The Effect of Thyroxine on (Na+, K+)-ATPase from the Heart and the Kidney of Rabbit

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Summary

The effect of thyroxine on membrane bound (Na+, K+)-ATPase isolated as a microsomal fraction from rabbit heart and kidney was investigated. In the heart, thyroxine administration produced an increased Ki value (a concentration of ouabain required for half maximal inhibition of (Na+, K+)-ATPase activity) without alteration of the specific activity of cardiac (Na+, K+)-ATPase, indicating that the digitalis sensitivity of cardiac (Na+, K+)-ATPase was decreased. On the contrary, a significant increase of the specific activity of renal (Na+, K+)-ATPase was observed without change in its digitalis sensitivity. These results suggest that (1) a decreased sensitivity of cardiac (Na+, K+)-ATPase to digitalis glycosides in thyrotoxic animals may contribute to the decrease in the inotropic and toxic effects of the digitalis glycosides in the hyperthyroid state, and that (2) there may be an organ difference in (Na+, K+)-ATPase.

Additional Indexing Words:
Microsomal fraction Digitalis resistance Ouabain sensitivity Thyrotoxicosis

There is broad species variation in the sensitivity to digitalis glycosides of cardiac membrane bound sodium-potassium adenosine triphosphatase, (Na+, K+)-ATPase. The (Na+, K+)-ATPase obtained from digitalis resistant animals such as rat and mouse is 100 times more resistant to the inhibitory effect of ouabain than the (Na+, K+)-ATPase from digitalis sensitive animals such as rabbit, dog, and human. This phenomenon could account for species difference in susceptibility to the inotropic and toxic effects of digitalis glycosides.

On the other hand, resistance to the action of digitalis is known to occur in hyperthyroidism. Recently, it has been reported that thyroxine treatment enhanced (Na+, K+)-ATPase activities of liver, kidney, and skeletal
Thus, one could expect that after thyroxine administration there was a change in the digitalis sensitivity as well as the activity of (Na⁺, K⁺)-ATPase in cardiac muscle. In this study, we have examined both the specific activity and the sensitivity to ouabain of the cardiac membrane (Na⁺, K⁺)-ATPase of thyroxine treated rabbit.

**METHODS**

**Animal experiments:** In 13 male rabbits, weighing 2.0–2.5 Kg, acute thyrotoxicosis was induced by daily subcutaneous injections of L-thyroxine (300 μg/Kg) for 14 days. Paired control animals were injected daily of an equal volume of the diluent (0.01% NaOH). A body weight loss was observed in all rabbits treated with thyroxine, in contrast to a heart weight gain. Thyroxine administration caused a 55% increase in heart weight to body weight ratio from 2.14±0.04 Gm/Kg to 3.32±0.01 Gm/Kg.

**Isolation of membrane vesicles:** Animals were killed by a sharp blow on the head. The heart and kidney were immediately removed and kept in ice-cold 0.25 M sucrose. All procedures were carried out at 0–4°C. Left ventricle including ventricular septum and renal cortex were used for the preparation of cardiac and renal membrane vesicles. After removing fat and connective tissue, the tissues were minced and homogenized with a Polytron PT 10 (Kinematica Gmbh. Lucerne, Switzerland) 3 times for 1 min, with 9 v of 0.32 M sucrose solution containing 5 mM EDTA at pH 7.0 adjusted with 1M Tris. and 0.1% sodium deoxycholate. The preparation of membrane vesicles was obtained by differential centrifugations of the supernatant as previously described: 15 min at 1,000 X g, 15 min at 20,000 X g, 60 min at 100,000 X g. The last pellet was washed twice with a 0.25 M sucrose solution containing 1 mM EDTA at pH 7.0 adjusted with 1 M Tris. and suspended in 4–7 ml of the same medium. The protein concentration of the suspension for enzyme assay was 0.5–0.8 mg/ml. The preparation thus obtained appeared to consist exclusively of vesicles with single and double membranes in electron microscopic pictures as previously reported.

**Determination of ATPase activity:** ATPase activities of the vesicles were measured by the method of Nakao et al within 2 hours after preparation. Assays lasted 30 min at pH 7.4 and 37°C. During this period, the rate of ATP hydrolysis was linear. The reaction was initiated by adding 0.06 mg of the membrane vesicles in 0.1 ml to a 1.9 ml reaction mixture to give a final protein concentration of 0.03 mg/ml. The reaction was stopped by adding 2 ml of 10% trichloroacetic acid and the precipitated protein was removed by centrifugation. ATPase activity was determined by measuring the liberation of inorganic phosphate (Pi) according to the method of Fiske and Subbarow. The routine reaction mixture contained 20 mM cysteine, 5 mM ATP, 0.5 mM EDTA, 40 mM Tris-HCl buffer (pH7.4). Variations in the assay mixtures are reported with the tables and figures. Protein was determined by the method of Lowry et al.
**RESULTS**

*ATPase activities of myocardial and renal vesicles:* The effects of Mg++, Na+, and K+ on ATPase activities in myocardial and renal vesicles are shown in Fig. 1. The rate of ATP hydrolysis by the membrane vesicles was activated in the presence of Mg++, usually referred to Mg++-activated ATPase activity. In the heart, the average of Mg++-ATPase activity was 69.1±3.6 μmoles Pi/mg protein/hr (Table I). This ATPase activity was partially inhibited by addition of 140 mM Na+. The degree of inhibition at this sodium concentration was about 36% in the heart. On the other hand, K+ at the standard concentration of 14 mM had little influence on this basal Mg++-ATPase activity. By the addition of K+ in the presence of Mg++ and Na+, the enzyme was further stimulated. Thus, the (Na+, K+)-dependent ATPase activity was estimated as the difference between the activity in the assay medium, including Mg++, Na+, and K+, and that in the presence of Mg++ plus Na+ in the reaction mixture. The (Na+, K+)-ATPase activity in the heart was 14.3±1.0 μmoles Pi/mg protein/hr (Table I). As shown in Fig. 1, both the specific activity of (Na+, K+)-ATPase and its ratio to total ATPase activity were higher in the kidney than in the heart. Renal Mg++-ATPase and (Na+, K+)-ATPase activities were 41.7±1.0 μmoles Pi/mg protein/hr and 48.9±7.0 μmoles Pi/mg protein/hr respectively (Table I).

![Fig. 1. Effects of Mg++, Na+, and K+ on ATPase activity in myocardial and renal vesicles: The routine reaction mixture contained 20 mM cysteine, 5 mM ATP, 0.5 mM EDTA, 40 mM Tris-HCl buffer (pH 7.4), and 0.1 ml of enzyme in a total volume of 2 ml. And 5 mM MgCl₂, 140 mM NaCl, and 14 mM KCl were added as indicated. The (Na+, K+)-ATPase activity was estimated as the difference between the activity in the presence of Mg++, Na+, and K+ in the assay medium and that in the presence of Mg++ and Na+. Values are means ± S.E. of 12 and 4 individual determinations for myocardial and renal vesicles respectively.](image-url)
Digitalis sensitivity of (Na⁺, K⁺)-ATPase: Inhibition of cardiac (Na⁺, K⁺)-ATPase by ouabain in various concentrations is shown in Fig. 2. (Na⁺, K⁺)-ATPase activity was progressively inhibited by increasing the ouabain concentration and 10⁻⁶ M ouabain completely inhibited cardiac (Na⁺, K⁺)-ATPase activity. Its half maximal inhibition by ouabain (Ki) was seen at approximately 1.9×10⁻⁶ M. The Ki value of renal (Na⁺, K⁺)-ATPase was

\[ \text{Ouabain M} \]

Fig. 2. The percentage of inhibition of cardiac (Na⁺, K⁺)-ATPase activity as a function of the concentration of ouabain: Myocardial vesicles of rabbit and rat were incubated in the reaction mixture containing 20 mM cysteine, 5 mM ATP, 0.5 mM EDTA, 40 mM Tris-HCl buffer (pH 7.4), 5 mM MgCl₂, 140 mM NaCl, and 14 mM KCl in the presence of various ouabain concentrations in a total volume of 2 ml. The concentrations of ouabain required for 50% inhibition of the enzyme activity (Ki) were 1.9×10⁻⁶ M in the rabbit and 2.9×10⁻⁴ M in the rat. Values are means ± S.E. of 4 individual determinations.

Table I. Effect of Thyroxine on Mg⁺⁺- and (Na⁺⁺, K⁺⁺)-ATPase of Heart and Kidney in the Rabbit

<table>
<thead>
<tr>
<th></th>
<th>(Na⁺⁺, K⁺⁺)-ATPase</th>
<th>Mg⁺⁺-ATPase</th>
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<tr>
<td></td>
<td>Specific Activity (µmoles Pi/mg/hr)</td>
<td>% Inhibition at 10⁻⁴ m Ouabain (%)</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (12)</td>
<td>14.3±1.0</td>
<td>43.6±2.7</td>
</tr>
<tr>
<td>T₄ (13)</td>
<td>12.6±1.4</td>
<td>26.8±3.7*</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (4)</td>
<td>48.9±7.0</td>
<td>40.9±1.9</td>
</tr>
<tr>
<td>T₄ (7)</td>
<td>75.9±3.5*</td>
<td>40.7±1.8</td>
</tr>
</tbody>
</table>

Rabbits were daily injected of L-thyroxine (300 µg/Kg) subcutaneously for 2 weeks (T₄). Controls (C) received diluent injections on the same schedule. Ki represents a concentration of ouabain required to achieve 50% inhibition of (Na⁺⁺, K⁺⁺)-ATPase activity. A number in parenthesis is the number of animals. All values are mean ± S.E. *=p<0.01 for control vs. thyrotoxic animals.
1.7×10^{-6} M, similar to that of the heart (Table I).

**Effect of thyroxine on membrane ATPase:** After thyroxine administration for 2 weeks both Mg^{++}-ATPase and (Na^+, K^+)-ATPase activities in the heart were not significantly changed compared with those found in the control (Table I). However, the inhibitory effect of ouabain on cardiac (Na^+, K^+)-ATPase was significantly decreased in the hyperthyroid state. The degree of inhibition of (Na^+, K^+)-ATPase activity by 10^{-6} M ouabain was decreased from 43.6±2.7% to 26.8±317%. Thus, the calculated Ki value was significantly increased from 1.9±0.31×10^{-6} M to 4.2±0.67×10^{-6} M, indicating that the digitalis sensitivity of cardiac (Na^+, K^+)-ATPase was decreased (Table I). However, it was not so changed as to be similar to that of rat. In our experiment as shown in Fig. 2, the rat cardiac (Na^+, K^+)-ATPase was markedly resistant to ouabain inhibition when compared with the rabbit cardiac (Na^+, K^+)-ATPase. The calculated Ki value in the rat heart was 2.9±1.1×10^{-4} M.

An organ difference was observed in the effect of thyroxine on (Na^+, K^+)-ATPase. In the kidney, (Na^+, K^+)-ATPase activity of thyrotoxic animal was markedly increased from 48.9±7.0 μmoles Pi/mg protein/hr to 75.9±3.5 μmoles Pi/mg protein/hr, whereas Mg^{++}-ATPase activity did not differ significantly from the control (Table I). However, in contrast to the heart, both the % inhibition at 10^{-6} M ouabain and the Ki value were the same as those of the control. The % inhibition at 10^{-6} M ouabain was 40.9±1.9% vs. 40.7±1.8%, the Ki value, 1.7±0.17×10^{-6}M vs. 1.7±0.15×10^{-6}M (control vs. thyrotoxic animals, Table I). These results show that the digitalis sensitivity of renal (Na^+, K^+)-ATPase was not changed after thyroxine treatment, although the ATPase activity was markedly enhanced.

**Discussion**

Our results revealed that thyroxine administration to rabbits produced a reduced digitalis sensitivity of cardiac (Na^+, K^+)-ATPase without alteration of the specific activity of cardiac (Na^+, K^+)-ATPase, whereas in the kidney, a significant increase in the specific activity of renal (Na^+, K^+)-ATPase was observed with no change in its digitalis sensitivity. A complex reorganization of cellular membrane densities in the experimental condition might alter (Na^+, K^+)-ATPase activity. However, our observation demonstrating no significant difference in the basal Mg^{++}-ATPase activities between experimental and control groups suggests that a reorganization of cellular membrane was unlikely, since Mg^{++}-ATPase activity served as a control for any procedures which might alter the yield of enzyme.
Recently, there has been an increasing accumulation of evidence in support of the idea that the specific inhibition of membrane bound \((\text{Na}^+, \text{K}^+)\)-ATPase of cardiac muscle might play a possible role in mediating the positive inotropic effects of cardiac glycosides.\(^2,5,18-20\) In fact, rat is a resistant species to cardiac glycosides and rat cardiac \((\text{Na}^+, \text{K}^+)\)-ATPase, as confirmed by our present observation, is also insensitive to the ouabain induced inhibition.\(^3,4\) Thus, it is of interest that in thyrotoxic animals, cardiac \((\text{Na}^+, \text{K}^+)\)-ATPase showed a significant reduction of digitalis sensitivity, as expressed by a greater concentration of ouabain required to achieve 50\% inhibition, without changes of a specific activity in contrast to the case of kidney. This result suggests that a decreased sensitivity of cardiac \((\text{Na}^+, \text{K}^+)\)-ATPase in thyrotoxic animal to digitalis glycosides may contribute to the decrease in the inotropic and toxic effects of the drug in the hyperthyroid state.

Recently, we have observed a marked difference in enzymatic characteristics of cardiac myosins obtained from rat and rabbit. The rat cardiac myosin had a higher ATPase activity in the presence of calcium ions than the rabbit cardiac myosin, and showed distinctive enzymatic characteristics.\(^21\) The different properties of these cardiac myosins could be converted by thyroid hormone. The rabbit cardiac myosin showed a pattern of activity similar to that of the rat cardiac myosin after thyroxine treatment.\(^22\) The present study revealed that a different sensitivities to digitalis glycosides between rat and rabbit cardiac \((\text{Na}^+, \text{K}^+)\)-ATPase shown in Fig. 2, could be also partially converted, but not so completely as observed in the case of cardiac myosin.

We observed an organ difference between heart and kidney in the effect of thyroxine on \((\text{Na}^+, \text{K}^+)\)-ATPase. It has been reported from other laboratories that after thryoxine administration, the \((\text{Na}^+, \text{K}^+)\)-ATPase activities of liver and skeletal muscle were increased but not in the brain.\(^11,12\) Recently, the study, using antisera to purified \((\text{Na}^+, \text{K}^+)\)-ATPase, has revealed antigenic differences in \((\text{Na}^+, \text{K}^+)\)-ATPase preparations isolated from various organs and species. These results together with our present data suggest that there may be organ and species differences in \((\text{Na}^+, \text{K}^+)\)-ATPase.

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