Thyrotropin-Releasing Hormone Decreases Leptin and Mediates the Leptin-Induced Pressor Effect

Silvia I. García, María S. Landa, Patricia I. Porto, Azucena L. Alvarez, Mariano Schuman, Samuel Finkielman, Carlos J. Pirola

Abstract—Leptin, an adipocyte-released hormone, modifies food intake and energy expenditure regulating hypothalamic-pituitary-thyroid axis function. We previously reported that thyrotropin-releasing hormone (TRH) precursor gene overexpression induces hypertension in the normal rat and that spontaneously hypertensive rats have central TRH hyperactivity with increased TRH synthesis and release and an elevated TRH receptor number. In both models, intracerebroventricular antisense (AS) treatment against the TRH precursor produced a dose-dependent reduction of the increased diencephalic TRH content while normalizing high arterial blood pressure. In this article, we report that male Wistar rats that were made hypertensive by intracerebroventricular injection of a eucaryotic expression plasmid containing the pre-TRH cDNA showed decreased leptin plasma levels and that pre-TRH AS treatment reversed this phenomenon. In addition, male and female spontaneously hypertensive rats showed lower levels of circulating leptin than did sex-matched Wistar-Kyoto control rats. This difference also was abated by the pre-TRH AS treatment. Conversely, 20 μg ICV leptin induced a long-lasting pressor effect (18±5 mm Hg, n=6, P<0.01, >60 minutes) that was not observed in pre-TRH AS pretreated rats (2±3 mm Hg, n=6) but persisted in rats used as controls that were treated with inverted oligonucleotide (20±6 mm Hg, n=4, P<0.01). These data suggest that in rats with TRH-induced hypertension, leptin is decreased, inducing compensatory adiposity. We propose that because leptin produces central TRH synthesis and release, obesity may induce hypertension through TRH system activation and that the TRH-leptin interaction may thus contribute to the strong association between hypertension and obesity. (Hypertension. 2002; 39[part 2]:491-495.)

Key Words: antisense elements ■ blood pressure ■ hypertension, obesity ■ hormones ■ rats, spontaneously hypertensive

Obesity is a major risk factor for essential hypertension. Conversely, hypertensive patients tend to be more obese than do normotensive people.1,2 Weight reduction is an effective way to decrease arterial blood pressure (ABP) in obese hypertensive patients, suggesting an important association between weight and ABP homeostasis.3 The cumulative body of evidence also suggests that obesity-induced hypertension may be due to an increased sympathetic outflow, among other factors.4 The mechanisms of this association are poorly understood, however.

Leptin is an adipocyte-derived hormone that is involved in the regulation of food intake and body weight, with the hypothalamus being a primary target of its action.5 In addition, leptin effects include an increase in overall sympathetic activity.6 As Ahima et al.7 reported, leptin also counteracts the starvation-induced suppression of thyroid hormone, apparently by upregulating the expression of the thyrotropin-releasing hormone (TRH; pyro-Glu-His-Proamide) precursor gene.

Besides its endocrine function, TRH also serves as a neurotransmitter in the central nervous system, and its presence in brain nuclei involved in cardiovascular regulation, such as in the periventricular region and the preoptic area, suggests that this tripeptide may modulate cardiovascular function.8 In fact, several groups have reported that brain microinjections of TRH produce sympathetically mediated dose-dependent pressor effects.9

Recently, we reported that the overexpression of the TRH precursor in areas surrounded by the third ventricle of the central nervous system in normal rats induces a long-lasting elevation of ABP as well as an increase in diencephalic TRH content in a dose-dependent manner.10 These effects were specifically reversed by a pre-TRH antisense (AS) treatment, indicating that the extrahypothalamic TRH system effectively participates in cardiovascular regulation in the rat. Accordingly, we demonstrated that spontaneously hypertensive rats (SHRs) present with an increase in both pre- and postsynaptic TRH system activities.11 These findings indicate that in

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addition to the regulation of the cardiovascular system in the normal rat, extrahypothalamic TRH may participate in the pathogenesis of hypertension in this genetic model. Subsequently, we found that a single intracerebroventricular (ICV) AS infusion dose-dependently decreases both the elevated dienecephalic TRH content and the ABP only in the SHR strain, independently of any change in thyroid status.12

In this study, we measured plasma leptin levels in male Wistar rats made hypertensive by periventricular TRH overproduction induced by ICV injection of the eucaryotic expression plasmid containing the pre-TRH cDNA (pCMV-TRH). We show that pCMV-TRH decreased leptin plasma levels and that pre-TRH AS treatment reversed this effect, whereas AS oligodeoxynucleotide with an inverted sequence (ODNinv) did not. Both male and female SHRs displayed lower levels of circulating leptin than did sex- and age-matched WKY control rats. This difference was abated by the pre-TRH AS treatment. Conversely, in Wistar-Kyoto (WKY) rats, ICV leptin induced a long-lasting pressor effect that was not observed in pre-TRH AS-pretreated rats, but it was still present in ODNinv-treated animals.

These data indicate that leptin is decreased in TRH-induced hypertension, which may cause compensatory adiposity. We propose that as leptin increases central TRH synthesis and release, obesity may increase ABP through TRH system activation, and that the TRH-leptin interaction thus may contribute to the strong association between hypertension and obesity.

Methods

Animals

Adult male Wistar rats (age, 16 weeks old; weight, 250 to 300 g), male and female SHRs of the Okamoto-Aoki strain and sex- and age-matched WKY control rats were housed in a room maintained at a control temperature of 23 ± 1 °C on a 12-hour light-dark schedule. Food and water were administered ad libitum. Animal experimentation protocols were approved by our Institutional Animal Care and Use Committee.

During ICV infusions, the rats were anesthetized with pentobarbital 45 mg/kg. A 25-gauge stainless steel cannula was directed to the third ventricle through a burr hole in the skull for injection. Coordinates were 1.3 mm posterior to the bregma on the midline and 4.5 mm below the dura. At the end of each experiment, the position of the cannula was assessed by histological examination. All substances were dissolved in phosphate-buffered saline, and a total infusion volume of 5 μl (1 μl/min) was used. Control rats received vehicle only.

Three groups of male Wistar rats were treated intracerebroventricularly with vehicle, AS, or ODNinv, respectively. Simultaneously, they were transfected with a eucaryotic expression vector (pCMV-TRH) containing the human CMV gene promoter, the bovine growth hormone polyadenylation signal, and 1322 bp pre-TRH cDNA, as described previously.10 SHRs and WKY rats received a single injection of vehicle, AS, or ODNinv. After 24 hours, we measured ABP by a tail-cuff method, then the animals were killed by decapitation, their brains were removed rapidly for diencephalic TRH determination, and blood samples were collected from the tail for plasma leptin determination at the end of each experimental period.

Acute ICV Leptin Infusion

Male rats were anesthetized with pentobarbital (45 mg/kg), and mean ABP (MABP) was recorded throughout the experiment by a polyethylene cannula previously inserted into the left carotid artery connected to a Statham transducer (Gulton-Statham Products) coupled with a polygraph (Grass Instrument Co). The analog signal was converted to a digital recording by a standard integration system installed in a personal computer. Data are expressed as mean ± SD of 1 determination/s during a 10-minute period. Basal values correspond to MABP readings obtained 30 minutes before ICV injections. After a basal period, each rat received a first ICV infusion with AS, ODNinv, or vehicle, and after 1 hour, a second ICV infusion of either 20 μg leptin (Sigma Co) or vehicle was administered. MABP was recorded during an additional period of 60 minutes, then the animals were killed by decapitation. Brain tissue was rapidly removed for diencephalic TRH determination, and blood samples were collected from the tail for plasma leptin determination at the end of each experimental period.

Assay of Plasma Leptin

Tail blood samples were collected with sodium EDTA, and plasma leptin levels were measured using an enzyme-linked immunosassay kit (Assay Designs, Inc).

Diencephalic TRH Content Determination by Radioimmunoassay

The diencephalic region of each animal was dissected rapidly with the aid of a stereotaxic atlas. TRH content determination was performed by a previously published method.13

Statistical Analysis

Results are expressed as mean ± SD from independent experiments. Statistical significance between means for the effects of treatment on MABP was determined by two-way ANOVA with repeated measurements on one factor. Where pairwise comparisons were made after ANOVA, the Tukey test for individual differences was used.

Results

In this study, we confirmed our previous observations that ICV injection of a eucaryotic expression plasmid containing the pre-TRH cDNA (pCMV-TRH) induced the expected increase in diencephalic TRH content along with a hypertensive state, with both effects being blocked by a pre-TRH AS treatment (data not shown). In this condition, after 24 to 48 hours, 100 μg pCMV-TRH also produced a significant decrease in leptin plasma levels (Table). This effect was reversed by pre-TRH AS but not by vehicle or ODNinv, which were used as controls (Table).

Because SHRs have increased central TRH system activity in basal conditions, we investigated the circulating leptin levels in adult male and female SHRs and compared them with sex- and age-matched WKY control rats. We observed that in both strains, males presented significantly higher basal levels of plasma leptin than did females (Table). At any rate, SHRs showed significantly lower basal levels of circulating leptin than did WKY rats, regardless of sex (Table). This difference was suppressed by pre-TRH AS but not by vehicle
Plasma Leptin Concentrations in Control and pCMV-TRH-treated Wistar Rats and Female and Male SHRs and WKY Normotensive Rats: Effect of Pre-TRH AS and Pre-TRH Inverted AS (ODN<sub>inv</sub>)

<table>
<thead>
<tr>
<th>Group</th>
<th>Vehicle</th>
<th>Pre-TRH AS</th>
<th>ODN&lt;sub&gt;inv&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar control</td>
<td>9.4±1.5</td>
<td>9.1±2.0</td>
<td>7.9±2.1</td>
</tr>
<tr>
<td>Wistar pCMV-TRH</td>
<td>5.3±1.3</td>
<td>9.0±1.2</td>
<td>4.8±1.4*</td>
</tr>
<tr>
<td>Female WKY</td>
<td>7.2±1.2</td>
<td>8.2±1.3</td>
<td>7.5±2.2</td>
</tr>
<tr>
<td>Male WKY</td>
<td>12.0±1.4</td>
<td>10.8±1.8</td>
<td>11.6±2.0</td>
</tr>
<tr>
<td>Female SHR</td>
<td>4.8±0.9</td>
<td>8.9±1.5</td>
<td>5.1±1.8</td>
</tr>
<tr>
<td>Male SHR</td>
<td>8.7±0.9</td>
<td>13.7±1.6</td>
<td>9.4±1.4</td>
</tr>
</tbody>
</table>

*Results are expressed as mean±SD, n=6, ANOVA. pCMV-TRH, plasmid vector that encodes pre-TRH under cytomegalovirus promoter; WKY, Wistar-Kyoto; SHR, spontaneously hypertensive rats; TRH, thyrotropin-releasing hormone; AS, antisense; ODN<sub>inv</sub>, oligodeoxynucleotide.

P<0.05 vs Wistar control rats.
#P<0.05 vs females.
†P<0.05 vs sex-matched rats.
‡P<0.05 vs vehicle.

or by ODN<sub>inv</sub> (Table). These results suggest that increased diencephalic TRH may downregulate plasma leptin levels.

Because leptin produces central TRH synthesis and release, the question arises whether TRH mediates the pressor effect of ICV leptin. To answer this question, we infused 20 μg leptin in 5 μl of saline ICV for 5 minutes in anesthetized Wistar rats. We found that in comparison with saline alone, which lacked any effect, ICV leptin induced a significant, long-lasting (>60 minutes) increase in ABP (18±5 mm Hg, n=6, P<0.01) that was not observed after 60 minutes of pretreatment with a pre-TRH AS (2±3 mm Hg, n=6) but persisted in rats treated for the same length of time with ODN<sub>inv</sub> used as a control (20±6 mm Hg, n=4, P<0.01) (Figure). No differences were observed in the ABP of control Wistar rats infused intracerebroventricularly with saline in place of leptin among AS, ODN<sub>inv</sub>, and vehicle pretreatments throughout the experiments (data not shown). As expected, after the animals were killed after 60 minutes of leptin infusion, a significant twofold increase in diencephalic TRH content was observed in control and ODN<sub>inv</sub>-pretreated animals (Figure). To the contrary, the effect of leptin on diencephalic TRH content was not observed when TRH AS was infused intracerebroventricularly 60 minutes before ICV leptin (Figure).

**Discussion**

Obesity is the commonest nutritional disorder in Western societies, making it an important public health problem because of its association with hypertension and other conditions. Adipose tissue plays an important role in energy regulation via hormonal signals acting at multiple sites to control food intake and energy expenditure. Using this model, we were able to show that a specific pre-TRH AS ODN made resistant to nuclease by phosphorothioation blocked the increase in both the diencephalic TRH content and ABP for up to 72 hours. Next, we were able to show that pCMV-TRH reduced by 45% the basal leptin plasma levels. Furthermore, a single injection of a pre-TRH AS ODN 24 hours before the experiment reversed this effect. The observed actions of the TRH AS ODN are sequence-specific and seem not to be due to a nonspecific toxicity, because treatment with ODN<sub>inv</sub> with an identical percentage base composition showed no effects.

In physiological studies, intravenous or ICV injections of TRH were found to increase ABP. This effect was blocked by the destruction of the sympathetic system, indicating that the pressor effect could be mediated by catecholamines involving the modulations of diverse neurotransmitter system activities. Leptin gene expression and secretion are nutritionally as well as hormonally regulated; they are increased by overfeeding, high-fat diet, insulin, and glucocorticoids, and they are decreased by fasting and catecholamines.

Therefore, it is tempting to speculate with regard to whether a reciprocal interaction exists between periventricular TRH and leptin levels mediated by the sympathetic outflow. To further test this hypothesis, we evaluated circulating leptin levels in the SHRs. In 1995, we reported for the first time that SHRs show significant hyperactivity in the extrahypothalamic TRH system, with a twofold increase in the preoptic area TRH content and TRH receptor number as well as significantly increased TRH concentration in the cerebrospinal fluid. These findings indicate that SHRs develop increases in TRH synthesis and release in the central nervous system. A significant hyperactive TRH system was also apparent at the prehypertensive stage in these rats, indicating that alterations in this system may participate in both the development and the maintenance of hypertension in SHRs.

In this study, male and female SHRs showed lower levels of circulating leptin than their sex-matched WKY controls in basal conditions, a finding that is in line with the evidence...
indicating that SHR s have a higher sympathetic tone.\textsuperscript{19} Although the increase in leptin levels is a well-characterized phenomenon in Koletsky SHR s carrying a leptin receptor mutation,\textsuperscript{20} we found no reports in the literature of this leptin decrease in the SHR strain with respect to the WKY strain. In addition, we observed that females have lower leptin levels than males in either strain, which can be explained by females’ lower body weight. In any case, because we were able to demonstrate the effectiveness of the AS ODN treatment in decreasing the elevated diencephalic content and ABP in the SHRs for up to 72 hours, we measured leptin levels after 24 hours of ICV TRH AS treatment. Regardless of sex, the decrease in plasma leptin in SHRs as compared with that in WKY rats was abated by the pre-TRH AS but not by the ODN\textsuperscript{18} used as a control.

These results, taken together, suggest that increased periventricular TRH activity induced hypertension and decreased plasma leptin levels. In fact, Komorowski et al\textsuperscript{21} reported that acute TRH administration reduced plasma leptin levels in lean as well as obese patients.

That spontaneous mutations in the leptin receptor gene in \textit{db/db} mice and \textit{fa/fa} rats produce defective leptin receptors and lead to severe obesity could imply that diminished leptin levels in the TRH-induced hypertensive state may cause an increase in food intake and a decrease in leptin-mediated energy expenditure, hence producing a compensatory increase in adipose tissue. The effects of leptin on food intake and body weight balance are mediated, at least in part, however, by neuropeptides such as neuropeptide Y, agouti-related peptide, melanin-concentrating hormone, proopiomelanocortin, cocaine- and amphetamine-regulated transcript, and \(\alpha\)-melanocyte-stimulating hormone, among others. In this sense, the effect of leptin on metabolic rate seems to be mediated by thyroid status through TRH gene activation. In addition, although obesity-related hypertension may be secondary to insulin resistance and/or hyperinsulinemia, the enhanced sympathetic outflow induced by leptin may also play a main pathophysiological role in this form of hypertension.

Therefore, we explored whether the hypertensive effect of the acute intracerebroventricularly administered leptin may be mediated by the periventricular TRH system. In Wistar rats, as little as 20 \(\mu\)g ICV leptin induced a long-lasting pressor effect with no change in heart rate (data not shown). These results support the finding that acute and chronic leptin treatments can increase ABP in conscious rats and in \textit{ob/ob} mice.\textsuperscript{6} At any rate, the pressor effect was not observed in vehicle-infused rats, demonstrating that the ABP elevation was not due to the infused volume. Interestingly, the pressor action of leptin occurred in the presence of a significant twofold increase in the diencephalic TRH content.

Our results are in agreement with those reported by Harris et al\textsuperscript{22} indicating that leptin upregulates TRH gene expression by acting on its promoter, through the activation of either a cAMP response element or a Stat-response element. Even though the pre-TRH AS was applied 1 hour before leptin infusion, the AS treatment reversed the effects of leptin on diencephalic TRH content and ABP. The effect of TRH AS on leptin-induced pressor action might be specific for two reasons: Leptin actions were still present in ODN\textsuperscript{18}-treated animals, and another potent central hypertensive agent such as angiotensin II (1 \(\mu\)g) produced a similar pressor response in vehicle and TRH AS-treated animals (data not shown).

These data may indicate that a short period of time such as 1 hour is enough to inhibit leptin-induced TRH gene activation. This rapid onset suggests a high turnover of the periventricular TRH in ICV leptin-treated rats. The effect of ICV leptin seems to be centrally mediated because no elevation in circulating leptin was apparent (data not shown).

Although our experiments were not focused on the TRH activity of the hypothalamic-pituitary-thyroid axis, another possible site of action of AS ODN against TRH is the hypothalamus, where alterations in the TRH synthesis might affect thyroid function, thereby indirectly influencing cardiovascular function. Our previous findings, however, indicate that the TRH AS-induced hypotensive effect does not seem to be explained by changes in thyroid status in either normal rats with TRH overproduction induced by pCMV-TRH transfection or in SHRs.

Additional studies are necessary to delineate the complex interactions that may take place with regard to the effect of ICV leptin on cardiovascular regulation. The TRH-dependent cardiovascular effects of ICV leptin might be mediated somewhat by the sympathetic system, however, because TRH injections produce an increase in plasma catecholamine levels, and adrenalectomy avoids its hypertensive effects.\textsuperscript{16}

Because TRH is a potent prolactin releaser,\textsuperscript{23} it can be hypothesized that AS treatment against TRH may decrease ABP by affecting prolactin levels. We cannot reject that possibility, because we have not measured prolactin; however, this hypothesis seems unlikely, because prolactin does not alter ABP directly and may require a week to potentiate the pressor effect of norepinephrine.\textsuperscript{24} In any case, the participation of prolactin in the inhibitory effects of TRH AS on leptin pressor action remains to be explored, because prolactin may have a major role in determining the deposition and mobilization of fat.\textsuperscript{25}

Our data suggest that in TRH-induced hypertension, leptin is decreased, a metabolic state that may induce compensatory adiposity. In addition, because leptin produces central TRH synthesis and release, we propose that obesity-related leptin elevation may induce hypertension through TRH system activation, which in turn increases sympathetic nerve activity. Accordingly, although more experiments are necessary to delineate this complex TRH-leptin interaction, it may contribute at least in part to the strong association of hypertension and obesity.

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