Thyroid-stimulating hormone receptor in brown adipose tissue is involved in the regulation of thermogenesis

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Submitted 11 May 2008; accepted in final form 12 June 2008

Endo T, Kobayashi T. Thyroid-stimulating hormone receptor in brown adipose tissue is involved in the regulation of thermogenesis. Am J Physiol Endocrinol Metab 295: E514–E518, 2008. First published June 17, 2008; doi:10.1152/ajpendo.90433.2008.—C.RF-Tshrhyt/hyt mice have a mutated thyroid-stimulating hormone receptor (TSHR), and, without thyroid hormone supplementation, these mice develop severe hypothyroidism. When hypothyroid Tshrhyt/hyt mice were exposed to cold (4°C), rectal temperature rapidly dropped to 23.9 ± 0.4°C at 90 min, whereas the wild-type mice temperatures were 37.0 ± 0.15°C. When we carried out functional rat TSHR gene transfer in the brown adipose tissues by plasmid injection combined with electroporation, there was no effect on the serum levels of thyroxine, although rectal temperature of the mice transfected with pcDNA3.1/Zeo-rat TSHR 90 min after cold exposure remained at 34.6 ± 0.34°C, which was significantly higher than that of Tshrhyt/hyt mice. Transfection of TSHR cDNA increased mRNA and protein levels of uncoupling protein-1 (UCP-1) in brown adipose tissues, and the weight ratio of brown adipose tissue to overall body weight also increased. Exogenous thyroid hormone supplementation to Tshrhyt/hyt mice restored rectal temperature 90 min after exposure to cold (36.8 ± 0.10°C). These results indicate that not only thyroid hormone but also thyroid-stimulating hormone (TSH)/TSHR are involved in the expression mechanism of UCP-1 in mouse brown adipose tissue. TSH stimulates thermogenesis and functions to protect a further decrease in body temperature in the hypothyroid state.

Thyroid-stimulating hormone receptor (TSHR) is a key molecule in the regulation of thyroid functions, including hormone synthesis, secretion, and cell growth (10). TSHR expression has long been considered to specifically occur in the thyroid gland. However, Vizek et al. reported that thyroid-stimulating hormone (TSH) stimulated glycerol release from human adipose tissue of a newborn but not of an adult (22). Marcus et al. also demonstrated that TSH is the dominating lipolytic hormone in vitro during the human neonatal period (3), suggesting the existence of functional TSHR in human adipocytes in the neonatal period.

Subsequently, we cloned functional TSHR cDNA from rat adipose tissues (4, 7), proposing that this extrathyroidal TSHR may play important roles in the pathogenesis of extrathyroidal manifestations of Graves’ disease. At present, it has been reported that TSHR is detectable in a variety of cell types, including the thymus, bone, heart, lymphocytes, and retroperitoneal fibroblasts (3). TSHR expression levels in these tissues are relatively low, and in some cases only detectable by highly sensitive methods such as RT-PCR. In contrast, rat adipose tissues expressed high level of TSHR, a level that is almost comparable to that of the thyroid (4). Why such a large amount of TSHR is expressed in the adipose tissues remains to be elucidated.

C.RF-Tshrhyt/hyt mice are hypothyroid with severely hypoplastic thyroid glands because of a mutation in the fourth transmembrane domain of TSHR (20). A proline-to-leucine mutation at codon 556 results in plasma membrane targeting (6), but defective TSH binding and receptor function (6, 20) provides a suitable model for studying the role of extrathyroidal TSHR. Through in vivo electroporation of functional rat TSHR gene into the brown adipose tissues (BAT) of C.RF-Tshrhyt/hyt mice, we studied the role of TSHR in the regulation of BAT thermogenesis.

MATERIALS AND METHODS

Animals. All studies were approved by the Animal Research Committee of the University of Yamanashi. C.RF-Tshrhyt/hyt mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and bred to generate experimental animals. The mice were kept in a specific pathogen free mouse room void of thyroid hormone supplementation. Supplementation of Tshrhyt/hyt mice with thyroid hormones was carried out by intraperitoneal injection of thyroxine (T4: 0.1 μg in 50 μl of saline) for 3 wk according to the methods of Green et al. (5). All mice were 70–84 days old at the start of the experiments. To determine TSHR genotype, we carried out PCR using tail DNA with the following primers: 5'-GGCAATATCCGGTCCGTCGTC-3' and 5'-GATGATCTTCAGATGATGGG-3' (12). The nucleotide at codon 556, CCG or CTG, was determined by directly sequencing the PCR products. Rectal temperature of the mice was measured using a Digital Thermometer (model TD-300; Shibaura Electronics, Tokyo, Japan). Triiodothyronine (T3) in BAT was extracted with methanol according to the method of Morreale de Escobar et al. (12). Serum free T4 and T3 were assayed by the ECLusis system (Roche Diagnostic, Tokyo, Japan). Values are expressed as means ± SE, and statistical analysis was carried out using the Student’s t-test.

Gene transfer in BATs by plasmid injection combined with electroporation. pCMV-enhanced green fluorescent protein (EGFP) was constructed by inserting a DNA fragment containing cytomegalovirus (CMV) promoter (Bgl II/Bam HI) from pcDNA3.1/Zeo (Invitrogen, Carlsbad, CA) into pEGFP-1 (CLONTECH Laboratories, Palo Alto, CA). pcDNA3.1/Zeo-rat TSHR was made by inserting rat TSHR cDNA [EcoRI fragment, donated from Dr. Kohn L.D. (Ohio Univer-
**Table 1. Body mass of C.RFhyt/hyt mice and serum-free T4 and cAMP T4 contents in BAT**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Height, cm</th>
<th>Weight, g</th>
<th>Serum-freeT4, ng/dl</th>
<th>cAMP, pmol/mg tissue</th>
<th>Tissue T4, pg/mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>7</td>
<td>8.12 ± 0.07</td>
<td>17.9 ± 0.46</td>
<td>1.47 ± 0.09</td>
<td>9.8 ± 0.48</td>
<td>1.89 ± 0.22</td>
</tr>
<tr>
<td>Hty/hyt</td>
<td>6</td>
<td>7.57 ± 0.35*</td>
<td>16.5 ± 0.78*</td>
<td>0.10 ± 0.02</td>
<td>7.7 ± 0.29*</td>
<td>0.97 ± 0.04*</td>
</tr>
<tr>
<td>Hty/hyt + rTSHR</td>
<td>6</td>
<td>7.56 ± 0.27*</td>
<td>16.6 ± 0.46*</td>
<td>0.092 ± 0.02</td>
<td>15.6 ± 1.25</td>
<td>0.95 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. T₄, thyroxine; T₃, triiodothyronine; hyt, hypothyroid; rTSHR, rat thyroid-stimulating hormone receptor. Height was measured from the tip of the nose to the base of the tail. *P < 0.001 vs. wild-type (*) and vs. hyt/hyt (†)
Fig. 2. Effects of TSHR gene transfer on uncoupling protein (UCP)-1 expression in BAT. A: Northern blot analysis of mouse UCP-1 mRNA from BAT. mRNA from the wild-type rat (1), Tshrhyt/hyt + rat TSHR (2), and Tshrhyt/hyt mice (3). Lower bands are β-actin. B–E: Immunoreactivity of UCP-1 after 3 wk of in vivo electroporation of pcDNA3.1/Zeo to the wild-type mice (B, magnification ×400), to the Tshrhyt/hyt mice (C, ×400), and pcDNA3.1/Zeo-rat TSHR to the Tshrhyt/hyt mice (D, ×400; E, ×100). Arrows indicate the speckled UCP-1-positive areas in BAT. Yellow bars: 100 μm, blue bar: 400 μm.

RESULTS AND DISCUSSION

We exposed Tshrhyt/hyt and wild-type mice to a cold environment (4°C) and measured time-dependent changes of their rectal temperature. Rectal temperature of the wild-type (n = 6) and Tshrhyt/hyt (n = 6) mice before exposure to cold was 38.3 ± 0.11°C and 35.8 ± 0.18°C, respectively, the latter being significantly lower than the former (P < 0.001). After 90 min exposure to cold, the temperature of the wild-type mice slightly decreased to 37.0 ± 0.15°C, but that of the Tshrhyt/hyt mice rapidly dropped to 23.9 ± 0.40°C.

We then carried out functional rat TSHR gene transfer in the BATs by plasmid injection combined with electroporation. At first, we evaluated electroporation efficiency of DNA in the BAT of wild-type C.RF mice using EGFP expression plasmids. In vivo after electroporation (3 wk), we could observe speckled EGFP fluorescence driven by CMV promoter in the tissue but not when we used a promoterless pEGFP-1 plasmid (Fig. 1, Aa and Ab). The green fluorescent protein-positive area was 13.3 ± 2.5% of the total area (n = 4). Northern blot analysis showed a faint 0.8-kb transcript of EGFP, the amount of which was much less than that of β-actin (Fig. 1Ac).

Next, we transfected pcDNA3.1/Zeo in BAT of wild-type mice (n = 7) and Tshrhyt/hyt mice (n = 6) and pcDNA3.1/Zeo-rat TSHR in BAT of Tshrhyt/hyt mice (n = 6). Later (3 wk), those mice were also exposed to a cold environment (4°C). Rectal temperature of the wild-type and Tshrhyt/hyt mice before exposure to cold was 38.4 ± 0.17°C and 35.2 ± 0.15°C, respectively, the latter being significantly lower than the former (P < 0.001). After 90 min exposure to cold, the temperature of the wild-type mice slightly decreased to 36.5 ± 0.38°C, but that of the Tshrhyt/hyt mice rapidly dropped to 24.0 ± 0.40°C. Rectal temperature of Tshrhyt/hyt mice transfected with rat TSHR (rTSHR) cDNA [Tshrhyt/hyt + rTSHR mice, (n = 6)] was 37.1 ± 0.22°C, which was significantly higher than that of Tshrhyt/hyt mice (P < 0.001). Furthermore, even after a 90-min exposure to 4°C, temperature remained at 34.6 ± 0.34°C (Fig. 1B). Figure 1Ca shows the results of Northern blot analysis of TSHR in the wild-type and Tshrhyt/hyt C.RF mice. Because Roselli-Rehfuess et al. detected the TSHR message in BAT from guinea pig (18), we could also detect TSHR mRNA (4.2 kb) in BAT from the wild-type mouse, the levels of which were nearly the same as those from Tshrhyt/hyt mice. In BAT from Tshrhyt/hyt + rTSHR mice, we could detect a faint but definite 2.4-kb transcript from pcDNA3.1/Zeo-rat TSHR. By RT-PCR, we could amplify rat TSHR cDNA only from Tshrhyt/hyt + rTSHR mice, not from the wild-type and Tshrhyt/hyt mice (Fig. 1Cb).

Figure 1D shows the weight ratio of the BAT to the whole body (BAT/BW × 10³) in these three groups. BAT of Tshrhyt/hyt are very atrophic. BAT/BW of the wild-type and Tshrhyt/hyt
mice is 6.7 ± 0.3 and 3.2 ± 0.2, respectively. In contrast, BAT/BW of the Tshrhyt/hyt + rTSHR mice is 4.7 ± 0.4, indicating that BAT relative weight is increased, not only maintained.

Serum free T₄ levels of the wild-type mice were 1.47 ± 0.09 ng/dl (n = 7). The values of Tshrhyt/hyt and Tshrhyt/hyt + rTSHR mice were 0.10 ± 0.02 ng/dl (n = 6) and 0.092 ± 0.02 ng/dl (n = 6), respectively (Table 1). No significant difference was observed between the latter two groups. At the start of this experiment, the height of Tshrhyt/hyt mice was significantly shorter than that of wild-type mice. However, no difference was observed in body weight between the groups (Table 1). These results suggest that the increase of the rectal temperature in Tshrhyt/hyt + rTSHR mice in a cold environment was not due to the difference in serum T₄ levels or in animal surface area but the result of transfection functional TSHR in BAT.

Figure 2A is the result of Northern analysis for UCP-1 mRNA in the wild-type, Tshrhyt/hyt, and Tshrhyt/hyt + rTSHR mice. When compared with the wild-type mice, it is evident that the expression of UCP-1 in the BAT of Tshrhyt/hyt mice was suppressed. However, UCP-1 mRNA in BAT from Tshrhyt/hyt + rTSHR mice increased about threefold compared with that of Tshrhyt/hyt mice. Figure 2, B–D, shows the results of immunostaining UCP-1 in those BAT. Adipose cells of the wild-type mice accumulated fat droplets and were strongly positive for UCP-1 (Fig. 2B). In the cells of Tshrhyt/hyt mice, however, fat droplets almost completely disappeared, and UCP-1 antibody did not stain the cells (Fig. 2C). In adipose cells of Tshrhyt/hyt + rTSHR mice, small amounts of fat droplets were observed, and UCP-1-positive speckled areas were located in these tissues (Fig. 2D).

Finally, we supplied exogenous thyroid hormone to Tshrhyt/hyt mice. Serum-free T₄ levels (1.64 ± 0.05 pg/ml) were nearly the same as those of the wild-type mice (1.51 ± 0.1 pg/ml), and rectal temperature after a 90-min exposure to 4°C (36.8 ± 0.10°C) reached that of the wild-type mice (Fig. 3). This suggests that TSH/TSHR play important roles in BAT thermogenesis when mice are in a hypothyroid state.

BAT is the primary site of adaptive thermogenesis, heat production in response to lowering temperature, in small animals. This thermogenic capacity of BAT largely depends on UCP-1, which can dissipate the proton gradient across the mitochondrial inner membrane (13). Responsive elements for thyroid hormone and cAMP (CRE) have been identified in the 5’-flanking region of UCP-1 gene, and both catecholamines and thyroid hormone positively regulate the expression of UCP-1 gene (15, 16). Adaptive thermogenesis of BAT has been thought to be initiated by catecholamines but must have thyroid hormone present (8, 17). In the present study, we demonstrate that in vivo electroporation of functional TSHR in BAT of Tshrhyt/hyt mice did not affect the serum T₄ level; alternatively, it increased UCP-1 expression, which partially restored rectal temperature of the mice. Because T₃ content levels in BAT from Tshrhyt/hyt and Tshrhyt/hyt + rTSHR mice were nearly the same, but cAMP content in BAT from Tshrhyt/hyt + rTSHR mice was significantly higher than those from Tshrhyt/hyt (Table 1), it is likely that TSH/TSHR stimulated UCP-1 expression via CRE.

Human TSH/TSHR has been reported to play important roles in lipolysis in neonatal periods (11, 22). We previously reported TSH-induced lipolysis in isolated rat fat cells (7). The data presented here indicate that TSHR is involved in the regulation of thermogenesis in mouse BAT, which functions to protect a further drop in body temperature of hypothyroid mice.

A controversy exists concerning the physiological significance of BAT in humans. There is, however, a large amount of evidence to suggest BAT is present in young infants and in adults with certain pathological and nonpathological situations (2, 14), where its functions are closely related to food intake, energy expenditure, and control of body weight. Further study is needed to clarify the roles of TSHR in humans, but the present data suggest that TSH and thyroid hormone might contribute to the control of energy expenditure and metabolic rate.

REFERENCES
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