Deterioration of Mitochondrial Function in Heart Muscles of Rats with Hypothyroidism

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Summary In rats with hypothyroidism caused by partial thyroidectomy, a significant decrease in mitochondrial electron-transport activity was found 6 weeks after the thyroidectomy. The level of acetylcholine in heart muscle cells of the left ventricle was found to be increased at the same time after the operation. Norepinephrine in the same cells increased even earlier; significant increase was found 3 weeks after the operation. Supplementation with triiodothyronine lessened these impairments. The relationship among these changes was discussed in relation to hypothyroid cardiomyopathy.

Key Words: mitochondrial function, acetylcholine, norepinephrine, heart muscle, hypothyroidism

Cardiovascular function is greatly influenced by hormonal factors; and cardiac failure, associated with endocrine disorders such as hypothyroidism, is often observed in clinical investigations [1]. The mechanism of hypothyroid cardiomyopathy has attracted much interest because of its variety of symptoms stemming from various morphological and biochemical alterations [2]. Since these alterations revert back to normal by supplementation with thyroid hormone [3], lack of this hormone has been considered to be responsible for these symptoms. Concerning cardiomyopathy, recent advances revealed that cardiac mitochondrial abnormality is closely related to its development. Nevertheless, cardiac mitochondrial function in hypothyroidism has not been well studied. On the other hand, alterations in the autonomic nervous system are suggested to be involved in the genesis of mitochondrial dysfunction [4, 5]. Taking these points into consideration, we

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decided to examine the mitochondrial electron-transport activity and the levels of acetylcholine and norepinephrine in the heart muscle of rats with hypothyroidism.

MATERIALS AND METHODS

Animals. Male specific pathogen-free Wistar rats weighing 60-70 g and 4 weeks of age were used. Animals were maintained in our animal facility until the time of the study.

Surgical procedure. Hypothyroidism was achieved by partial thyroidectomy immediately following transplantation of the parathyroid glands to neck muscles [6]. Briefly, rats were anesthetized with ethylether, and under a stereomicroscope the parathyroid glands were transplanted into the neck muscles. Thereafter, partial thyroidectomy was performed. Hypothyroidism caused by thyroidectomy was confirmed by the radioimmunoassay of triiodothyronine as described below.

Radioimmunoassay of triiodothyronine. Blood was collected via the inferior vena cava from sacrificed animals for analysis of triiodothyronine. Plasma triiodothyronine levels were measured by using radioimmunoassay kits (Dainabot, Tokyo).

Supplementation with triiodothyronine. Supplementation was carried out by daily subcutaneous injection of 10 mg/ml/kg body weight of triodo-L-thyronine dissolved in 0.9% NaCl containing 4 mM NaOH for 3 weeks starting from 3 weeks after surgery.

Preparation of heart mitochondria. Rats were cervically dislocated, and the hearts were removed rapidly and washed with cold saline. The cardiac mitochondria were prepared by differential centrifugation according to the method of Hatefi et al. [7], and finally suspended in 0.25 M sucrose/10 mM Tris-HCl (pH 7.8) buffer. Due to the large amount of protein required for measurement of mitochondrial electron-transport activity, three hearts were combined for preparation of the mitochondria.

Measurement of electron-transport activity. The specific activity of NADH-cytochrome c reductase was determined by a modification of the method of Hatefi and Rieske [8]. The reaction mixture consisted of 0.06 ml of potassium phosphate (1.0 M, pH 8.0), 0.1 ml of NaN₃ (0.1 M), 0.06 ml of ethylenediaminetetraacetic acid (EDTA; 1 mM), 5 ml of 1% deoxycholic acid (pH 8.0), 0.18 ml of 1% ferricytochrome c, and 2.6 ml of distilled water. The reaction was started by the addition of 10 μl of mitochondrial suspension and 75 μl of NADH (0.01 M). After a 15-s incubation at 30°C, the reaction rate was followed for 1 min by recording of the increase in absorbance of cytochrome c at 550 nm. The activity of NADH-cytochrome c reductase was deduced from the rate of increase in the absorbance.

The specific activity of succinate-cytochrome c reductase was determined by the method of Tisdale [9]. The reaction mixture consisted of 0.3 ml of potassium phosphate (0.1 M, pH 7.4), 0.03 ml of NaN₃ (0.1 M), 0.06 ml of EDTA (0.01 M), 0.15 ml of 10% bovine serum albumin, 0.3 ml of potassium succinate (0.1 M), 0.3 ml of
1% ferricytochrome c, and 1.86 ml of distilled water. The reaction was started by the addition of 10 μl of mitochondrial suspension. After a 15-s incubation at 30°C, the reaction rate was followed for 1 min by recording of the increase in absorbance of cytochrome c at 550 nm. The activity of succinate-cytochrome c reductase was deduced from the rate of increase in the absorbance.

The specific activity of cytochrome c oxidase was determined by a modification of the method of Wharton and Tzagoloff [10]. For preparation of ferrocyanochrome c, 1% ferricytochrome c was reduced completely by dithionite, and excess dithionite was removed by passage of the solution through a column of Sephadex G-25 (fine). A mixture of 2.67 ml of potassium phosphate buffer (50 mM, pH 7.0) and 30 μl of 10% Triton X-100 was added to 0.1 ml of ferrocyanochrome c solution. Immediately after the addition of 0.1 ml of mitochondrial suspension, the reaction rate was followed for 10 s by recording of the decrease in absorbance of cytochrome c at 550 nm. The activity of cytochrome c oxidase was deduced from the rate of decrease in the absorbance.

Quantification of acetylcholine and norepinephrine. The left ventricles were isolated and frozen in liquid nitrogen and stored at -70°C until quantification could be made. Tissue was prepared according to the modified method described previously [11]. Acetylcholine was separated from choline by reverse phase high performance liquid chromatography (HPLC), and was subjected to a post-column reaction system with acetylcholinesterase and choline oxidase. A modification [11] of Potter’s method [12] using an immobilized enzyme column as a post-column reactor was used. Hydrogen peroxide produced was determined by electrochemical detection (ECD). The norepinephrine assay was conducted by the HPLC-ECD method described previously [11].

RESULTS

The effects of partial thyroidectomy on blood triiodothyronine level, heart weight, body weight, and heart/total body weight ratio are shown in Table 1. Significant decrease in the level of triiodothyronine was observed either 3 or 6 weeks after the operation. Supplementation with the thyroid hormone resulted in a lesser decrease. Significant decreases in heart and total body weight were also observed, and the heart/total body weight ratio was decreased as well. Administration of the thyroid hormone resulted in a lesser decrease of each value.

The activities of the electron-transport chain in heart mitochondria before and after thyroidectomy are shown in Table 2. NADH-cytochrome c reductase activity was decreased 6 weeks after thyroidectomy, though no significant decrease was seen 3 weeks after the operation. Supplementation with the thyroid hormone prevented the decrease. Similar change was observed in the activity of cytochrome c oxidase. On the other hand, there was no significant change in the activity of succinate-cytochrome c reductase following the operation.

The acetylcholine and norepinephrine levels in the left ventricle of the heart
The level of acetylcholine was increased 6 weeks after thyroidectomy, though no significant change was seen 3 weeks after thyroidectomy. Supplementation with triiodothyronine resulted in a lesser increase. Thyroidectomy increased the norepinephrine level in heart muscles significantly when measured either 3 or 6 weeks after thyroidectomy. Exogenously supplied triiodothyronine prevented this increase as well.

Table 1. Effects of thyroid status on triiodothyronine, heart weight, and body weight.

<table>
<thead>
<tr>
<th>Operation</th>
<th>Time after operation (weeks)</th>
<th>Supplementation with T3</th>
<th>T3 (mg/ml)</th>
<th>HW (mg)</th>
<th>BW (g)</th>
<th>HW/BW (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>3</td>
<td>-</td>
<td>1.02±0.11</td>
<td>709±58</td>
<td>215±10</td>
<td>3.3±0.2</td>
</tr>
<tr>
<td>Thyroidectomy</td>
<td>3</td>
<td>-</td>
<td>0.55±0.07†</td>
<td>267±41†</td>
<td>155±9†</td>
<td>1.7±0.2†</td>
</tr>
<tr>
<td>Sham</td>
<td>6</td>
<td>-</td>
<td>0.93±0.06</td>
<td>908±38</td>
<td>333±9</td>
<td>2.7±0.1</td>
</tr>
<tr>
<td>Thyroidectomy</td>
<td>6</td>
<td>-</td>
<td>0.47±0.08†</td>
<td>358±49†</td>
<td>174±17†</td>
<td>2.1±0.3†</td>
</tr>
<tr>
<td>Thyroidectomy</td>
<td>6</td>
<td>+</td>
<td>0.77±0.15*‡</td>
<td>692±58*‡</td>
<td>273±23*‡</td>
<td>2.5±0.1*‡</td>
</tr>
</tbody>
</table>

T3, triiodothyronine; HW, heart weight; BW, body weight; HW/BW, heart/total body weight ratio. Mean±SD is given. n=6. Significant difference: *p<0.05, versus respective sham operation; †p<0.01 versus respective sham operation; ‡p<0.01 versus thyroidectomy (6 weeks).

Table 2. Effects of thyroid status on electron-transport activity.

<table>
<thead>
<tr>
<th>Operation</th>
<th>Time after operation (weeks)</th>
<th>Supplementation with T3</th>
<th>NADH-cytochrome c reductase</th>
<th>Succinate-cytochrome c reductase</th>
<th>Cytochrome c oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>3</td>
<td>-</td>
<td>420±54</td>
<td>368±44</td>
<td>2,214±174</td>
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<tr>
<td>Thyroidectomy</td>
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<td>-</td>
<td>396±70</td>
<td>376±49</td>
<td>2,293±260</td>
</tr>
<tr>
<td>Sham</td>
<td>6</td>
<td>-</td>
<td>439±55</td>
<td>364±43</td>
<td>2,333±244</td>
</tr>
<tr>
<td>Thyroidectomy</td>
<td>6</td>
<td>-</td>
<td>334±72*</td>
<td>358±39</td>
<td>1,932±211*</td>
</tr>
<tr>
<td>Thyroidectomy</td>
<td>6</td>
<td>+</td>
<td>442±57†</td>
<td>384±57</td>
<td>2,347±254†</td>
</tr>
</tbody>
</table>

T3, triiodothyronine. Figures represent nmol/min/mg protein. Mean±SD is given. n=6. Significant difference: *p<0.05 versus sham operation; †p<0.01 versus thyroidectomy (6 weeks).

Table 3. Effects of thyroid status on acetylcholine and norepinephrine levels.

<table>
<thead>
<tr>
<th>Operation</th>
<th>Time after operation (weeks)</th>
<th>Supplementation with T3</th>
<th>Acetylcholine</th>
<th>Norepinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>3</td>
<td>-</td>
<td>1.42±0.33</td>
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<td>Thyroidectomy</td>
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<td>1.64±0.50</td>
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<tr>
<td>Sham</td>
<td>6</td>
<td>-</td>
<td>1.70±0.22</td>
<td>4.07±0.52</td>
</tr>
<tr>
<td>Thyroidectomy</td>
<td>6</td>
<td>-</td>
<td>3.79±0.39*</td>
<td>8.56±0.63*</td>
</tr>
<tr>
<td>Thyroidectomy</td>
<td>6</td>
<td>+</td>
<td>2.48±0.39*‡</td>
<td>4.11±0.07†</td>
</tr>
</tbody>
</table>

T3, triiodothyronine. Figures represent nmol/g wet weight. Mean±SD is given. n=6. Significant difference: *p<0.05 versus sham operation; †p<0.01 versus thyroidectomy (6 weeks).
DISCUSSION

The present results clearly indicate that in hypothyroidism mitochondrial dysfunction occurs in the cells of heart muscle and that the dysfunction is reversed by the administration of triiodothyronine. Since we have already demonstrated that cardiac electron-transport reflects cardiac function [13, 14], hypothyroid cardiomyopathy is considered to be ascribed to mitochondrial dysfunction in heart muscle cells.

It is well known that a decrease in peripheral adrenergic function, alteration in responsiveness to catecholamines, and decreases in the density and responsiveness of beta adrenergic receptors are seen in hypothyroidism [15-17]. These changes were reversed by thyroid hormone replacement [17]. In the present study, a significant increase in the left ventricular norepinephrine level was observed either 3 weeks or 6 weeks after thyroidectomy. Supplementation with thyroid hormone reduced the increase significantly. Accordingly, this observed increase in norepinephrine in the left ventricle was caused by thyroidectomy. Recently, we demonstrated that norepinephrine-iron complex induced lipid peroxidation [18]. Lipid peroxides are known to provoke changes in mitochondria. Lipid peroxides were found to bring about a decrease in the electron density of mitochondria of arterial smooth muscle cells and inhibit the respiration of these cells [19].

In the present study, a significant increase in the left ventricular level of acetylcholine was also found, but only at 6 weeks after thyroidectomy. We already reported that an acetylcholinesterase inhibitor, pyridostigmine, impaired mitochondrial electron-transport activity by causing an elevation of the acetylcholine level [4, 5]. The increase in acetylcholine observed in the present study was comparable to that seen in rats administered pyridostigmine [4, 5]. Supplementation with thyroid hormone lessened the increase. These results suggest that the increase in the acetylcholine level might also be involved in the genesis of mitochondrial dysfunction in rats with hypothyroidism.

REFERENCES


