to the endocrine pancreas. It should be mentioned that while vehi-

TABLE 1

Basal hormonal and metabolic parameters in lean rats chronically intracerebroventricularly infused with leptin or vehicle

	Vehicle-infused rats	Leptin-infused rats	Vehicle-infused pair-fed rats
Leptinemia (ng/ml)	2.5 \pm 0.2	1.4 \pm 0.4*	1.3 \pm 0.1*
Insulinemia (pmol/l)	237 \pm 10	81 \pm 12*	99 \pm 6*
Corticosteronemia (nmol/l)	162 \pm 65	165 \pm 23	187 \pm 46
Glycemia (mmol/l)	6.7 \pm 0.3	6.1 \pm 0.2	6.0 \pm 0.2
Basal R_d ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	6.4 \pm 0.3	5.8 \pm 0.2	6.0 \pm 0.2
Insulin-stimulated R_d ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	16 \pm 0.5	21.6 \pm 2*	19.4 \pm 0.6*

Data are means \pm SE of 5–6 animals per group. Infused leptin was 12 μ g per rat per day. * P at least <0.05.

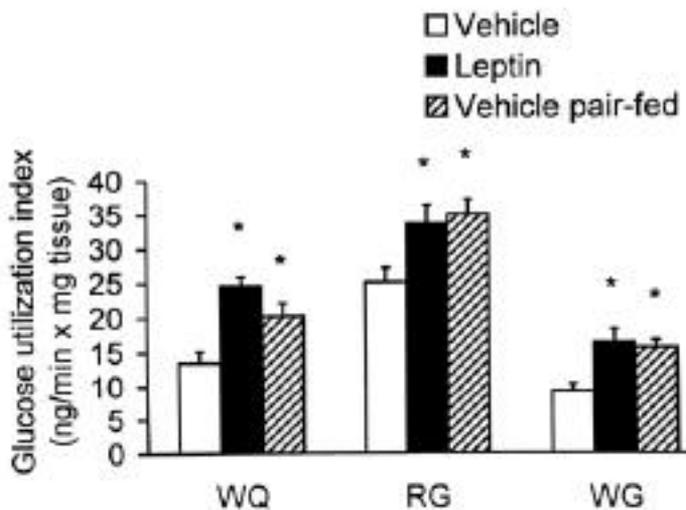


FIG. 2. In vivo glucose utilization index of various muscles measured by the labeled 2-deoxyglucose technique during euglycemic-hyperinsulinemic clamps in intracerebroventricular vehicle-infused control rats fed ad libitum, leptin-infused (12 μ g/day) rats, and vehicle-infused rats pair-fed to the amount of food consumed by the leptin-infused group. Continuous vehicle or leptin infusion over 4 days. Data are means \pm SE of 5–6 animals per group. * P < 0.05 vs. fed ad libitum control rats.

cle-infused pair-fed rats had marked (though not quantifiable) behavioral changes characteristic of intense food seeking, such as was not the case for leptin-infused rats.

Experiments carried out under both basal conditions and during euglycemic-hyperinsulinemic clamps showed that continuous intracerebroventricular leptin administration did not alter basal HGP, nor did it change the effect of insulin in suppressing HGP. It is therefore concluded that central leptin infusion to normal rats fails to alter the in vivo liver glucose output. This is in contrast with data obtained by others in awake mice in which acute leptin infusion has been demonstrated to increase basal HGP (25).

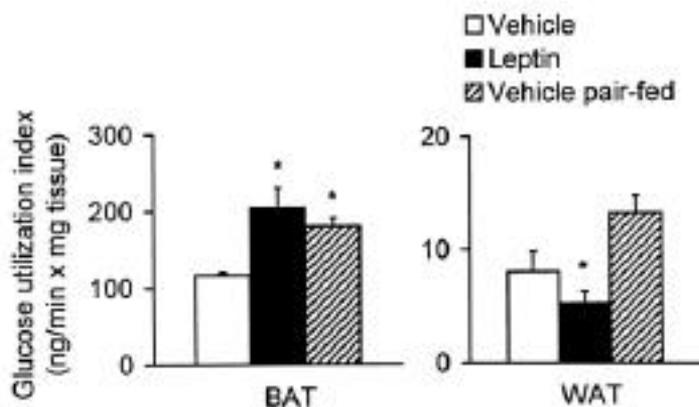


FIG. 3. In vivo glucose utilization index of BAT and epididymal WAT measured by the labeled 2-deoxyglucose technique during euglycemic-hyperinsulinemic clamps in intracerebroventricular vehicle-infused control rats fed ad libitum, leptin-infused (12 μ g/day) rats, and vehicle-infused rats pair-fed to the amount of food consumed by the leptin-infused group. Continuous vehicle or leptin infusion over 4 days. Data are means \pm SE of 5–6 animals per group. * P < 0.05 vs. fed ad libitum control rats in BAT and vs. pair-fed control rats in WAT.

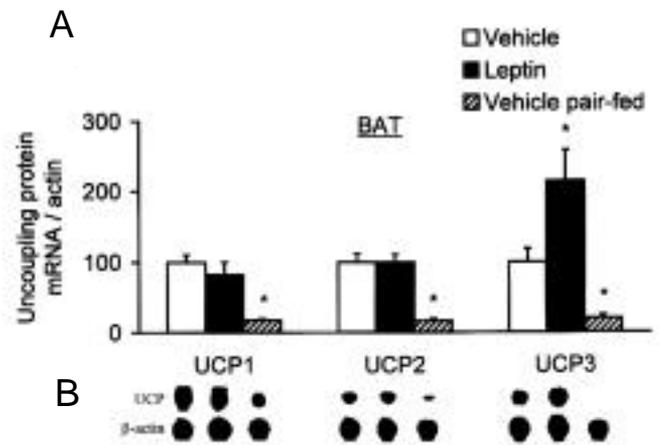


FIG. 4. UCP1, UCP2, and UCP3 mRNA levels in BAT in intracerebroventricular vehicle-infused control rats fed ad libitum, leptin-infused (12 μ g/day) rats, and vehicle-infused rats pair-fed to the amount of food consumed by the leptin-infused group. Continuous vehicle or leptin infusion over 4 days. **A:** Data are means \pm SE of 5 animals per group. * P at least < 0.05 vs. fed ad libitum control rats. **B:** Representative UCP1, UCP2, UCP3, and β -actin mRNA signals under the various conditions studied.

Additionally, this study demonstrates that continuous central leptin administration was able to further increase the in vivo insulin stimulatory effect on overall R_d measured during euglycemic-hyperinsulinemic clamps. The further increase in R_d brought about by intracerebroventricular leptin administration was also observed after the pair-feeding regimen. The potentiation of the insulin effect on R_d by both leptin and pair-feeding was measured in specific insulin-responsive tissues, such as muscles and BAT. In these tissues, the glucose utilization index was increased beyond the values reached by the insulin stimulus alone, both by intracerebroventricular leptin infusion and by the pair-feeding regimen. The only tissue in which the effect of leptin was not identical to that of the pair-feeding regimen was WAT. In this tissue, while chronic intracerebroventricular leptin brought about a decrease in the insulin-stimulated glucose utilization index, the pair-feeding regimen failed to do so.

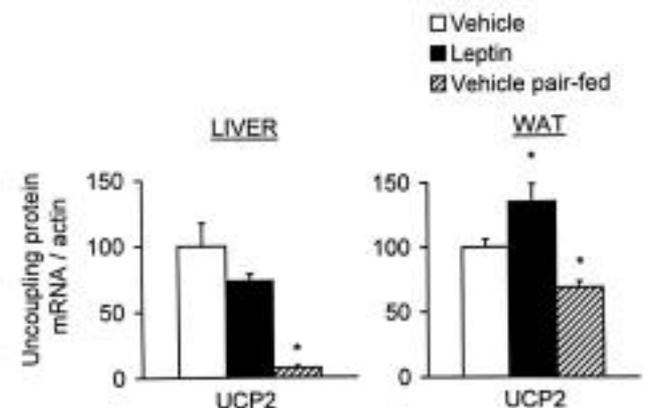


FIG. 5. UCP2 mRNA levels in liver and WAT in intracerebroventricular vehicle-infused control rats fed ad libitum, leptin-infused (12 μ g/day) rats, and vehicle-infused rats pair-fed to the amount of food consumed by the leptin-infused group. Continuous vehicle or leptin infusion over 4 days. Data are means \pm SE of 5 animals per group. * P at least < 0.05 vs. fed ad libitum control rats.

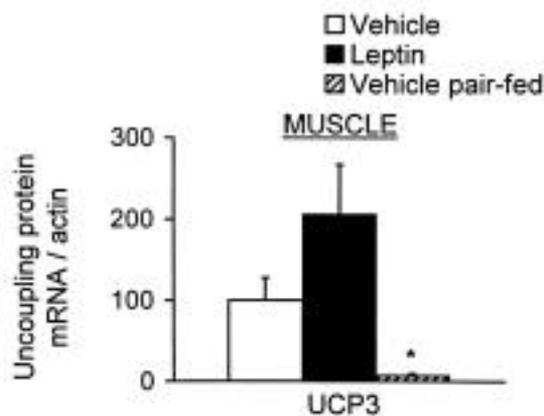


FIG. 6. UCP3 mRNA levels in muscle (RO) in intracerebroventricular vehicle-infused control rats fed ad libitum, leptin-infused (12 μ g/day) rats, and vehicle-infused rats pair-fed to the amount of food consumed by the leptin-infused group. Continuous vehicle or leptin infusion over 4 days. Data are means \pm SE of 5 animals per group. **P* at least <0.05 vs. fed ad libitum control rats.

Although all the changes in glucose handling elicited by the continuous intracerebroventricular infusion of leptin cannot yet be explained mechanistically, it is worthwhile noting that they are almost the exact opposite of those elicited by chronic intracerebroventricular infusion of the orexigenic neuropeptide, neuropeptide Y (23). This substantiates the notion that leptin and neuropeptide Y belong to a tight loop system connecting the hypothalamus and the periphery aimed at regulating glucose handling together with body weight homeostasis.

Despite the apparent analogy between leptin's effects and those of pair-feeding, their respective underlying mechanisms differ. Indeed, the pair-feeding regimen seems to be accompanied by a decrease in sympathetic tone. This is in keeping with the observed marked decrease in BAT UCP1, UCP2, and UCP3 mRNA levels, the decreased liver and WAT UCP2 mRNA levels, and those of muscle UCP3, suggesting the occurrence of a decrease in energy dissipation as heat in the pair-fed group. The decreased expression of UCP3 mRNA expression in skeletal muscle induced by food restriction (50%) has already been reported (27) and is in contrast with the effect of fasting (28) or severe food restriction (29).

In contrast, intracerebroventricular leptin administration is characterized by the maintenance of adequate UCP expression in BAT (UCP1 and UCP2) and liver (UCP2), i.e., a maintenance at levels similar to those measured in fed ad libitum control rats. Intracerebroventricular leptin infusion results in an actual increase in UCP2 expression in WAT, in keeping with data reported by others (30), and in an increase in UCP3 expression in BAT and muscle. This may indicate that leptin brings about a state of fuel depletion while maintaining the activity of thermogenic processes, as reported in mice via the measurement of actual energy expenditure (7). Such leptin-controlled processes (i.e., UCPS status and actual energy expenditure derived thereof) are potentially sympathetic nerve-mediated and occur at the level of the several target tissues mentioned above (31). Thus, although the thermogenic activity favored by intracerebroventricular leptin does not appear to affect the fuel refurbishment of most tissues, as

measured during the euglycemic-hyperinsulinemic clamps, it may be of importance to prevent excessive fuel storage after cessation of leptin administration, while such may not be the case after cessation of the pair-feeding regimen.

In conclusion, it is proposed that 1) the chronic intracerebroventricular leptin-elicited behavioral, hormonal, and metabolic effects are the direct consequences of the presence of this hormone within the central nervous system, presumably acting at the hypothalamic Ob-Rb receptor level; 2) both intracerebroventricular leptin administration and the pair-feeding regimen result in increased insulin-stimulated glucose turnover in all tissues except for WAT, which indicates that intracerebroventricular leptin does not affect insulin-stimulated glucose utilization when its effect on food intake is controlled for; 3) leptin's effects differ from those of a pair-feeding regimen, as it maintains a normal and even a supra-normal expression of UCPS in BAT, liver, WAT, and muscle, while this expression is markedly decreased by the pair-feeding regimen; and 4) the observation that leptin decreases WAT glucose utilization while it produces an increase in UCP2 expression might be in keeping with a role of leptin in favoring lipolysis in this tissue.

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