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

Patricia Cristina Lisboa, Karen Jesus Oliveira, Adriana Cabanelas, Tania Maria Ortiga-Carvalho, and Carmen Cabanelas Pazos-Moura

Laboratório de Endocrinologia Molecular, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, 21949-900 Rio de Janeiro, Brazil

Submitted 21 November 2002; accepted in final form 13 February 2003

Acute cold exposure, leptin, and somatostatin analog (octreotide) modulate thyroid 5'-deiodinase activity. Am J Physiol Endocrinol Metab 284: E1172–E1176, 2003. First published February 25, 2003; 10.1152/ajpendo.00513.2002.—We investigated the effect of acute cold exposure, leptin, and the somatostatin analog octreotide (OCT) on thyroid type I (D1) and II (D2) deiodinase activities. Microsomal D1 and D2 activities were measured by the release of 125I from 125I-reverse triiodothyronine (rT3) under different assay conditions. Rats exposed to 4°C (15, 30, 60, and 120 min) showed progressive reduction in thyroidal D1 and D2, reaching 30% at 2 h (P < 0.05) despite increased circulating TSH (P < 0.05) associated with the higher thyroid D1 and D2 in hypothyroid rats. A single injection of leptin (8 μg/100 g body wt sc) induced increased thyroid and liver D1 (P < 0.05), but not thyroid D2, activities at 30 and 120 min, independently of the serum TSH rise shown only at 2 h. OCT (1 μg/kg body wt sc) increased D1 and D2 activity significantly 24 h after a single injection, with no changes in serum TSH. Therefore, leptin and somatostatin are potential physiological upregulators of thyroid deiodinases, and their low secretion during acute cold exposure may be a potential mechanism contributing to cold-induced reduction in thyroid deiodinase activity.

Address for reprint requests and other correspondence: Patricia Cristina Lisboa, Laboratório de Endocrinologia Molecular, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, CCS, Bloco G, Ilha do Fundão, Rio de Janeiro, 21949-900, RJ, Brazil (E-mail: cpazosm@biof.ufrrj.br).

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), and data are expressed in nanograms of the reference preparation, RP-3].

Serum total T4 and T3 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 Kruskall-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 (Fig. 3, inset), whereas at 3 h there was a small transient rise in serum TSH; however, as we had discussed in a previous paper (12), the mechanism is not known.

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 liver D1 activity of rats (13). Here, we showed that an acute rise of serum leptin is also able to increase liver D1 activity, which may be the result of leptin’s local or central action. Leptin is likely to be a physiological regulator of liver D1, because leptin-deficient ob/ob mice have been reported to have decreased liver D1 activity (20). Although others (9) had suggested that the higher serum T3 would be responsible for the increase in deiodinase activity of animals chronically treated with leptin, our data suggest that D1 activity is upregulated by leptin even in the presence of unchanged serum T3.

**Fig. 2.** Thyroid D1 (A) and D2 (B) activities and liver D1 (C) activity in control rats (open bars) and rats treated with leptin (L; 8 μg/100 g body wt) and killed 30 or 120 min later (solid bars). Inset: serum TSH of the same groups. Values are means ± SE; n = 5–7 animals/group. *P < 0.05.

**Fig. 3.** Thyroid D1 (A) and D2 (B) activities in control rats (open bars) and rats treated with a single octreotide injection (1 μg/kg body wt sc) 3 or 24 h before they were killed (solid bars). Inset: serum TSH of the same groups. Values are means ± SE; n = 5–7 animals/group. *P < 0.05.
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.

The authors are grateful to Dr. A. F. Parlow, Scientific Director, National Hormone and Pituitary Program, NIH, for providing us with rat TSH kit reagents, as well as mouse recombinant leptin. We also thank Norma Lima, Advaldo Bezerra, and Wagner Nunes for excellent technical assistance.

This research was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro, and Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior.

Present address of P. C. Lisboa: Departamento de Ciências Fisiológicas, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, 20551-030, Rio de Janeiro, RJ, Brasil.

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