Acute cold exposure, leptin, and somatostatin analog (octreotide) modulate thyroid 5’-deiodinase activity

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The thyroid gland has a very high D1 activity, and recently the presence of D2 activity and mRNA was detected in both human (24, 29, 30) and rat (4, 14) thyroid gland, although in the normal thyroid, D2 has a much lower expression than D1 (4, 14). Although in humans most T3 has extrathyroidal origin, in rats the thyroid contributes ~50% of serum T3 (11), coming not only from the thyroid biosynthesis but also from thyroid T4-to-T3 conversion, catalyzed predominantly by D1. Therefore, the regulation of the deiodination process in the thyroid gland may have consequences for serum T3 production in the rat. Despite that, except for the well-demonstrated stimulatory effect of TSH (15, 33), very little is known about the regulation of thyroid deiodination. To enhance the knowledge of in vivo regulation of thyroidal D1 and D2 activity, we studied the response of these enzymes to acute cold exposure and to acute administration of leptin and of the long-acting somatostatin analog octreotide.

Acute cold exposure is a model of interest, because higher T3 production is essential for the increased thermogenesis necessary for cold adaptation. Low temperatures induce modifications in thyroid function, as well as, in peripheral thyroid hormones, metabolism. Cold exposure is associated with a rapid activation of the hypothalamus-pituitary-thyroid axis (3, 18, 19), with an early increment in serum TSH followed by an increase in serum thyroid hormone levels after a lag of a few hours (18). In addition, the extrathyroidal generation of T3 is increased mainly in brown adipose tissue, due to the large sympathetic nerve-mediated increment in D2 activity (31). Cold exposure has also induced an increase in liver D1 (28), but no information is available regarding the thyroidal enzyme. Because TSH upregulates thyroidal D1 (15, 33), we reasoned that, during acute cold exposure, the higher rate of TSH secretion may change the activity of thyroid deiodinases. Leptin has recently been shown to act as a positive regulator of hepatic deiodinase activity in ad libitum-fed rats (13) and also of the enzyme in brown adipose tissue (9). However, no information is available regarding the thyroidal enzyme. Previously, we showed that liver and pituitary D1 are regulated by the long-acting

A majority of biological responses to thyroid hormones require the enzymatic conversion of thyroxine (T4) to 3,3’,5’-triiodo-L-thyronine (T3), which is the biologically more active hormone. Two distinct deiodinating enzymes are responsible for this reaction, namely type I and type II iodothyronine deiodinases (D1 and D2). Based on several functional criteria, D1 and D2 have major kinetic differences and distinct tissue distribution (6). In the rat, D1 activity is predominantly expressed in the liver, kidney, and thyroid, whereas D2 activity is expressed in the pituitary, brain, and brown adipose tissue (6). According to current concepts, derived from studies employing compartmental analyses of T4 to T3 conversion, D1 and D2 make a similar contribution to the generation of serum T3 (6, 25).

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somatostatin analog octreotide (OCT) (12); therefore, the thyroid deiodinase might also have its activity modulated through the activation of somatostatin receptors.

In addition, both somatostatin and leptin have their secretion rapidly and profoundly reduced by acute cold exposure (3, 7, 17, 32). Therefore, the relative deficiency of these hormones may potentially, at least partly, influence thyroid deiodinase activity in response to cold, in case they prove to be physiological modulators of the enzymes, which is not known at the present time.

Therefore, acute cold exposure, activation of somatostatin receptors, and leptin have been shown to modulate deiodinase activity in tissues other than the thyroid. In the present study, our aim was to investigate whether they are also able to regulate thyroid D1 and D2 deiodinase in vivo.

MATERIALS AND METHODS

Treatment of Animals

Adult male Wistar rats (weighing 250–300 g) were maintained in a room under a 12:12-h light-dark cycle and controlled temperature (23 or 24°C). The use and handling of experimental animals followed the principles described in the Guide for the Care and Use of Laboratory Animals (5).

Acute cold model. Rats were divided into five groups: control rats (room temperature) and rats maintained below 4°C for 15, 30, 60, and 120 min. They were killed by decapitation in the cold room.

Hypothyroidism. Hypothyroidism was induced by giving one group of rats 0.03% methimazole (MMI; Sigma, St. Louis, MO) in the drinking water for 3 wk before they were killed.

Leptin administration. Animals were separated into three groups, two of which were given a subcutaneous injection of 8 µg/100 g body wt mouse recombinant leptin [National Hormone and Pituitary Program, National Institutes of Health (NIH), Torrance, CA]. The control group received 0.2 ml of saline (vehicle). The rats were killed 30 or 120 min after the injection.

OCT treatment. Two groups of rats received a single injection of 1 µg/kg body wt OCT, a long-acting somatostatin analog (Sandostatin; Sandoz, NJ), diluted in saline 3 and 24 h before they were killed. A third group was used as control (saline only).

In different sets of experiments, all rats were killed by decapitation, trunk blood was collected to determine serum TSH, and the thyroid gland and liver were dissected out and immediately processed for deiodinase activity measurement.

Deiodinase Activity Measurements

Thyroid and liver were homogenized in 50 mM Tris-HCl buffer (pH 6.8) and centrifuged at 1,500 g at 4°C for 30 min. The supernatants of both tissues were centrifuged at 190,000 g for 90 min, and the pellets, containing the microsomal fractions, were resuspended and stored at −70°C until assayed. Assays for deiodinase activity were performed by the release of [125I] from the 125I-labeled reverse T3 (rT3), with minimal modifications, as previously described (12, 22, 27). D1 activity in thyroid and hepatic microsomal fractions was assayed in phosphate buffer containing 1 mM EDTA, pH 6.9, in the presence of 1.5 µM rT3, 10 mM DTT, and 100 nM T4 (to suppress thyroid D2). To evaluate thyroid D2 activity, an assay was performed in the same buffer by using 2 nM rT3, 40 mM DTT, and 1 mM 6-n-propyl-2-thiouracil (PTU; a D1 inhibitor; Sigma). Equal volumes of the [125I]T3 (1.07 mCi/µg; DuPont-New England Nuclear, Boston, MA), purified before each set of assays by paper electrophoresis, were added to each assay tube. Incubations, in a shaking water bath at 37°C, were stopped after 30 min by the addition of a mixture of 8% BSA and 10 mM PTU, followed by cold 20% TCA. The samples were then centrifuged (2,000 rpm, 4°C, 5 min), and 200 µl of the supernatant were applied to Dowex 50W-x2 (100–200 mesh hydrogen from Bio-Rad, Richmond, CA) columns. 125I, eluted from the column with 10% acetic acid, was measured in a gamma counter. The specific enzyme activity was expressed in nanomoles or picomoles of rT3 deiodinated per hour per milligram of protein. Protein was measured by the Bradford method (8).

Quantification of Serum Hormones

Serum TSH concentrations were measured by specific RIA, using reagents supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (NIH), and data are expressed in nanograms of the reference preparation, RP-3.

Serum total T3 and T4 concentrations were determined by radioimmunoassay with Coat-A-Count kits (DPC, Los Angeles, CA).

Statistical Analysis

Data are reported as means ± SE. A Student’s t-test or one-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls multiple comparison test or Dunnett’s multiple comparison test was employed for assessment of data significance. Serum TSH was analyzed by the Kruskal-Wallis multiple comparison test followed by Dunn’s multiple comparison test. Differences were considered to be significant at P < 0.05.

RESULTS

Acute Cold Exposure and Hypothyroidism Experiment

Euthyroid rats exposed to cold showed a progressive reduction in thyroid D1 for the period of 30 min to 2 h (P < 0.05) of cold exposure. Reduction at 30 min was −24% and reached 44% at 2 h, as depicted in Fig. 1A. Liver D1 activity was 45% higher in animals exposed to cold for 2 h (Fig. 1C). As expected, serum TSH was increased by acute cold exposure, reaching a peak at 30 min (5.8 times higher in relation to animals at room temperature). Serum T4 was not changed significantly; however, serum T3 was higher at 60 and 120 min in the cold (Table 1).

Thyroid D2 activity (Fig. 1B) also decreased significantly at all times of 4°C exposure (15 min: 20%, 30 min: 32%, 60 min: 36%, 120 min: 40%). Hypothyroidism increased serum TSH and concomitantly increased thyroid D1 activity (Fig. 1D).

Leptin Experiment

A single administration of leptin induced an ~40% increase in thyroid D1 activity (P < 0.05%) observed 30 and 120 min after the injection (Fig. 2A) but did not alter D2 activity (Fig. 2B). Serum TSH was normal at 30 min but was double that of controls at 120 min (Fig. 2), as already demonstrated in a previous paper (26a). Serum T4 and T3 were not changed significantly at either time point (data not shown).
Liver D1 activity (Fig. 2C) was also increased at both times after leptin injection (Control = 2.1 ± 0.17; leptin at 30 min = 3.0 ± 0.20; leptin at 120 min = 2.80 ± 0.28 nmol 125I·h⁻¹·mg protein⁻¹; P < 0.05).

**Long-Acting Somatostatin Analog (OCT) Experiment**

After a single injection of the long-acting somatostatin analog OCT, the activity of thyroid D1 was 22 and 48% higher than that of the saline-injected group at 3 and 24 h, respectively, although only at 24 h did the values reach statistical significance (P < 0.05; Fig. 3A). Thyroid D2 activity (Fig. 3B) presented an increment only after 24 h of OCT administration (43%). Serum TSH at 24 h was not altered by OCT administration (43%). Serum TSH at 24 h, respectively, although only at 24 h did the values reach statistical significance (P < 0.05). Thyroid D2 activity (Fig. 3B) presented an increment only after 24 h of OCT administration (43%). Serum TSH at 24 h was not altered by OCT administration (43%). Serum TSH at 24 h, respectively, although only at 24 h did the values reach statistical significance (P < 0.05). Thyroid D2 activity (Fig. 3B) presented an increment only after 24 h of OCT administration (43%). Serum TSH at 24 h was not altered by OCT administration (43%). Serum TSH at 24 h, respectively, although only at 24 h did the values reach statistical significance (P < 0.05).

**DISCUSSION**

During acute cold exposure there is an early rise in serum TSH, demonstrated by several previous papers (3, 18) and confirmed in the present study. Therefore, it was initially expected that thyroid deiodinase would be increased, because in hypothyroid animals, the high levels of serum TSH are associated with a higher thyroid D1 activity, and also, as first demonstrated here, with higher thyroid D2 activity. Surprisingly, the cold environment induced a rapid and progressive reduction in thyroid D1 and D2 activity. This seems to be an acute adjustment to cold, because previously (24) it has been reported that rats exposed to 4°C for 6 days presented a higher thyroid D1 specific activity and an increase in total deiodination because of the cold-induced growth of the gland (27). The response in the liver was the opposite; the hepatic D1 was activated by cold exposure, in agreement with a previous study (28). The higher serum T3 observed at 60 and 120 min of cold exposure may result from increased liver D1 activity, but it is likely to be consequent to the marked increase in brown adipose tissue (BAT) D2 activity induced by the sympathetic nervous system, as previously suggested by the finding that PTU treatment, an inhibitor of thyroid hormone biosynthesis and D1 activity, did not prevent the cold-induced rise in serum T3 (31).

The scope of the present paper does not allow us to elucidate the precise mechanism by which thyroid deiodinase is acutely decreased in cold-exposed animals. The activation of the adrenergic system may be a possibility, since norepinephrine has been reported to inhibit TSH-induced thyroid hormone secretion (1). However, to the best of our knowledge, the influence of the adrenergic system on deiodinase activity is not known.

Here, we first demonstrated that an acute rise in serum leptin rapidly changes thyroid D1, independently of changes in serum TSH. Leptin, therefore, may act directly on the thyroid to stimulate D1 activity. This suggestion is supported by the recent characterization of leptin receptors in the thyroid and by the demonstration that prolonged treatment with leptin induced thyroid growth and increased serum thyroid hormones, albeit serum TSH was slightly reduced (26). On the other hand, D2 activity was not altered by leptin injection, suggesting that thyroidal enzymes have some independent mechanisms of regulation in vivo. Acute cold exposure induces a rapid and profound decrease in leptin production and liberation by the adipose tissue (7, 17, 32). Therefore, although it needs experimental demonstration, it is possible that, during acute cold exposure, the lack of the stimulatory effect of leptin may contribute, at least partly, to reduce thyroid D1 activity.

Leptin administered for 3 days into the central nervous system produced a rise in BAT D2 (10) and in the
Acute administration of the long-acting somatostatin analog OCT induced an increase in thyroid D1 and D2 activity after 24 h. Previously, it was demonstrated that OCT promoted a reduction in liver and pituitary D1 activities, without changing pituitary D2 (12). After chronic somatostatin infusion, hepatic D1 activity was also decreased (16). Therefore, it is possible that somatostatin is a positive regulator of thyroid deiodinase. This effect is also independent of serum TSH, since D1 activity was increased regardless of normal serum TSH. A direct effect in the thyroid gland is supported by the detection of injected OCT in the thyroid (21), the presence of thyroidal somatostatin receptors (2), and reports of somatostatin effects in the gland (23). In addition, although it cannot be directly deduced from the present study, it is possible that the decrease in hypothalamic somatostatin release, an early event during acute cold exposure (3), may make some contribution to the lower thyroid D1 and D2 activities of cold-exposed animals.

In conclusion, in the present paper we first showed that D1 and D2 activity in the thyroid gland is suppressed by acute cold exposure despite high serum TSH. We also are the first to report that acute administration of leptin and the somatostatin analog octreotide stimulates thyroid D1, whereas thyroid D2 is stimulated only by octreotide. In addition, we raise the...
hypothesis that, during acute cold exposure, the lower production of leptin and somatostatin is a potential mechanism contributing to the reduction of thyroid deiodinase activity.

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