Thyroid hormone-catecholamine interrelationship during cold acclimation in rats
Compensatory role of catecholamine for altered thyroid states

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Abstract. Effects of hyper- and hypothyroidism on catecholamine (CA) metabolism in the brain, adrenal glands, liver, and brown adipose tissue (BAT) were studied in adult rats during cold acclimation. Hypothyroidism was induced by the administration of propylthiouracil (PTU) and hyperthyroidism by the injection of thyroxine (T4). After 2 weeks of treatment, they were exposed to cold (5°C) and sacrificed after 1 or 4 weeks. Although the body weight gain of PTU-treated rats were markedly impaired, the body temperature was maintained within normal range. They had increased cerebral dopamine, adrenal CA and BAT norepinephrine (NE) contents, enhanced cerebral tyrosine hydroxylase and adrenal dopamine β-hydroxylase (DBH) activities and elevated [3H]dihydroalprenolol (DHA) binding to liver plasma membranes (P < 0.01 vs controls). T4-treated rats showed an increased brain and adrenal CA only after cold exposure. The BAT NE content, DHA binding to liver plasma membranes, and [3H]guanosine diphosphate binding to BAT mitochondria were reduced by 30 to 50% from control values after 4 weeks of cold exposure. These results indicate that during cold acclimation, 1) thyroid hormone deficiency is associated with an accelerated CA synthesis and release, which results in an enhanced BAT thermogenesis, and 2) the hyperthyroid state suppresses CA release, hepatic DHA binding, and BAT heat production. Thus, there is a close metabolic interrelationship between thyroid hormone and CA during exposure to cold. CA appears to ameliorate thyroid hormone excess or deficiency.

The calorigenic action of thyroid hormone and catecholamine (CA) is well established. Both arise from the common precursor, tyrosine, and play a pivotal role in the homeotherm. Thyroid hormone controls the basal metabolic rate (BMR) by stimulating oxidative phosphorylation of the mitochondrial respiratory chain. Thyroid hormone-induced changes in BMR usually occur slowly and tend to persist. On the other hand, CA increases heat production above BMR by enhancing lipolysis and glycolysis in response to various metabolic demands, which are rapidly switched on and off.

On exposure to cold, an elevation of heat production is brought about by the enhanced norepinephrine (NE) secretion from the sympathetic nervous system (Hsieh et al. 1957b), which is the underlying mechanism of non-shivering thermogenesis. Recently, the major site of non-shivering thermogenesis is recognized to be brown adipose tissue (BAT) (Foster & Frydman 1978). Although a line of evidence indicates that cold exposure evokes a rapid rise in plasma thyrotropin (TSH) (Knigge 1960; Itoh et al. 1966) and a stimulation of thyroid secretion, the interrelationship between thyroid hormone and CA still remains to be elucidated. In our study, we examined effects of altered thyroid states on the CA metabolism in the central nervous system (CNS), adrenal glands, liver, and BAT during cold acclimation. We show that there is a close metabolic interrelationship between thyroid hormone and CA.
Materials and Methods

Animals and tissue preparations

Adult Wistar rats weighing 200–250 g were orally administered 0.05% propylthiouracil (PTU) solution or injected 2 μg/100 g body weight of thyroxine (T₄) sc on alternate days for 2 weeks at room temperature (22°C). Then, they were exposed to cold (5°C) for 4 weeks with continuous PTU or T₄ administration. Before and after 1 or 4 weeks of cold acclimation, body weight and body temperature were measured, and they were sacrificed by decapitation under ether anaesthesia. The brain was immediately removed and dissected into the left and right cerebrum and cerebellum. One cerebrum and cerebellum were homogenized in 2 ml of 0.1 mol/l perchloric acid (PCA). After centrifugation at 2500 rpm for 10 min at 4°C, the supernatant was stored at −20°C for subsequent CA determination. One of the adrenal glands and 100 mg of interscapular BAT were also homogenized in 1.0 ml of 0.1 mol/l PCA, and the extract was treated in the same way as the brain. The remaining cerebrum and adrenal gland were homogenized in 0.25 mol/l sucrose containing 5 mmol/l EDTA and used for the assay of tyrosine hydroxylase (EC 1.14.16.2) and dopamine β-hydroxylase (DBH, EC 1.14.2.1) activities. Liver plasma membranes were separated by sucrose gradient centrifugation as described by Ray (1970). BAT mitochondrial fraction was prepared by the method described by Hitelimal et al. (1969) and stored at 0°C in 0.25 mol/l sucrose for not more than 5 h before use.

CA and enzyme assays

CA was measured by high performance liquid chromatography (HPLC) (Shimazu LC-5A) with an electrochemical detector (Coulochem 5100A, ESA Inc, USA), after absorption and extraction from activated alumina (Wako Chem Co, Osaka). The CA recovery, calculated from the added internal standard, dihydroxybenzylamine (DHBA), was 66 ± 7%. Intra-assay coefficient of variance was within 5%. Tyrosine hydroxylase activity in the brain and DBH activity in the adrenal glands were determined by the methods described by Nagatsu et al. (1979) and Nagatsu & Udenfriend (1972), respectively. Protein concentration was measured with a commercially available kit, tonein TP (Otsuka Assay Lab, Tokyo) using human serum albumin as standard.

[3H]guanosine diphosphate (GDP) and [3H]dihydroalprenolol (DHA) binding assay

GDP binding sites in BAT mitochondria were assessed from the binding of [3H]-labelled GDP (New England Nuclear) in the presence of 100 μmol/l GDP (Sigma Chem Co), essentially as described by Nicholls (1976). BAT mitochondria pooled from 4 to 6 rats (protein concentration 0.5 g/l) were added to medium containing 100 mmol/l sucrose, 20 mmol/l N-tris(hydroxymethyl)-2-aminoethane sulfonate, sodium salt, 10 mmol/l choline chloride, 1 mmol/l EDTA, and 5 μmol/l rotenone (pH 7.1) with or without 100 μmol/l GDP. The reaction was started by the addition of 0.25 μCi [3H]GDP (specific activity, 10 Ci/mmol, final concentration 25 nmol/l) and incubation at 23°C for 10 min. Mitochondria were trapped on a glass fiber filter (Whatman GF/C) under vacuum aspiration and washed twice with incubation medium. After complete evaporation of the medium, the filter was placed in 5 ml toluene containing 0.7% PPO and bound GDP activity was counted with a liquid scintillation spectrophotometer.

[3H]DHA binding was measured by the method described by Bukowiecki et al. (1978). Crude membranes (0.5–1.0 mg of protein) were incubated in a total volume of 1 ml of buffer (50 mmol/l Tris-HCl/10 mmol/l MgCl₂, pH 7.4) containing 2.4 nmol/l [3H]DHA (specific activity 103.8 Ci/mmol, New England Nuclear) for 20 min at 25°C. Incubation was terminated by rapid vacuum filtration of the mixture through Whatman GF/C glass fiber filter followed by a wash with 3 ml of ice-cold incubation buffer. Bound DHA activity was counted with a liquid scintillation spectrophotometer. Specific binding was determined from the difference

Table 1. Body weight gain and body temperature during cold acclimation.

<table>
<thead>
<tr>
<th></th>
<th>Body weight gain (g/day)</th>
<th>Body temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22°C</td>
<td>5°C 1 w</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>6.9 ± 1.5</td>
</tr>
<tr>
<td>T₄</td>
<td>10</td>
<td>6.2 ± 1.2</td>
</tr>
<tr>
<td>PTU</td>
<td>10</td>
<td>2.3 ± 1.7</td>
</tr>
</tbody>
</table>

Values are mean ± sd. *P < 0.05, **P < 0.001 vs controls.
Table 2.
Brain catecholamine content before and after cold acclimation.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No.</th>
<th>Forebrain + midbrain</th>
<th>Cerebellum + brain stem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NE (ng/g-tissue)</td>
<td>DA (ng/g-tissue)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>298 ± 53</td>
<td>558 ± 23</td>
</tr>
<tr>
<td>T4</td>
<td>8</td>
<td>298 ± 35</td>
<td>519 ± 37</td>
</tr>
<tr>
<td>PTU</td>
<td>8</td>
<td>328 ± 45</td>
<td>706 ± 107*</td>
</tr>
<tr>
<td>After 5°C, 1 w</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>191 ± 26</td>
<td>521 ± 71</td>
</tr>
<tr>
<td>T4</td>
<td>6</td>
<td>220 ± 18</td>
<td>572 ± 42</td>
</tr>
<tr>
<td>PTU</td>
<td>6</td>
<td>170 ± 26</td>
<td>465 ± 61</td>
</tr>
</tbody>
</table>

Values are mean ± sd. *P < 0.01, **P < 0.001 vs control.

between total binding and binding in the presence of 10 µmol/l propranolol. Statistical analysis was carried out by the Student's unpaired t-tests.

Results

Body weight gain and body temperature
Body weight gain of PTU-treated rats was significantly reduced throughout the experimental period (P < 0.01 vs controls), but they did not show any hypothermia during cold exposure (Table 1). The T4-treated rats also showed an impaired growth rate at the first week of cold exposure, but it was improved later, exceeding the control rate by 4 weeks. A significant hyperthermia was not observed in the T4-treated group.

Changes in brain CA and tyrosine hydroxylase activity
At room temperature, the PTU-treated rats had an increased cerebral dopamine (DA) and a decreased cerebellar DA content, which was abolished after exposure to cold (Table 2). The brain

Table 3.
Brain tyrosine hydroxylase (TH) and adrenal dopamine β-hydroxylase (DBH) activity during cold acclimation.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Experimental group</th>
<th>22°C</th>
<th>5°C, 1 w</th>
<th>5°C, 4 w</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TH</td>
<td>Control</td>
<td>25.8 ± 7.2 (4)</td>
<td>25.3 ± 7.7 (6)</td>
<td>25.9 ± 4.3 (4)</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>11.3 ± 4.3 (4)*</td>
<td>37.5 ± 15.2 (6)</td>
<td>34.1 ± 10.1 (4)</td>
</tr>
<tr>
<td></td>
<td>PTU</td>
<td>19.4 ± 7.5 (4)</td>
<td>67.3 ± 11.2 (6)**</td>
<td>14.3 ± 1.4 (4)*</td>
</tr>
<tr>
<td>DBH</td>
<td>Control</td>
<td>~</td>
<td>1.9 ± 0.7 (6)</td>
<td>5.6 ± 1.4 (4)</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>~</td>
<td>1.6 ± 0.4 (6)</td>
<td>16.7 ± 112.4 (4)</td>
</tr>
<tr>
<td></td>
<td>PTU</td>
<td>~</td>
<td>3.7 ± 2.1 (6)</td>
<td>16.7 ± 3.8 (4)*</td>
</tr>
</tbody>
</table>

Values are mean ± sd. TH: pmol/min/mg protein. DBH: nmol/min/mg protein. Number in parentheses: number of rats. *P < 0.01, **P < 0.001 vs controls.
Changes in adrenal norepinephrine (NE) and epinephrine (E) contents before and after exposure to cold. Adult rats were administered orally 0.05% PTU solution or injected 2 µg/100 g body weight of T4 on alternate days for 2 weeks and exposed to cold (5°C) for 1 to 4 weeks. The treatments were continued during cold exposure. Number in parentheses: number of rats. Vertical bar: ± 1 SD. Asterisks: significant difference vs controls (O). T4-treated rats: (●). PTU-treated rats: (▲).

Changes in brown adipose tissue (BAT) weight and norepinephrine (NE) content in BAT after exposure to cold for 1 week (white column) or 4 weeks (shaded column). The treatments were the same as described in Fig. 1. Number in parentheses: number of rats. Vertical bar: ± SD. Asterisks: significant difference vs controls. Symbols are the same as in Fig. 1.
Changes in BAT weight, CA content and GDH binding

The PTU-treated rats had a reduced BAT weight and a concomitant increase in NE content at 1 week of cold exposure, whereas the T4-treated rats had a decreased NE content in BAT ($P < 0.01$ vs controls) (Fig. 2). These changes persisted throughout cold acclimation, although the PTU-treated rats had a restored BAT weight by 4 weeks. These results suggest that an enhanced NE release and lipolysis occur in PTU-treated rats, whereas both processes are suppressed in T4-treated rats during cold acclimation. Fig. 3 shows the [3H]GDP binding to BAT mitochondria before and after cold exposure. Although the half maximal binding (ED50) was the same in the 3 groups ($3.2 \times 10^{-7}$ mol/l), both T4- and PTU-treated rats had a slightly reduced GDP binding before cold exposure. Four weeks after cold acclimation, GDP binding in the T4-treated rats was reduced by 30% from the control value, whereas there was no significant difference between the PTU-treated rats and controls.

Changes in DHA binding to liver plasma membranes

At 1 week of cold exposure, [3H]DHA binding to hepatic plasma membranes did not differ in the 3 groups. However, at 4 weeks, the PTU-treated rats exhibited a 50% higher and the T4-treated rats a 30% lower binding than did the controls (Fig. 4).

Changes in adrenal CA and DBH activity

Adrenal NE in the PTU-treated rats was elevated before cold acclimation ($P < 0.01$ vs controls), declined rapidly at 1 week and rose again to the level of the T4-treated group (Fig. 1). Adrenal NE in the T4-treated rats gradually increased during exposure to cold. Adrenal epinephrine (E) and DBH activity were markedly elevated in both PTU- and T4-treated rats 4 weeks after cold exposure (Table 3). These results indicate that adrenal CA synthesis is enhanced in both thyroid hormone excess and deficiency.
Discussion
Thyroid hormone plays an important role in the adaptive phenomena associated with changes in environmental temperature. It has been well established that exposure to cold induces and accelerated turnover of thyroid hormone as well as thyroid hyperactivity (Cottle & Carlson 1956). This is brought about through stimulation of thyrotropin (TSH) from the pituitary gland (Itoh et al. 1966). Furthermore, thyroidectomized rats or rats given PTU show a decreased cold resistance (Seller & You 1950). However, the elevated TSH rapidly falls to its previous level following cold exposure (Itoh et al. 1966). The initially reduced plasma thyroid hormone level also returns to normal after 4 weeks of cold acclimation (Van Hardeveld et al. 1979). After full adaptation to cold there is no significant difference in the level of thyroid function between cold- and warm-acclimated animals (Cadot et al. 1969), whereas an increased oxygen consumption persists. As shown in our results, hypothyroid rats still responded to cold by an increase in non-shivering heat production. This indicates that the enhanced thermogenesis in cold-acclimated rats is not a direct product of the circulating level of thyroid hormone.

The increased thermogenesis in cold-acclimated rats is most likely due to the enhanced non-shivering heat production induced by the calorigenic action of CA liberated from the sympathetic nervous endings and adrenal glands (Hsieh & Carlson 1957a,b). Exposure to cold elicits an immediate increase in NE excretion, which persists as long as the rats are kept in the cold (Lebuc 1961). Our data demonstrate that a marked increase in adrenal CA content and DBH activity occurred during cold acclimation (Fig. 1, Table 3). This was associated with a rise in the weight and NE content of BAT. These changes were observed in both hyper- and hypothyroid rats, supporting the concept that CA plays a more essential role in the non-shivering thermogenesis than thyroid hormone.

The magnitude of the CA responses to cold exposure was increased in the hypothyroid rats and decreased in the hyperthyroid ones. A synergistic action has been reported between thyroid hormone and CA on the calorigenic response to NE administration (Leblanc & Villemaire 1970). A recent observation indicates that during cold exposure, plasma NE concentration is increased 6- to 12-fold, which accelerates peripheral conversion of T4 to T3 (Storm et al. 1981). Thyroid hormone increases the sensitivity to NE by the interaction between ß-adrenergic receptor and the guanine nucleotide binding component of adenylyl cyclase (Malbon et al. 1984). We could not substantiate this mechanism during cold acclimation. DHA binding to liver plasma membranes was not altered at 1 week after cold exposure and reduced in the T4-treated rats at 4 weeks. This was also the case for the GDP-binding to BAT mitochondria, which is in accordance with the observation reported by Sundin (1981). Therefore, thyroid hormone-induced cold resistance cannot be explained on the basis of the alterations in ß-adrenergic receptor or in mitochondrial proton channels. A plausible explanation is that the thyroid hormone-induced rise in BMR reduces the demand for non-shivering thermogenesis, whereas thyroxine deficiency enhances the heat production by CA.

Although a line of evidence suggests the involvement of CNS in thermoregulation during cold acclimation, the underlying mechanism is not clear. In addition to the stimulation of TSH release, thyrotropin-releasing hormone (TRH) accelerates CA turnover (Keller et al. 1974) and produces a rise in plasma NE and E in rats (Brown 1981). Furthermore, we have observed that intracereally administered TRH produced hyperthermia in young rats (Sato et al. 1983). These findings led to the concept that TRH may play a regulatory role in thermogenesis. Reichlin et al. (1972) found an increase in hypothalamic TRH synthetase activity following cold exposure, although no significant changes in hypothalamic TRH content was observed in rats during acute and chronic exposure to cold (Jobin et al. 1975). If an increased TRH release from the hypothalamus occurs during cold exposure, as suggested by the pituitary-thyroid stimulation, it may be compensated for by an equal rise in synthesis. This hypothesis must be studied in the future.

In conclusion, there is a close metabolic interrelationship between CA and thyroid hormone in the adaptation to cold. The calorigenic action of CA plays a major role in this process, which is increased in the hypothyroid state and decreased in the hyperthyroid state. The regulatory mechanism of this interaction in CNS as well as in peripheral tissues awaits further investigations.
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References


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