Endotoxin-Induced ATP Depletion in Thyrotoxic Rats

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Abstract

Effect of endotoxin from E. coli on the ATP content in heart muscle, the liver and the kidney of thyrotoxic rats was studied. When endotoxin (200-400 µg) was intravenously injected to rats taking drinking water containing 2-7.5 µg T3 per ml, body temperature rose and the heart rate increased. At the same time, a marked decrease in the ATP content in heart muscle and the kidney was observed together with an increase in Na+-K+-ATPase activity. Such changes were not observed or seen only to a small extent in euthyroid rats after endotoxin administration. Endotoxin-induced ATP depletion in T3-treated rats was prevented by administration of 5 mg hydrocortisone just prior to endotoxin injection. These findings indicate that endotoxin easily causes ATP depletion in some tissue or organs in thyrotoxicosis, even if the dose of endotoxin is not enough to produce such an effect in the euthyroid. These observations are of interest in relation to thyroid storm associated with bacterial infection.

It is well known that thyroid storm occurs in untreated or poorly treated hyperthyroid patients most commonly in association with bacterial infection (Nelson and Becker, 1969), but its pathogenesis is yet unknown. We observed a patient with thyroid storm. The patient was transferred to our hospital because of fever, congestive heart failure with atrial fibrillation and unconsciousness. He died on the second hospital day in spite of intensive treatment for thyroid storm. Autopsy and histological examination revealed no specific findings other than acute renal tubular necrosis and central necrosis of the liver, which might have resulted from relatively acute hypoxemia (Robbins, 1974). Whereas these findings are not so common, similar observations have been reported in some cases of thyroid storm (Lamberg et al., 1964; Nelson and Becker, 1969). These histological changes can be explained by ATP deficiency as well as by hypoxia, since ATP is not produced without oxygen. Metabolism of many substances is enhanced in thyrotoxicosis. ATP metabolism may also be accelerated, since Na+-K+-ATPase (ATPase) activity in some tissues or organs is reported to be elevated in thyrotoxic rats (Ismail-Beigi and Edelman, 1971). If there are some factors inhibiting ATP production or accelerating ATP consumption, tissue ATP depletion may readily occur in thyrotoxicosis. This hypothesis would provide an explanation for the patho-

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genesis of thyroid storm. In this paper we describe a study on ATP depletion in some organs caused by endotoxin from E. coli in thyrotoxic rats.

Materials and Methods

Male Wistar rats, weighing 90–100 g, were maintained on drinking water containing 0.5–7.5 µg T₃ per ml. T₃ was dissolved in drinking water by adding a few drops of 1 N NaOH which was also added to control drinking water. On the 6th day of T₃ administration, 50–400 µg of endotoxin from E. coli (0111: B4, Difco Lab.) suspended in 200 µl of saline was injected into the jugular vein under ether anesthesia. Control animals were given the same volume of saline not containing endotoxin. To study the effect of hydrocortisone (Solu-cortef®, Upjohn), 5 mg of the glucocorticoid was also injected into the jugular vein 1 min before endotoxin administration.

The second ether anesthesia was induced 48 min after endotoxin injection. Care was taken that the animals did not get wet with ether to avoid obtaining an abnormally low body temperature. As the body temperature rectal temperature was measured with an electronic thermometer. The heart rate was calculated after recording an electrocardiogram with a portable electrocardiograph recorder. Then the abdomen of the rats was cut open by median section. A flat piece (15–25 mg) of tissue was taken from the kidney with scissors, weighed and then put into a tube containing 3 ml of distilled water kept at 95°C. These procedures were carried out within 45 sec. Then a wedge-shaped piece of the liver was taken and processed in the same way. Immediately after a blood sample was drawn by cardiac puncture, a piece of heart muscle was taken from the apex of the heart. Blood and all the tissue samples were taken within 6 min after the induction of the second anesthesia. These pieces of tissue were boiled in distilled water at 95°C for 10 min, and the supernatant was kept frozen at −30°C for several days until the ATP assay. The remaining kidney, liver and heart muscle were frozen as quickly as possible, and kept at −30°C for several days until the assay of ATPase activity.

Serum T₃ was determined in diluted samples using an Amerlex T₃ RIA® (Amersham). Tissue ATP extracted into distilled water was assayed by the luciferin-luciferase method (Strehler and McElroy, 1957) using an ATP photometer. The reagent used was Picozyme F® (Packard). ATPase in tissue homogenate was determined by the method of Brunberg and Halmi (1956). Homogenate was incubated at 37°C for 10 min with 3 mM ATP in the presence and absence of 1 mM ouabain. Inorganic phosphate (P_i) released from ATP was determined.

Statistical analysis was carried out by Student’s t-test. The results are presented as the means ± SD. The numbers of experimental animals are shown in the table and figures.

Results

When both T₃ (7.5 µg/ml in drinking water) and endotoxin (200 µg) were administered, the rats were continuously moving around and occasionally jumped to the cage cover after endotoxin injection. Irritation was usually observed and some of the animals died in other similar experiments. In contrast, such irritation was not observed in the groups of rats receiving either one or neither of these substances. Changes in serum T₃, the heart rate, body temperature and the tissue ATP content are shown in Table 1. Serum T₃ was much higher in the two T₃-treated groups than the untreated groups. Endotoxin did not cause any change in serum T₃ in either T₃-treated or untreated groups. The heart rate increased slightly, when endotoxin or T₃ was administered separately. A further increase in the heart rate was observed when endotoxin and T₃ were administered together. Endotoxin did not change body temperature, and T₃ raised it slightly. Endotoxin together with T₃ caused a marked rise in body temperature. The ATP content in heart muscle and that in kidney changed in parallel with each other. They were not changed by endotoxin alone. A decrease in the ATP content was observed in the T₃-treated group. A further decrease was exhibited in the group given
Table 1. Changes in the serum T₃, heart rate, body temperature and the tissue ATP content after administration of T₃ (7.5 µg/ml in drinking water) and endotoxin (200 µg intravenously).

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Endotoxin</th>
<th>T₃</th>
<th>T₃ + endotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Serum T₃ (ng/ml)</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.2ᵃ</td>
<td>32.9 ± 5.9ᵇ</td>
<td>28.9 ± 3.5ᵇ,ᵉ</td>
</tr>
<tr>
<td>Heart beats/min</td>
<td>417 ± 30</td>
<td>479 ± 5ᵇ</td>
<td>583 ± 53ᵇ</td>
<td>704 ± 36ᵇ,ᵈ</td>
</tr>
<tr>
<td>Body temp (°C)</td>
<td>36.6 ± 0.3</td>
<td>36.5 ± 0.2ᵃ</td>
<td>38.4 ± 0.2ᵇ</td>
<td>39.8 ± 0.5ᵇ,ᵈ</td>
</tr>
<tr>
<td>ATP content (nmol/mg tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>1.38 ± 0.2₀</td>
<td>1.30 ± 0.2ᵃ</td>
<td>0.91 ± 0.1₄ᵇ</td>
<td>0.37 ± 0.06ᵇ,ᵈ</td>
</tr>
<tr>
<td>Liver</td>
<td>1.11 ± 0.1₆</td>
<td>1.10 ± 0.2ᵃ</td>
<td>0.38 ± 0.0₅ᵇ</td>
<td>0.31 ± 0.0₅ᵇ,ᵉ</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.15 ± 0.2₀</td>
<td>0.99 ± 0.0₄ᵃ</td>
<td>0.72 ± 0.1₁ᵇ</td>
<td>0.40 ± 0.0₅ᵇ,ᵈ</td>
</tr>
</tbody>
</table>

ᵃNot significant vs. control, ᵃᵖ<0.01 vs. control
ᵇNot significant vs. T₃, ᵇᵖ<0.01 vs. T₃

Fig. 1. Changes in the ATP content in heart muscle (A), the liver (B) and the kidney (C) after T₃ treatment and/or endotoxin injection. The rats were given drinking water containing T₃ (5 µg/ml). On the 6th day of T₃ treatment, they were given endotoxin (200 µg) intravenously. C, control; E, endotoxin only; T₃, T₃ only; T₃E, T₃ plus endotoxin.

*not significant, **p>0.05, ***p>0.01.

Fig. 2. Changes in ATPase activity in heart muscle (A), the liver (B) and the kidney (C) after T₃ treatment and/or endotoxin injection. The experimental conditions are indicated in Fig. 1. See Fig. 1 for specifications.
endotoxin after T\textsubscript{3} treatment. The ATP content in the liver was not changed by endotoxin. It decreased following T\textsubscript{3} treatment and no further decrease was demonstrated with T\textsubscript{3} plus endotoxin.

Figures 1 and 2 show the ATP content and ATPase activity in heart muscle, the liver and the kidney. In this experiment the T\textsubscript{3} concentration in drinking water was 5 µg/ml and the dose of endotoxin was 200 µg. The ATP content in heart muscle and that in the kidney changed in parallel with each other as shown in Table 1. T\textsubscript{3} and endotoxin, when given separately, did not cause a significant change in the ATP content compared to the control. A significant decrease was exhibited when endotoxin was given to the animals treated with T\textsubscript{3}. In the liver, a significant fall in the ATP content was observed in the T\textsubscript{3}-treated group. But T\textsubscript{3} treatment plus endotoxin did not cause a further decrease. T\textsubscript{3} administration resulted in an increase in ATPase activity in heart muscle, the liver and the kidney. Endotoxine administration did not change the ATPase level in either T\textsubscript{3}-treated or untreated animals.

As shown in Fig. 3, a decrease in the ATP content in heart muscle was dose-responsive to T\textsubscript{3}. The ATP content was not decreased by the administration of 400 µg of endotoxin alone. But a significant decrease was observed paralleling the increase in the dose of T\textsubscript{3} in addition to endotoxin. Such a decrease in the ATP content was also dose-responsive to endotoxin, when the rats were treated with a constant dose of T\textsubscript{3} (Fig. 4). T\textsubscript{3} (2 µg/ml in drinking water) did not cause an apparent decrease in the ATP content, but the decrease became significant when the dose of endotoxin increased.

Fig. 5 shows the effect of hydrocortisone on ATP depletion in heart muscle induced by T\textsubscript{3} plus endotoxin. T\textsubscript{3} (2 µg/ml in drinking water) together with endotoxin (400 µg) significantly decreased the ATP content. The decrease was prevented by hydrocortisone (5 mg) injection just prior to endotoxin administration. While irritation was observed in rats taking T\textsubscript{3} and endotoxin, it was not observed when they were given the glucocorticoid in addition to endotoxin and T\textsubscript{3}.

Fig. 3. Changes in the ATP content in heart muscle according to the dose of T\textsubscript{3}. The rats were given drinking water containing various amounts of T\textsubscript{3} (0, 0.5, 1, 2 and 5 µg/ml). On the 6th day, a constant amount of endotoxin (400 µg) was injected. For specifications, see Fig. 1.
Fig. 4. Changes in the ATP content in heart muscle according to the dose of endotoxin. The rats were given drinking water containing a constant amount of T₃ (2 µg/ml). On the 6th day, an increasing dose of endotoxin (0, 50, 100, 200 and 400 µg) was injected. For specifications, see Fig. 1.

Fig. 5. Effect of hydrocortisone on ATP depletion in heart muscle induced by T₃ and endotoxin. The rats were given drinking water containing T₃ (2 µg/ml). On the 6th day, endotoxin (400 µg) was injected to one group of rats (T₃E), and to another group (T₃EH) 1 min after hydrocortisone (5 mg) administration. See Fig. 1 for specifications.

Discussion

Thyroid storm is a well known life-threatening complication of hyperthyroidism, characterized by symptoms such as severe tachycardia or arrhythmia, fever, sweating and unconsciousness, but its pathogenesis and biochemical basis are yet unknown. As far as we know, there is only one report presenting an experimental model of thyroid storm (Roberts et al., 1956), although it does not refer to ATP metabolism. We discuss here endotoxin-induced ATP depletion in thyrotoxic rats from the standpoint that thyroid storm possibly results from such ATP deficiency, although several other hypotheses may also exist.

In this study using rats, the additive effect of T₃ and endotoxin to elicit irritation with a marked increase in body temperature and heart rate is obvious. A small dose of endotoxin intensifies the symptoms of thyrotoxicosis. These findings resemble symptoms which are observed in patients with thyroid storm. At the same time in these rats, the ATP content decreased in some organs that are the targets of thyroid hormones. It is not known how ATP depletion relates to the symptoms in the rats. It is to be determined whether such ATP depletion also occurs in patients with thyroid storm in association with bacterial infection.

Thyroid hormones enhance calorigenesis and oxygen consumption. It was once pro-
posed that thyroid hormones act as an uncoupler of oxidative phosphorylation (Hoch, 1962), but Stocker et al., (1968) observed a coupled oxidative phosphorylation in muscle of patients with hyperthyroidism. Therefore ATP production may well be enhanced to the level to which oxygen consumption increases in thyrotoxicosis. On the other hand, thyroid hormones increase ATPase activity in some organs (Ismail-Beigi and Edelman, 1971), as confirmed in this study. Therefore, ATP consumption may also be accelerated in thyrotoxicosis. Production and consumption of ATP may be enhanced in thyrotoxicosis without a change in the tissue ATP content. The decrease in ATP content in the liver in T₃-treated (5 and 7.5 µg/ml in drinking water) rats may be explained as follows: (1) the liver was probably exposed to a much higher concentration of T₃ than the heart and the kidney, since the rats were given T₃ in drinking water; (2) ATP production in the liver may have stopped due to anoxia after taking the tissue sample, while rapid ATP consumption may have continued thereafter until boiling. The slight but significant decrease in ATP content in the animals taking drinking water containing 7.5 µg T₃ per ml, which was not observed in the rats taking drinking water with 5 µg T₃ per ml, may also be explained by a similar mechanism.

Endotoxin is a well known pyrogen related to bacterial infection. The pyrogen may act directly on the central nervous system controlling body temperature. In addition, it may act on leukocytes to produce a leukocytic pyrogen which acts on the central nervous system (Atkins, 1960). There are some other theories explaining the induction of fever by endotoxin. In any case, these theories agree with each other in maintaining that the mediation of the central nervous system is necessary for fever production. Although it is not yet known whether endotoxin acts directly on peripheral tissue to cause fever and to decrease the tissue ATP content, there is a report that E. coli endotoxin acts directly on human erythrocytes to decrease the ATP content (Wallas et al., 1979). It is certain on the basis of the present study that even a small dose of endotoxin is pyrogenic and decreases the tissue ATP content in thyrotoxic rats. The dose of endotoxin (400 µg per rat at maximum) is small compared to the minimal lethal dose of E. coli endotoxin in rats (20–60 mg/kg) reported by Berczi (1966). Thyrotoxicosis may increase sensitivity to endotoxin.

How does endotoxin decrease the ATP content? Although there are not so much data on endotoxin-induced ATP depletion, it probably acts directly on peripheral tissues resulting in ATP depletion. Endotoxin, a lipopolysaccharide, has hydrophilic and hydrophobic portions in its molecule like phospholipids. It is easily incorporated into phospholipid micelles (Rothfield et al., 1966). Therefore, it is probable that it interacts with biomembranes and phospholipid bilayers, resulting in leakage of many solutes through the membranes or uncoupling of oxidative phosphorylation. This idea is supported by the observations in an in vitro study on the effect of E. coli endotoxin on the isolated mitochondria of the rat liver (Mela et al., 1970). Endotoxin has an inhibitory effect on mitochondrial respiration as well as some uncoupling activity. The idea is also supported by our observation (Ando et al., 1986) of similar ATP depletion in thyrotoxic rats administered a small dose of 2,4-dinitrophenol, a commonly used uncoupler of oxidative phosphorylation.

The ATP content decreases according to the amount of increase in the doses of T₃ and endotoxin. This phenomenon shows that the serum T₃ concentration in patients with thyroid storm is not necessarily higher than that in uncomplicated hyperthyroidism (Brooks et al., 1975). Even if hyperthyroidism is not very severe, thyroid storm
may occur in the presence of any factor which acts strongly in causing ATP depletion as well as endotoxin.

It is clear from the results of the present study that hydrocortisone blocks the effect of T₃ plus endotoxin to decrease the content of tissue ATP. This effect of the glucocorticoid is exhibited within a short period of time. Such an effect of hydrocortisone therefore seems unlikely to be mediated by a protein produced after nuclear binding of the glucocorticoid, but probably by its direct action on biomembranes or interaction with endotoxin. It is known that glucocorticoids can protect several biological reactions to endotoxin. The lethal dose of endotoxin in normal mice is 6,000 times higher than that in adrenectomized mice (Chedid et al., 1963). In any case, the life-saving effect of glucocorticoids is well known from clinical experience, and the use of steroids is recommended in thyroid storm.

In conclusion, endotoxin readily decreases the ATP content in some organs in thyrotoxic rats, even if the dose of endotoxin is not enough to induce ATP depletion in euthyroid rats. In other words, thyrotoxicosis may increase susceptibility to endotoxin. Although the biochemical mechanism of ATP depletion is yet to be elucidated, the observation is of interest in relation to thyroid storm associated with bacterial infection.

References


