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Association between diencephalic thyroliberin and arterial blood pressure in agouti-yellow and ob/ob mice may be mediated by leptin

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Abstract

Leptin, a hormone secreted by the adipose tissue, stimulates anorexigenic peptides and also inhibits orexigenic peptides in hypothalamic arcuate nuclei-located neurons. It also counteracts the starvation-induced suppression of thyroid hormones by up-regulating the expression of preproTRH gene. On the other hand, in addition to its role as a modulator of the thyroid-hypothalamic-hypophysial axis, thyrotropin-releasing hormone (TRH) acts as a modulator of the cardiovascular system. In fact, we reported that overexpression of diencephalic TRH (dTRH) induces hypertension. We have recently shown that, in rats with obesity-induced hypertension, hyperleptinemia may produce an increase of dTRH together with an elevation of arterial blood pressure (ABP) through an increase of sympathetic activity and that these alterations were reversed by antisense oligonucleotide and small interfering RNA against *preproTRH* treatments. Here we explore the possible role of dTRH as a mediator involved in leptin-induced hypertension in 2 obesity mouse models: agouti-yellow mice, which are hyperleptinemic and hypertensive, and ob/ob mice, which lack functional circulating leptin. These 2 models share some characteristics, but ob/ob mice show lower ABP and plasma catecholamines levels. Then, for the first time, we report that there is a clear association between ABP and dTRH levels in both mouse models, as we have found that dTRH content was elevated in agouti-yellow mice and diminished in ob/ob mice compared with their controls. We also show that, after 3 days of subcutaneous leptin injections (10 μg/12 hours), ABP and dTRH increased significantly in ob/ob mice with no alterations of thyroid hormone levels. These results add evidence to the putative molecular mechanisms for the strong association between obesity and hypertension.

1. Introduction

The leptin hormone is mainly produced and secreted by adipose tissue [1]. As a consequence, plasma leptin levels are directly proportional to body fat content [2,3]. This hormone acts in 2 ways: (1) stimulating neurons from hypothalamic arcuate nuclei that express and release anorexigenic peptides such as α-melanocyte–stimulating hormone (α-MSH) and cocaine- and amphetamine-regulated transcript peptide and (2) inhibiting other neurons that coexpress the orexigenic peptides neuropeptide Y and agouti-yellow–related protein (AgRP) [4-6]. AgRP acts as an antagonist of α-MSH for the binding to 3 of the 5 types of the α-MSH receptors: MC1R in the skin and MC3R and/or MC4R in the central nervous system [7]. A high leptin level reduces AgRP and increases α-MSH release. As a result, leptin causes a decrease in energy intake and an increase in energy expenditure [8,9].

As reported by Ahima et al [10], leptin also counteracts the starvation-induced suppression of thyroid hormones by up-regulating the expression of the thyrotropin-releasing hormone precursor gene (*preproTRH*). In other words, leptin can regulate the expression of thyrotropin-releasing hormone (TRH) directly or indirectly by increasing the production of α-MSH [11-14] and enzymes that are necessary for the processing of proTRH in TRH [12,15].

Apart from the classic role of TRH in the thyroid-hypothalamic-hypophysial axis, it acts as a neuropeptide modulating cardiovascular function among several other physiological functions [16]. We have previously reported that overexpression of diencephalic TRH (dTRH) induces hypertension in the normal rat [17]. Furthermore, in rats with obesity-induced hypertension, high levels of leptin produce an increase in dTRH along with an elevation in systolic arterial blood pressure (SABP) through an increase of

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sympathetic activity, an alteration that can be reversed by intracerebroventricular antisense or interfering RNA treatments [18].

Agouti-yellow mice characterized by an agouti protein overexpression are hyperleptinemic but obese, a consequence of a selective leptin resistance with loss of leptin-reducing appetite and weight responses but preservation of the leptin-induced sympathetic activation [19], which is a fact that seems to explain the elevation of ABP in this model [20].

On the other hand, ob/ob mice lack functional circulating leptin. Thus, they resemble some but not all of the characteristics of agouti-yellow mice. For instance, obesity in ob/ob mice is not associated with hypertension but with lower blood pressure levels owing to low levels of plasma catecholamines [21]. As expected, leptin treatment reversed the ob/ob phenotype, producing a decrease in body weight and food intake [21].

Bearing this background in mind, we propose that cardiovascular leptin actions are, in part, mediated by the dTRH system. We therefore studied the expression of dTRH and its possible association with ABP values in 2 obesity models: agouti-yellow mice and ob/ob mice. We report here for the first time that there is a clear association between ABP and dTRH levels in both mouse models and show that leptin can increase both blood pressure and dTRH in ob/ob mice. These results add evidence to the putative molecular mechanisms for the strong association between obesity and hypertension.

2. Materials and methods

2.1. Animals

We studied 12-week-old male agouti-yellow (C57BL/6J-A^y), *Ob/Ob* (C57BL/6J-ob/ob), and their wild-type control (C57BL/6J) mice from The Jackson Laboratory (Bar Harbor, ME) (n = 4 for each group). They were housed individually in environmentally controlled conditions (temperature, 20°C ± 2°C; light/dark cycle of 12/12 hours) and had access to food (standard laboratory diet) and water ad libitum.

In some experiments, control and ob/ob mice (5 per group) were treated with a subcutaneous injection of either 10 μg recombinant mouse leptin (Sigma-Aldrich, St Louis, MO) dissolved in phosphate-buffered saline (100 μL) or vehicle twice a day for 3 days. Arterial blood pressure was measured every 24 hours for at least a week before the treatment (basal) and during the experiment as described below. At the end of the experiments, the animals were killed and the brains were removed for dTRH determination.

2.2. Arterial blood pressure measurements

Systolic ABP was measured in awake animals by a tail-cuff plethysmographic method for 5 consecutive days after a period of at least 7 days of animal adaptation to the procedure.

2.3. Determination of dTRH and plasma leptin, T_3, and T_4 contents

The animals were killed by decapitation, their brains were rapidly removed, and the diencephalic region of each animal was dissected. Diencephalic TRH content was measured using a previously published radioimmunoassay method [22].

Blood samples were collected in tubes containing sodium EDTA and centrifuged. Plasma was immediately frozen. Leptin levels were measured using an enzyme-linked immunoassay kit (Assay Designs, Ann Arbor, MI), and T3 and T4 concentrations were measured using electrochemiluminescence immunoassay (kits 2010 and 1010, Roche, Buenos Aires, Argentina).

2.4. Statistical analysis

Results are expressed as mean ± SD from independent experiments. Statistical significance between means was determined by 2-way analysis of variance with repeated measures on one factor when variables were measured for several consecutive days. Spearman rank order correlation coefficient was used to analyze correlations between variables.

3. Results

As expected, the body weight of both obese strains was significantly greater than that of wild-type controls (Fig. 1,

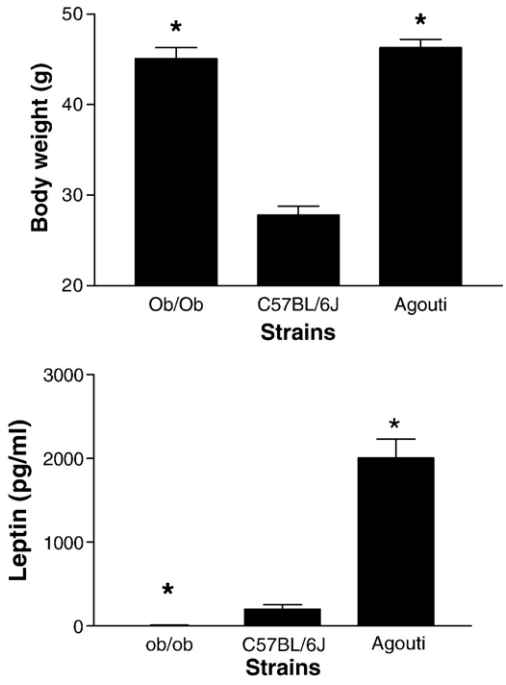

Fig. 1. The upper panel shows body weight of control, ob/ob, and agouti-yellow mice. The lower panel confirms that plasma leptin content in all strains used was as expected: agouti-yellow mice had much higher plasma leptin than controls, and ob/ob showed no detectable level. Results are expressed as mean ± SD. * $P < .001$ vs control group.

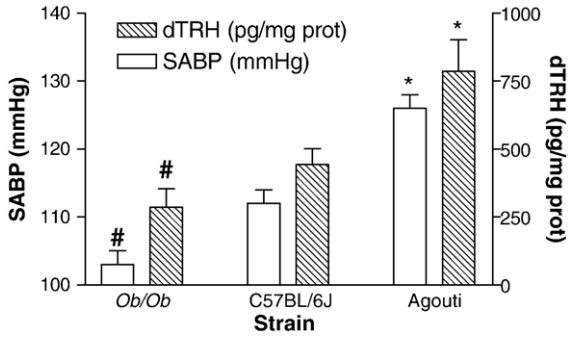

Fig. 2. Diencephalic TRH content (right Y axis) and SABP (left Y axis). Results are expressed as mean ± SD. # $P < .05$ vs control and agouti-yellow mice and * $P < .001$ vs control and ob/ob mice.

upper panel). Consistent with their well-known hyperleptinemic state, agouti-yellow mice had a 10-fold increase in plasma leptin level with respect to control animals. In contrast, ob/ob mice had no detectable leptin levels owing to the null mutation that they carry in the leptin (*Lep*, formerly *ob*) gene (Fig. 1, lower panel).

As hypothesized, ob/ob mice showed significantly lower SABP and dTRH levels than the control group; in contrast, obese hyperleptinemic agouti-yellow mice had significantly higher SABP and dTRH than the control and ob/ob groups (Fig. 2). As shown in Fig. 3, the whole experiment showed a highly significant correlation between dTRH content and SABP (Spearman R: 0.89, n = 12, $P < .001$). Despite the differences in dTRH content, ob/ob, agouti-yellow, and control mice had similar levels of plasma T3 and T4 (Table 1).

To further explore whether leptin deficiency is the cause of the decreased levels of both ABP and dTRH levels in the ob/ob mice, they were treated with subcutaneous recombinant mouse leptin. The administration of leptin (10 μg/12 hours) to ob/ob mice and their C57BL/6J control strain for 3 days produced a significant decrease in food intake at 48 and 72 hours after treatment ($P < .001$ vs basal and 24 hours) (Fig. 4, upper panel) along with a significant reduction of body weight in ob/ob at 72 hours

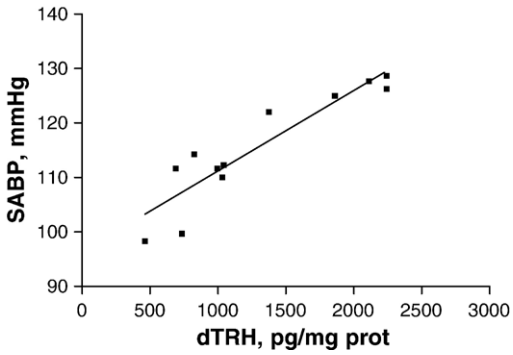

Fig. 3. Correlation (Spearman R: 0.89, n = 12, $P < .001$) between SABP (in millimeters of mercury) and dTRH (in picograms per milligram protein) in the whole set of animals.

Table 1
Plasma T3 and T4 levels in obese ob/ob and agouti-yellow vs control lean (C57BL/6J) mice

Strain	T_4 (ng/mL)	T_3 (μg/dL)
ob/ob	4.32 ± 1.02	0.94 ± 0.20
C57BL/6J	4.90 ± 0.69	1.16 ± 0.20
Agouti-yellow	4.49 ± 0.76	1.09 ± 0.14

Results are expressed as mean ± SD.

after treatment ($P < .04$ vs basal weight) (Fig. 4, lower panel), in accordance with results from other groups [21].

Leptin treatment significantly increased SABP 24 to 72 hours after injection in ob/ob mice ($P < .04$ vs ob/ob saline group) (Fig. 5 upper panel). Finally, dTRH content (Fig. 5, lower panel), lower in ob/ob control mice under basal condition ($P < .05$ vs C57BL/6J), was normalized by leptin treatment and reached the same values as those of lean control mice after 72 hours of treatment ($P < .03$ vs ob/ob saline group). No changes in SABP or dTRH were found in lean control mice treated with leptin compared with those treated with saline.

4. Discussion

By using 2 different strains of obese mice, we show evidence of the relation between plasma leptin levels, dTRH content, and blood pressure levels. First, ob/ob mice lack

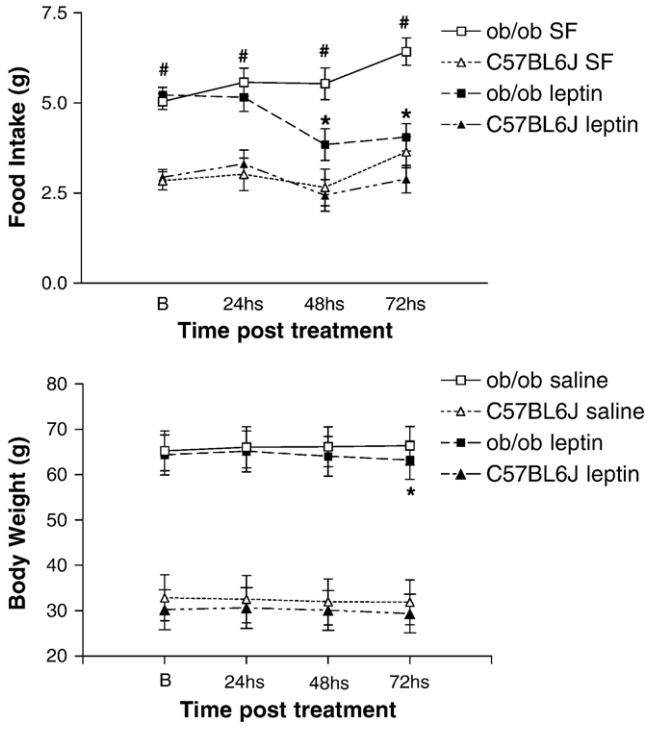

Fig. 4. Effect of leptin or phosphate-buffered saline (SF) injections on food intake (upper panel) and body weight (lower panel) of ob/ob mice and their lean control mice (C57BL/6J). Results are expressed as mean ± SD. * $P < .04$ vs basal in the same strain (B) and # $P < .001$ vs C57BL/6J saline.

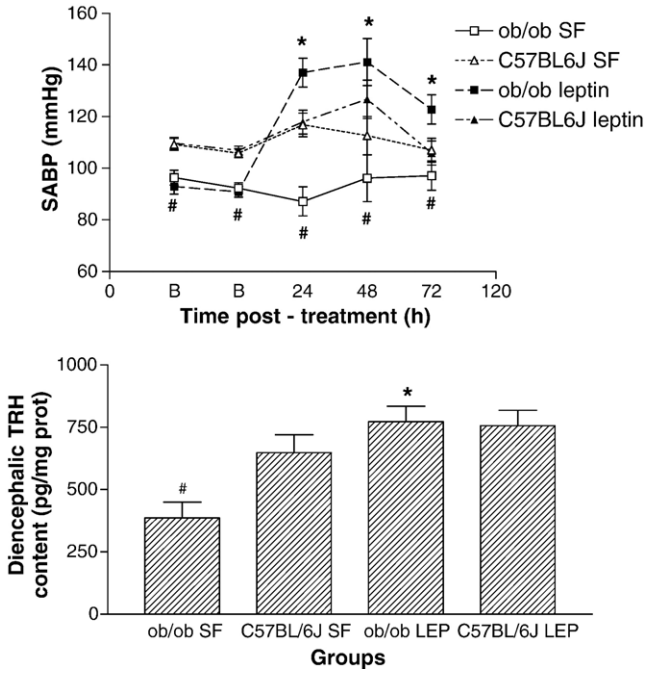

Fig. 5. Effects of leptin (LEP) or phosphate-buffered saline (SF) injections on SABP at 24, 48, and 72 hours (upper panel) and dTRH content at the end of the experiment in ob/ob mice and their lean control (C57BL/6J). Results are expressed as mean ± SD. * $P < .04$ vs ob/ob saline and # $P < .05$ vs C57BL/6J saline.

functional leptin and show low levels of ABP and dTRH content. On the other hand, hyperleptinemic and hypertensive agouti-yellow mice present high dTRH content. These results show a highly significant correlation between dTRH content and SABP in all the animals studied, suggesting that dTRH may be mediating leptin cardiovascular effects in mice. In fact, the increased blood pressure and dTRH levels observed in agouti-yellow mice is consistent with our previous observations showing that these characteristics can be reverted by intracerebroventricular treatment with antisense or small interfering RNA against preproTRH [23].

To further explore whether leptin deficiency in ob/ob mice is the cause of the decreased levels of both blood pressure and dTRH levels, mice were treated with subcutaneous recombinant mouse leptin. After 48 hours, the ob/ob mice treated with exogenous leptin showed all the expected hormone-induced effects such as loss of appetite and weight as previously reported [21]. Besides, and in accordance with our hypothesis, leptin increased both the low dTRH content and the blood pressure levels found in the ob/ob strain in basal condition. No effects were seen in leptin-treated control mice, indicating that the high leptin levels for 3 days are not able to induce anorexigenic leptin effects in normoleptinemic mice. These results were similar to the ones published by Harris et al [21].

Basing our conclusions on the knowledge that cardiovascular effects of dTRH are mediated by the sympathetic system [24,25], we propose that the leptin-induced sympathetic tone may be mediated by a dTRH increase. In fact, these results are in agreement with those that we found in an obese rat model induced by a high-fat diet in which the elevation of body weight is accompanied by hyperleptinemia, dTRH elevation, and hypertension with elevation of the sympathetic tone. All these features can be reversed by antisense or small interfering RNA against *preproTRH* [26].

Despite the differences in dTRH content found in ob/ob and agouti-yellow mice, there were no changes in the thyroid status. Although a plausible explanation could be that there are different TRH pools in the hypothalamic region involved in nonhypophysiotropic actions, as recently reported by other authors [13,27], more experiments are necessary to solve this issue. Nevertheless, these findings are in agreement with reported work in rats from our laboratory and others showing that variations in the hypothalamic TRH are not always accompanied by changes in thyroid status [13,28-30].

It is to be noted that another possible explanation for our results may be that leptin administration is known to affect locomotor activity levels, a fact that could be expected to have an independent effect on body weight and ABP [31].

In summary, our data support the view that TRH may mediate some of the effects of leptin not only on energy intake and energy expenditure but also on ABP regulation. This mechanism may be important to explain the prevalent association between obesity and hypertension, a heavy burden on public health.

Acknowledgment

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