Involvement of Thyroid Hormones in the Effect of Intracerebroventricular Leptin Infusion on Uncoupling Protein-3 Expression in Rat Muscle

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We have shown previously that continuous (6 days) intracerebroventricular (ICV) leptin infusion in normal rats resulted in decreases in food intake and body weight. A reduction of food intake imposed on control rats (pair-feeding), aimed at mimicking leptin-induced hyperphagia, produced a marked decrease in the expression of muscle uncoupling protein-3 (UCP-3), whereas ICV infusion of leptin prevented such a decrease in UCP-3. To investigate an involvement of thyroid hormones in this effect of leptin, plasma levels of these hormones were determined in ICV leptin-infused, ICV vehicle-infused ad libitum fed or pair-fed controls. ICV leptin infusion and pair-feeding resulted in decreased plasma thyroid-stimulating hormone (TSH) and T4 levels relative to ad libitum fed controls. ICV leptin infusion maintained plasma levels of T3, but the levels were decreased by pair-feeding. The activity of the enzyme (hepatic 5′-monodeiodinase) responsible for T4/T3 conversion was measured. In the leptin-infused group, the activity of 5′-monodeiodinase was maintained at the values measured in ad libitum fed rats; in pair-fed rats, activity was reduced. Thus, conversion of T4 to T3 is decreased by pair-feeding, whereas such is not the case during leptin infusion. To further substantiate an involvement of thyroid hormones in the effect of leptin on muscle UCP-3 expression, hypothyroid rats were ICV infused with leptin or vehicle. It was observed that in hypothyroid rats, ICV leptin was unable to maintain muscle UCP-3 expression at values measured in ad libitum fed controls. These results suggest that central leptin stimulates T3 production via an activation of T4 to T3 conversion, and that this stimulation could be responsible for the effect of leptin on muscle UCP-3 expression. Thyroid hormones could thus be important mediators of the effect of leptin on energy expenditure.
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BAT, brown adipose tissue; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; ICV, intracerebroventricular; TSH, thyroid-stimulating hormone; UCP, uncoupling protein; WAT, white adipose tissue.

Leptin is a hormone released by adipose tissue (1). After leptin binds to long-form hypothalamic receptors, it acts as a satiety factor (2–4). Leptin also plays an important role in increasing energy expenditure (4,5), an effect that could be mediated by uncoupling protein (UCP)-1 (6) and by the newly discovered UCP-2 (7) and UCP-3 (8). UCP-1 is abundantly expressed in rodent brown adipose tissue (BAT) and has been shown to have the property of dissipating energy as heat (6). While also expressed in BAT, UCP-2 is present in other tissues, such as white adipose tissue (WAT), lung, liver, and kidney (7). Its role in the heat dissipation process is still a matter of debate. Due to the presence of UCP-3 in both BAT and skeletal muscles, including human muscles (8), UCP-3 is a potential candidate in the regulation of body weight in large mammals, all the more so now that its role in energy dissipation as heat has recently been documented (9).

We have shown previously that chronic central or intravenous leptin infusion is able to maintain or increase the expression of UCPs in different tissues to values observed in ad libitum fed vehicle-infused controls. In contrast, a reduction of food intake imposed on control rats and aimed at mimicking the effect of leptin on this parameter resulted in massive decreases in the expression of the three UCPs (10,11). Such an effect of leptin is in keeping with data by others reporting increases in the expression of UCPs after chronic leptin administration in rats (12–14) or obese mice (15,16).

Thyroid hormones have also been demonstrated to be major regulators of UCPs. T3 treatment increases UCP-3 mRNA in muscle (16,17–19) as well as UCP-2 mRNA in muscle, BAT, and WAT (18,20,21). In contrast, a state of hypothyroidism induces a decrease in the expression of muscle UCP-3 (16,17).

Finally, it has been shown that leptin may modulate the plasma levels of thyroid hormones; as during fasting, leptin treatment prevents the drop of plasma T3 and T4 levels from occurring (22–24).

In view of the above-mentioned considerations, the purpose of the present study was to determine if thyroid hormones could be one of the mediators of the central effect of leptin on muscle UCP-3.

RESEARCH DESIGN AND METHODS

Animals. Eight- to 9-week-old male Sprague-Dawley rats purchased from IFFA Credo (L'Arbresle, France) were housed in individual cages under con-

ditions of controlled temperature (23°C) and illumination (7:00 A.M.–7.00 P.M.). They were allowed ad libitum access to water and standard laboratory diet (Provimi Lacta SA, Cossonay, Switzerland) unless otherwise stated. Food intake and body weight were measured daily.

Chronic intracerebroventricular infusions. 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 Corporation, Palo Alto, CA) delivering 12.5 µg of leptin per day (recombinant mouse leptin provided by Novartis, Basle, Switzerland) for 6 days or its vehicle (Tris 0.1 mol/l, pH 9) were connected to the intracerebroventricular (ICV) infusion cannula via a polyethylene catheter under ether anesthesia (25). Three groups of rats were investigated: 1 group of rats were ICV infused with leptin; 1 group of control rats were ICV infused with the vehicle and allowed to eat ad libitum; and 1 group of control rats were ICV 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.), and 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.

Chemical thyroidectomy. Rats were treated with methimazole (2-mercapto-1-methyl-imidazole; Fluka Chemie, Buchs, Switzerland) in their drinking water at a dose of 0.2 g/l. Eighteen days later, 1 group of rats was ICV infused with leptin for 6 days, and 2 groups of control rats, ad libitum fed or pair-fed respectively, were ICV infused with the vehicle.

5′-Deiodinase type I activity. Monodeiodinase type I activity was determined in liver homogenates using 1 µmol/l rT3 and 1 mmol/l dithiothreitol with 10 min incubation by measuring the release of radioiodine from [^{125}I]rT3 according to the method of Leonard and Rosenberg (26).

Northern blots. At the end of each experiment, skeletal muscles were removed and total RNA extracted (27). Aliquots of 10 µg were size-fractionated on 1.5% agarose gels. Blots were hybridized (Quikhyb, Stratagene) to random primed labeled cDNAs for UCP-3 (provided by D. Ricquier, Meudon, France; GenBank Accession U92069), β-actin (Clontech Laboratory, Palo Alto, CA) or glyceraldehyde-3-phosphate dehydrogenase (G3PDH) (Clontech Laboratory) (28). Autoradiographs (X-Omat-AR; Kodak, Rochester, NY) were quantified by densitometry with the Image Quant Software (Molecular Dynamics, Sunnyvale, CA). Abundance of UCP-3 mRNA relative to that of β-actin or G3PDH was expressed as a percentage of corresponding ad libitum fed vehicle-infused controls.

Measurements of plasma hormones and metabolites. Thyroid-stimulating hormone (TSH), T3, and T4 levels were measured by radioimmunoassay (DPC Technic, Los Angeles, CA; Immulite 2000, rat TSH application: LKRTS, T3: L2KT32, T4: L2KT42; Laboratoire de Chimie Clinique, Hopital Cantonal, Geneva, Switzerland).

Statistical analysis. Statistical analysis of the data was carried out by 1-way analysis of variance followed by the Turkey procedure for multiple comparisons. The calculations were performed using the Statistica software (Statsoft, Palo Alto, CA). A P value < 0.05 was considered statistically significant.

RESULTS

As expected, chronic (6 days) ICV leptin administration in normal rats resulted in a marked decrease in body weight (Table 1). Leptin-treated rats had a 50% decrease in food intake that was mimicked by the pair-feeding regimen (data not shown).

TABLE 1
Effects of ICV leptin administration on body weight changes in normal and hypothyroid rats

	Body weight changes over 6 days (g)
Ad libitum controls	11.7 ± 2.3
Leptin	–26.7 ± 3.3*
Pair-fed controls	–29.9 ± 1.2*
Hypothyroid leptin	–29.6 ± 1.7*
Hypothyroid pair-fed controls	–29.3 ± 4.4*

Data are means ± SE of 5 or 6 animals per group. Continuous vehicle or leptin infusion (12.5 µg/day) over 6 days. *$P \leq 0.05$ compared with ad libitum fed controls.

In euthyroid rats, both the ICV infusion of leptin and the pair-feeding regimen resulted in decreases in plasma TSH levels compared with values obtained in ad libitum fed controls (Fig. 1), although they failed to reach statistical significance. As further shown by Fig. 1, both the ICV leptin infusion and the pair-feeding regimen produced significant decreases in plasma T4 levels (28 and 44%, respectively; intergroup difference, NS) relative to ad libitum fed controls. In contrast, plasma T3 levels of the leptin-infused rats were maintained at values similar to those of ad libitum fed controls, whereas a 28% decrease in the T3 levels was measured in the pair-fed animals (Fig. 1). These results suggested a difference in the conversion of T4 to T3 between the leptin-infused rats and the pair-fed control group. Therefore, the activity and expression of hepatic 5′-mondeiodinase (type I), the main enzyme responsible for T4/T3 conversion, were measured. As shown by Fig. 2, the deiodinase activity was unaltered, but its mRNA levels were decreased by 50% in the leptin-infused group compared with the ad libitum fed one. Quite different was the situation observed in the pair-fed controls, in which the activity of the enzyme was significantly decreased and its expres-

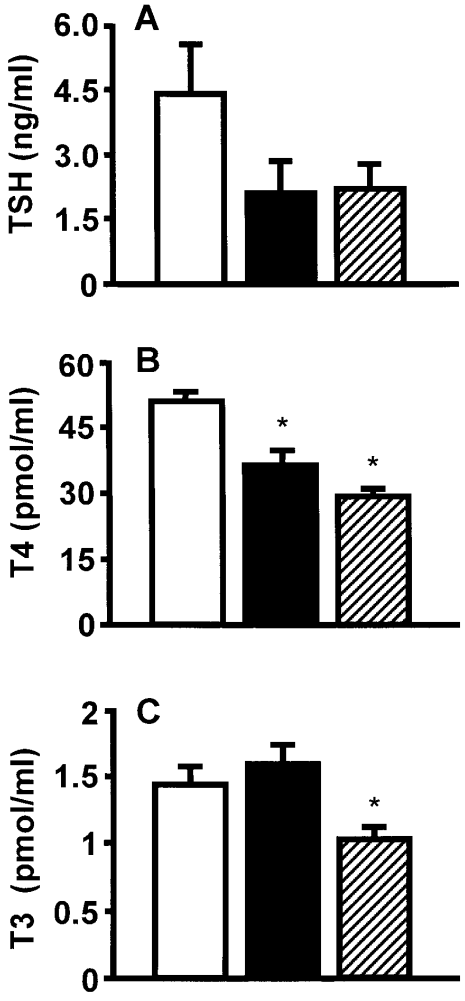

FIG. 1. Plasma TSH (*A*), T4 (*B*), and T3 (*C*) levels in ICV vehicle-infused control rats fed ad libitum (□), ICV leptin-infused rats (■), and ICV vehicle-infused rats pair-fed to the amount of food consumed by the leptin-infused group (▨). Continuous vehicle or leptin infusion (12.5 µg/day) over 6 days. Means ± SE of 5 or 6 animals per group. *$P \leq$ 0.01 vs. ad libitum fed controls.

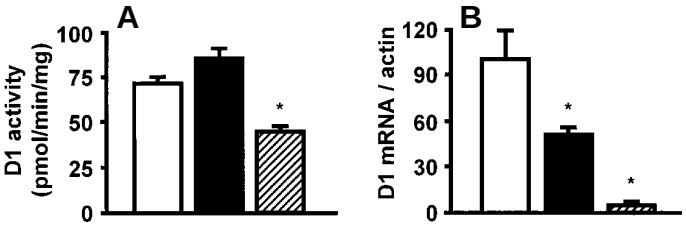

FIG. 2. Monodeiodinase type I (D1) activity (*A*) and mRNA (*B*) levels in liver of ICV vehicle-infused control rats fed ad libitum (□), ICV leptin-infused rats (■), and ICV vehicle-infused rats pair-fed to the amount of food consumed by the leptin-infused group (▨). Continuous vehicle or leptin infusion (12.5 µg/day) over 6 days. Means ± SE of 5 or 6 animals per group. *$P \leq 0.05$ vs. ad libitum fed controls.

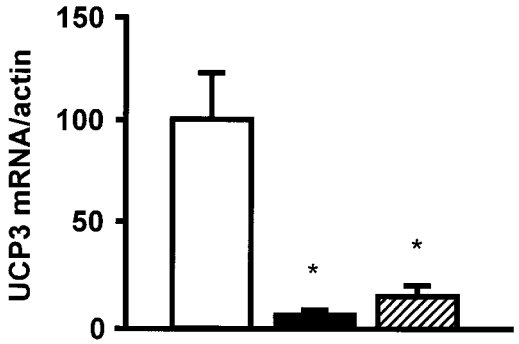

FIG. 3. UCP-3 mRNA levels in ICV vehicle-infused normal rats fed ad libitum (□), hypothyroid ICV leptin-infused rats (■), and hypothyroid ICV vehicle-infused rats pair-fed to the amount of food consumed by the hypothyroid leptin-infused group (▨). Continuous vehicle or leptin infusion (12.5 µg/day) over 6 days. Means ± SE of 5–7 animals per group. *$P \leq 0.01$ vs. ad libitum fed controls.

sion barely detectable (Fig. 2). These results are compatible with the hypothesis that leptin may play a role in maintaining a normal conversion of T4 to T3, a process that is markedly decreased in pair-fed animals by the reduction in food intake mimicking that brought about by leptin.

In earlier experiments, we have shown that leptin infusion to normal rats prevented the decrease in muscle UCP-3 expression that was due to the reduction of food intake elicited by pair-feeding (10). To investigate a possible involvement of thyroid hormones in this effect of leptin, central leptin infusion was carried out in hypothyroid rats, and its effect on muscle UCP-3 expression was compared with that measured in normal ad libitum fed controls and in hypothyroid pair-fed controls. Plasma TSH levels were increased relative to ad libitum fed controls in both groups of hypothyroid rats (147.5 ± 12.2 ng/ml in leptin-infused, 106.7 ± 23.8 ng/ml in pair-fed controls vs. 13.7 ± 2.2 ng/ml in ad libitum fed controls, n = 5–8; P at least < 0.05 vs. ad libitum controls). Plasma T3 levels were barely measurable in hypothyroid rats (0.15 ± 0.1 pmol/ml in leptin-infused and 0.48 ± 0.2 pmol/ml in pair-fed control rats, n = 5–8, NS), and plasma T4 levels were undetectable in these animals (<7 pmol/ml). As depicted in Fig. 3, compared with values of muscle UCP-3 mRNA measured in normal ad libitum fed rats, those obtained after a chronic ICV leptin infusion in hypothyroid rats were extremely low and comparable to the values measured in hypothyroid pair-fed controls. Thus, in hypothyroid animals, leptin fails to maintain the expression of UCP-3 as it does in normal rats. The body weight loss brought about by leptin and by pair-feeding in hypothyroid rats was identical to that measured in normal rats (Table 1).

DISCUSSION

We have shown previously that chronic ICV leptin infusion in normal rats maintained or even increased the expression of UCPs in different tissues—that of UCP-3 in muscle, in particular. This was observed in spite of the presence of a decreased food intake produced by the leptin treatment. Food restriction per se (produced by a pair-feeding regimen to mimic the leptin-induced hypophagia) resulted in a marked decrease in the expression of these proteins (10). Such an effect of leptin is in agreement with other studies reporting that leptin administration for several days leads to increased expression of UCP-2 in WAT (12,14) and of UCP-3 in BAT (12) as well as UCP-1 in normal rats when compared with pair-fed rats (12). It is also in keeping with the observation that in the leptin-deficient obese *ob/ob* mouse, UCP-3 expression in skeletal muscle is stimulated by chronic leptin administration (15,16). Noteworthy is the observation that in skeletal muscle of fasted rats, leptin injections do not alter the expression of UCP-3, probably because the latter is already overexpressed by the fasting condition (13,29). Finally, and in contrast to the chronic effects of leptin on the expression of UCPs, when short-term leptin infusion is carried out in normal mice, it has been shown to result in decreases in muscle UCP-3 and WAT UCP-2 expression (30).

The present study was focused on the effect of leptin on muscle UCP-3. Its regulation could be of importance for energy expenditure in large mammals, including humans (31,32), because UCP-3 is the UCP subtype present in skeletal muscle. More specifically, it was hypothesized that thyroid hormones could play a role as mediators of the central effect of leptin on muscle UCP-3 expression.

It was observed that the reduction of food intake per se (pair-feeding regimen), as well as the ICV leptin-induced hypophagia, resulted in decreases in plasma TSH and T4 levels. In contrast, whereas plasma T3 levels were decreased by pair-feeding, they were maintained at normal levels after the central infusion of leptin. This suggested that centrally administered leptin could bring about an increased conversion of T4 to T3. Such a possibility was supported by the observation that central leptin infusion prevented the drop in activity of hepatic 5′monodeiodinase type I observed in pair-fed rats, maintaining such an activity at a similar level to that measured in ad libitum fed controls. In contrast, 5′monodeiodinase type I mRNA levels were lower in the leptin-infused rats than in the ad libitum fed controls, whereas they were barely detectable in the pair-fed control animals. The difference in the effect of leptin on 5′monodeiodinase type I activity and mRNA suggests the existence of some posttranscriptional regulation. The effect of central leptin on thyroid hormones in normal rats is in keeping with results obtained by others showing that leptin prevents the drop in expression of proTSH in the paraventricular nucleus (23), as well as in plasma T3 and T4 levels measured during fasting, maintaining these parameters at normal values (22–24).

As mentioned previously, thyroid hormones have been shown to influence the expression of UCPs. In particular, UCP-3 expression is markedly decreased in skeletal muscle

in hypothyroid animals (16,17), whereas T3 administration to normal rats produces an increase in muscle UCP-3 expression (17–19). The expression of UCP-2 is also increased by T3 treatment in skeletal muscle, heart, cardiomyocytes, BAT, and WAT (18,20,21,33). Furthermore, administration of an inhibitor of the type II 5′-deiodinase leads to a decreased UCP-1 expression in BAT (34).

These data as well as those of the present study suggest that the maintenance of normal hepatic 5′-monodeiodinase activity and of T3 levels produced by the central administration of leptin (as opposed to the decreases thereof in the pair-fed controls) could be essential for the centrally elicited effects of leptin on muscle UCP-3 expression observed in this and in our previous study (10). This contention is supported by the observation that in hypothyroid rats, the ICV infusion of leptin is not able to maintain or stimulate muscle UCP-3 expression, which remains at a low level similar to that observed in pair-fed rats. Thus, thyroid hormones appear to be necessary for the central action of leptin on muscle UCP-3 expression, although other additional factors could be implicated as well. Among those, the sympathetic nervous system is known to be activated by the presence of leptin in the hypothalamus (35,36), and the effects of leptin on glucose metabolism seem to be dependent on the sympathetic tone (37,38). Also, it has been reported that UCP-3 expression is decreased in BAT when catecholamine synthesis is blocked (39) and is no more responsive to leptin in denervated muscles (30). Free fatty acids represent another candidate that may mediate the central effects of leptin on muscle UCP-3. Indeed, they seem to be important regulators of muscle UCP-3 expression (40,41) as, in fasting, UCP-3 expression in skeletal muscle is increased in correlation with the presence of high plasma free fatty acid levels.

Of note is the observation that leptin is able to decrease body weight in hypothyroid rats as it does in normal animals. The effect of leptin on muscle UCP-3 may therefore be important for preventing the rebound of body weight upon normalization of food intake rather than for the actual leptin-induced body weight loss.

In conclusion, the results of the present study strongly suggest that thyroid hormones are among the important mediators of the central effects of leptin on muscle UCP-3 expression. They also suggest that the effects of leptin on energy expenditure may depend on thyroid hormones.

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