Thyroid hormones modulate the endocrine and autocrine/paracrine actions of leptin on thyrotropin secretion

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Abstract

We investigated the influence of hypo- and hyperthyroidism on the ability of leptin to modulate TSH secretion. Two hours after receiving leptin (8 µg leptin/100 g BW; s.c.), hyperthyroid rats (10 µg thyroxine (T4)/100 g body weight (BW) for 5 days) showed a 1.7-fold increase in serum TSH ($P<0.05$); in hypothyroid rats, leptin had no effect. Hemi-pituitaries of hyperthyroid rats incubated with 10^{-9} and 10^{-7} M leptin showed reductions in TSH release of 40 and 50% respectively ($P<0.05$); incubation with 1:2000 and 1:500 dilutions of antiserum against leptin resulted in 3- and 4-fold higher TSH release ($P<0.05$

and $P<0.001$ respectively). However, in hypothyroid pituitaries leptin or the antiserum had no effect. The results suggest that the *in vivo* and *in vitro* responsiveness of TSH to leptin is abolished in hypothyroidism and is preserved in short-term hyperthyroidism, in comparison to previous reports in euthyroidism. In addition, the inhibitory action of pituitary leptin is enhanced in hyperthyroid glands, which may suggest a role for locally produced leptin in the suppression of TSH release associated with hyperthyroidism.

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Introduction

Leptin is a hormone secreted mainly by adipocytes which decreases caloric intake and increases energy expenditure (Ahima *et al.* 2000, Kordon 1998). In addition, leptin is an important neuroendocrine regulator, including regulation of the hypothalamus–pituitary–thyroid axis. It is well known that states of malnutrition are associated with a reduction in leptin production and thyroid axis activity. In fasting rats, replacement doses of leptin partially avoided the reduction of hypothalamic thyrotropin-releasing hormone (TRH) content, serum thyrotropin (TSH) and thyroid hormone concentrations induced by fasting (Ahima *et al.* 1996, Seoane *et al.* 2000). Also in humans, the marked suppression of TSH secretion induced by 72 h fasting was blunted with replacement doses of leptin, although leptin treatment did not reverse the fasting-induced changes in serum triiodothyronine (T3) and reverse T3 (rT3; Chan *et al.* 2003). Therefore, in states of nutritional deficiency the decrease in leptin contributes significantly to the reduction of thyroid hormone production, which represents an important adaptive mechanism aiming to reduce energy expenditure. The same may be occurring in some cases of euthyroid sick syndrome. It has been reported that among elderly patients with chronic non-thyroidal illnesses, those who presented low

serum free thyroxine (T4) and free T3 – as well as high reverse T3 – also had low concentrations of serum leptin (Corsonello *et al.* 2000). In addition, hypothyroid patients may show decreased serum leptin (Valcavi *et al.* 1996), although there is some disagreement in published reports (Zimmermann-Belsing *et al.* 2003) and so far a consistent relationship between serum concentrations of leptin and thyroid hormone has not been established.

Leptin may also be involved in modulating the set point of the thyroid axis under physiological conditions. Genetic leptin deficiency, both in rodents and humans, is not always associated with decreased thyroid hormone levels, but mild central hypothyroidism in children has been reported (Clément *et al.* 1998) as well as a stimulatory effect of leptin therapy on thyroid axis secretion (Faroqui *et al.* 2002). In addition, in normally fed rats, acute injection of low doses of leptin, was able to increase TSH secretion (Ortiga-Carvalho *et al.* 2002). The stimulatory effect of leptin on TSH release is probably due to positive regulation of TRH production and release by leptin (Ahima *et al.* 1996, Legradi *et al.* 1997), since we had shown before that the direct effect of leptin on the rat pituitary is to inhibit TSH release (Ortiga-Carvalho *et al.* 2002). Therefore, at least in rats, the augmentation of TSH secretion induced by acute administration of leptin is related to its hypothalamic action.

In addition to regulating TSH secretion by acting as a circulating hormone, leptin may also exert an autocrine/paracrine action within the anterior pituitary. Support for this mechanism is given by the presence of leptin, its receptor and their mRNAs in human and murine pituitary cells, including thyrotrophs (Morash *et al.* 1999, Jin *et al.* 2000, McDuffie *et al.* 2004). Furthermore, the immunoneutralization of pituitary-produced leptin, by adding leptin antiserum to the incubation medium of isolated pituitaries, led to an increase in TSH release (Ortiga-Carvalho *et al.* 2002). Therefore, these data strongly suggest that locally produced pituitary leptin acts as a local TSH-releasing inhibitor.

Thyroid hormones are the most important physiological inhibitors of TSH secretion. The strong negative regulation of thyroid hormones on TSH and TRH production and release determines the set point of the hypothalamic-pituitary-thyroid axis. Altered states of thyroid function are able to change the response of thyrotrophs to TRH and other regulators (DeLean *et al.* 1977, Gurr & Kourides 1985) and in the present work, we investigated whether hypo- and hyperthyroid rats exhibit changes in endocrine and local leptin actions on TSH release.

Materials and Methods

Animals

Adult male Wistar rats (270–300 g body weight (BW)) were kept under 12 h light:12 h darkness cycle (light onset at 0700 h) at $24 \pm 1^\circ\text{C}$; a standard diet and tap water were available *ad libitum*. All experimental protocols were approved by our institutional animal care committee (CAUAP).

The *in vivo* and *in vitro* experiments were performed with hypo- and hyperthyroid rats. Hypothyroidism was induced by treatment with methimazole (0.03%) in the drinking water for 21 days, as previously described (Engler *et al.* 1982). Hyperthyroidism was induced by s.c. daily single injections of thyroxine (T4-L-Thyroxine, Sigma), 10 $\mu\text{g}/100\text{ g BW}$ for 5 days. The rats exhibited moderate hyperthyroidism, with T4 serum concentrations around 55% higher than euthyroid rats (3.27 ± 0.1 vs $5.04 \pm 0.6\text{ }\mu\text{g}/\text{dl}$); serum T3 around 28% higher than normal rats (85.8 ± 1.2 vs $110.9 \pm 8.6\text{ ng}/\text{dl}$) and serum TSH levels reduced to approximately 25% of those of euthyroid rats (0.51 ± 0.04 vs $1.83 \pm 0.1\text{ ng}/\text{ml}$). With this short-term treatment with T4 we aimed to block predominantly the release of TSH and avoid more profound chronic effects of thyroid hormone on thyrotrophs.

In vivo experiments

Hypo- and hyperthyroid animals were divided in two groups that received a single s.c. injection of saline (control

group) or 8 $\mu\text{g}/100\text{ g BW}$ mouse recombinant leptin (National Hormone Pituitary Program (NHPP), NIH, Torrance, CA, USA). The rats were killed by decapitation 2 h after the injection. The time was chosen based on our previous report on euthyroid animals (Ortiga-Carvalho *et al.* 2002). Serum was obtained from trunk blood to measure TSH. Animals were killed before 1100 h.

In vitro experiments

Hypo- and hyperthyroid animals were killed by decapitation and their anterior pituitaries were quickly dissected out. Each hemi-pituitary was immediately transferred to a flask containing 1 ml Krebs–Ringer bicarbonate medium (pH 7.4) at 37°C in an atmosphere of 95% O_2 :5% CO_2 in a Dubnoff metabolic shaker. After a 30-min preincubation period, medium was changed to 1 ml medium alone (control) or medium containing mouse recombinant leptin to a final concentration of 10^{-11} , 10^{-9} or 10^{-7} M . At the end of 2 h incubation an aliquot was removed for TSH measurement.

In another set of experiments, using the same *in vitro* system, hemi-pituitaries were incubated in the presence of antiserum against leptin (rabbit anti-mouse leptin; NHPP) at 1:2000 or 1:500 dilutions. Control hemi-pituitaries were incubated with normal rabbit serum (NRS) at 1:500 dilution. After 2 h, an aliquot of medium was removed to measure TSH levels.

Hormone measurements

TSH concentration in the serum and in the incubation medium was measured by specific RIA, employing reagents supplied by NHPP as previously described (Ortiga-Carvalho *et al.* 1996, Chard 1987), and was expressed in terms of the reference preparation (RP3). Within-assay variation was 7.7%. The minimum assay detection value was 0.36 ng/ml. Serum T4 was detected by RIA (T4 Mab-ICN Pharmaceuticals, Costa Mesa, CA, USA) and within-assay variation was 1.4%.

Statistical analyses

Data are reported as means $\pm$ S.E.M. One-way ANOVA followed by a Student–Newman–Keuls multiple comparisons test was employed for assessment of significance of all data except for serum TSH, which was analyzed by ANOVA only after logarithmic transformation (Zar 1996). Differences were considered to be significant at $P < 0.05$.

Results

In vivo experiments

Hyperthyroid animals killed 2 h after receiving a single injection of leptin (8 $\mu\text{g}/100\text{ g BW}$) showed a 1.7-fold

Figure 1 Serum TSH concentration in hyper- and hypothyroid rats, 2 h after a single s.c. injection of mouse recombinant leptin (8 µg/100 g BW) or saline (Control). Data are presented as means ± S.E.M. * $P < 0.01$ vs control; $n = 12$ animals for hyperthyroid and 6–7 animals for hypothyroid per group.

higher serum TSH ($P < 0.01$) than the saline-injected group. However, in hypothyroid animals, leptin administration was ineffective in changing serum TSH (Fig. 1). Serum T4 concentrations of saline-treated and leptin groups respectively, were similar in hypothyroid (2.4 ± 0.11 vs 2.2 ± 0.11 µg/dl) and hyperthyroid rats (5.0 ± 0.59 vs 6.2 ± 0.94 µg/dl).

In vitro experiments

Leptin-incubated hemi-pituitary glands from hyperthyroid animals showed a dose-dependent decrease in TSH release, statistically significant at 10^{-9} and 10^{-7} M ($P < 0.05$), with a reduction of approximately 60% for both groups. However, leptin was not able to significantly change TSH release from glands of hypothyroid rats as compared with hypothyroid ones incubated with medium alone (Fig. 2).

In hyperthyroid hemi-pituitaries, incubation with antiserum against leptin at 1:2000 and 1:500 dilutions for 2 h resulted in approximately 3- and 4-fold increases in TSH release respectively, as compared with the group incubated with 1:500 dilution of NRS ($P < 0.05$ and < 0.001 respec-

Figure 3 TSH release from hemi-pituitary glands of hyper- and hypothyroid rats incubated with NRS at 1:500 dilution (Control) or in the presence of antiserum against mouse recombinant leptin at 1:2000 and 1:500 dilutions for 2 h. Data are means ± S.E.M. * $P < 0.05$, # $P < 0.001$ vs control; $n = 10$ –12 hemi-pituitaries for hyperthyroid and 11 hemi-pituitaries for hypothyroid per group.

tively). On the other hand, hemi-pituitaries from hypothyroid rats showed no response to the antiserum anti-leptin incubation (Fig. 3).

Discussion

The present study shows that leptin action on TSH secretion is modulated by thyroid function. In hypothyroidism the acute stimulatory effect of leptin on TSH release, previously reported in euthyroidism (Ortiga-Carvalho *et al.* 2002) could not be observed. This is in agreement with the report of Seoane *et al.* (2000), which showed that leptin was not able to stimulate TSH release in hypothyroid fasting rats. This is probably related to the already high rates of TSH secretion in hypothyroid rats that could not be further increased by acute leptin administration. Another possibility is that the cellular response to leptin may be changed at the level of the receptor or at the post-receptor level, resulting in resistance to leptin action.

However, in hyperthyroid animals the stimulatory effect of leptin on TSH secretion, although modest, is still

Figure 2 TSH release from hemi-pituitary glands of hyper- and hypothyroid rats incubated in the absence (Control) or presence of mouse recombinant leptin at different concentrations for 2 h. Data are presented as means ± S.E.M. * $P < 0.05$ vs control; $n = 7$ –8 hemi-pituitaries for hyperthyroid and 9–11 hemi-pituitaries for hypothyroid per group.

present. Even though the animals presented a mild, short-term hyperthyroidism, with TSH partially suppressed, this finding highlights the potency of leptin action on TSH release. The fact that serum T4 was similar in hyperthyroid groups treated with saline or leptin shows that the results are not due to changes in thyroid hormone serum levels. Leptin is probably acting at the hypothalamus, directly or indirectly stimulating TRH release, which in turn stimulates TSH secretion since the direct pituitary effect of leptin on TSH release is inhibitory, as shown by the *in vitro* results. Therefore, it can be suggested that the acute rise in serum leptin was able to override the potent inhibitory effect of thyroid hormones on TRH release. However, it is likely that with a chronic and higher degree of hyperthyroidism leptin would not be able to change TSH release because of the profound effects of thyroid hormone reducing not only the release but mostly the synthesis of TRH and TSH.

In agreement with *in vivo* studies, hemi-pituitaries from hypothyroid rats did not respond significantly to leptin, in regard to TSH release. In contrast, the direct pituitary effect of leptin on TSH release, previously reported in euthyroid glands, is maintained in hyperthyroid hemi-pituitaries. Although direct comparison is difficult, it seems that the magnitude of the leptin-induced rise in TSH release was slightly higher in hyperthyroid glands than that reported previously for euthyroid glands (Ortiga-Carvalho *et al.* 2002). Therefore, the direct action of leptin on TSH release is modified by thyroid hormones; hypothyroidism is associated with decreased or abolished responsiveness to leptin while hyperthyroidism seems to increase it.

In addition, thyroid status also modulated the action of pituitary leptin as an autocrine/paracrine regulator of TSH secretion, as suggested by the *in vitro* responses to the antiserum against leptin. In glands from hypothyroid rats, the antiserum had no effect, while in hyperthyroidism the increase in TSH release in the presence of the antiserum was higher than that observed for euthyroid glands. This can be deduced by comparing data from a previous report, in which the increase in TSH release from euthyroid pituitaries required a 4-fold higher concentration of the antiserum anti-leptin (Ortiga-Carvalho *et al.* 2002). The data suggest that endogenous leptin has a more important inhibitory action at hyperthyroid pituitaries than at euthyroid ones. This enhanced action of an inhibitory local factor may be contributing to the suppression of TSH release present in hyperthyroidism. Other important local TSH-releasing inhibitors, such as the bombesin-like peptide, neuromedin B has its effect regulated by thyroid state. Thyroid hormones act to increase the pituitary neuromedin B, expression (Jones *et al.* 1992, Ortiga-Carvalho *et al.* 1996), and probably release, which may explain the higher response to the immunoneutralization of the peptide (Rettori *et al.* 1992). However, it is not known if thyroid hormones are able to modulate leptin or

leptin receptor expression at the pituitary. This is a feasible hypothesis since pituitary leptin has been shown to be positively regulated by estrogen and growth-hormone-releasing hormone when added together to rat pituitary cell cultures (McDuffie *et al.* 2004).

Although we had used low dilutions of the antiserum against leptin, the physiological relevance of locally produced leptin on TSH release is supported by the fact that the antiserum action was dilution dependent and its effect was the opposite of that of leptin itself. Future studies will be necessary to determine the importance of pituitary leptin in relation to circulating leptin in the physiological regulation of TSH release.

Therefore, here we extended our previous observations in the euthyroid state to hypo- and hyperthyroid states, showing that the *in vivo* stimulatory effect of leptin on TSH release is still present in short-term hyperthyroidism, but disappears in hypothyroidism. In addition, thyroid state also modulates TSH responsiveness to direct pituitary leptin action that is abolished in hypothyroidism. Moreover, the action of pituitary leptin as a local inhibitor of TSH release also disappears in hypothyroidism and is enhanced in hyperthyroidism. The modulation of pituitary leptin action by thyroid hormones, the most important physiological inhibitor of TSH release, reinforces the hypothesis that pituitary leptin acts as a local regulator, modulating or fine-tuning the action of thyroid hormones on TSH release.

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