Effect of the Thyroid State on the Enzymatic Characteristics of Cardiac Myosin

A DIFFERENCE IN BEHAVIOR OF RAT AND RABBIT CARDIAC MYOSIN

By Yoshio Yazaki and Maurice S. Raben

ABSTRACT

The effect of thyroid state on the activity of myosin adenosinetriphosphatase (ATPase) was examined in the rat and the rabbit. Cardiac myosin from thyroxine-treated rabbits showed enzymatic properties characterized by high Ca
superscript 2+-activated ATPase activity, low activating energy, lower rate of inactivation at alkaline pH, and no activation by N-ethylmaleimide compared with the same properties in the normal rabbit; thyroidectomy did not affect the enzymatic properties of rabbit cardiac myosin. These findings suggest a difference in the myosin molecule at or near the active site, involving some sulfhydryl groups, between hyperthyroid and euthyroid rabbits. However, rat cardiac myosin showed a pattern of activity in the euthyroid state similar to that of the hyperthyroid rabbit and changed to the euthyroid type after thyroidectomy. These changes were specific for cardiac myosin, since no change was observed in skeletal myosin. It is unlikely that there are major differences in the myosin molecule associated with the two types of activity, since similar proportion and amino acid composition of the subunits of cardiac myosin were observed in the different thyroid states. Thus, we concluded that the administration of thyroxine to the rabbit stimulates the synthesis of new cardiac myosin with altered enzymatic properties and that synthesis of this type of cardiac myosin is maintained by the normal level of thyroid hormone in the rat.

KEY WORDS: muscle, myosin ATPase, thyroidectomy, N-ethylmaleimide, acrylamide gel electrophoresis, thyroxine, amino acid composition, myosin light chain, myosin heavy chain

Evidence suggests that the level of thyroid activity controls the enzymatic activity of cardiac myosin (1-7). An increase in the activity of cardiac myosin adenosinetriphosphatase (ATPase) from guinea pigs and rabbits treated with thyroxine has been observed (1-3). A decrease in the activity of myosin ATPase that correlates with cardiac function has been noted in hypophysectomized rats; thyroxine treatment restores the decreased cardiac myosin ATPase activity and the decreased cardiac performance to normal (4, 5). However, thyroxine treatment of normal rats fails to enhance the activity of cardiac myosin ATPase (4, 5). A difference in the enzymatic characteristics of cardiac myosins from different species of animals has been demonstrated (8), and the present study shows that the different properties of cardiac myosin can be converted by thyroid hormone (9). The experiments in the present study attempted to demonstrate the effect of thyroid hormone on the enzymatic properties of different types of cardiac myosin and to determine the mechanism of thyroxine-induced activation of myosin ATPase.

Methods

MODIFICATION OF THYROID ACTIVITY IN ANIMALS

Male rats (200-250g) and rabbits (2.0-2.5 kg) were studied. The hyperthyroid animals were given daily injections of l-thyroxine (300 µg/kg, sc) for 21 days. Normal control animals (euthyroid) were injected daily for 21 days with an equal volume of 0.9% saline solution. Weight loss was observed in all animals given thyroxine. After 21 days of thyroxine treatment, the body weights of the treated rats and rabbits decreased 35% and 42%, respectively, in contrast to a weight gain in normal rats and rabbits of 40% and 29%, respectively. About 25% of the thyroxine-treated animals died within 3 weeks. Hypothyroidism was produced by total resection of the thyroid glands. The animals were killed 21 days after surgery. The increase in body weight was less than 10% in the hypothyroid rats and rabbits. Serum thyroxine and cholesterol concentrations in normal control rabbits were approximately 1-2 µg and 40-
50 mg/100 ml, respectively. In a group of thyroidectomized rabbits, the values were 0.5 μg and 220–250 mg/100 ml, respectively.

**MYOSIN PREPARATION**

Fresh muscle tissue was obtained from animals killed by a sharp blow on the head. Ventricular heart muscle and back muscle were used for the extraction of cardiac and skeletal myosin, respectively. Myosin was prepared by a dilution technique described previously (8). All procedures were carried out at 4°C, and all solutions contained 1 mM ethylenediaminetetraacetic acid (EDTA). A final high-speed centrifugation with adenosine triphosphate (ATP)-magnesium to remove traces of actin was performed for 1 hour at 130,000 g. The ATPase assay was performed within 24 hours of the isolation of the myosin. Further purification of cardiac myosin was performed by elution from DEAE-Sephadex A25 equilibrated with 10 mM potassium phophate and 5 mM Tris-chloride buffer at pH 7.5. Myosin was eluted with a gradient to 0.3M KCl in the equilibrated buffer.

**DETERMINATION OF ATPase ACTIVITY**

Assays were run for 5 minutes at pH 7.5 and 25°C. During this period, the rate of ATP hydrolysis was linear. The reaction was initiated by the addition of myosin (0.4 mg in 1 ml for cardiac myosin, 0.2 mg in 0.1 ml for skeletal myosin) to a reaction mixture (1.9 ml) to give a final protein concentration of 0.2 or 0.1 mg/ml. The reaction was stopped by adding 10% trichloroacetic acid (2 ml), and the precipitated protein was removed by centrifugation. ATPase activity was determined by measuring the liberation of inorganic phosphate according to the method of Fiske and Subbarow (10). The routine reaction mixture contained 0.05M Tris buffer (pH 7.5) for the rat and 0.1 mM EDTA-activated and calcium (Ca²⁺)-activated ATPase activities were assayed in the presence of 1 mM EDTA with 0.6M KCl and of 10 mM CaCl₂, respectively. Protein was determined by the method of Lowry et al. (11) using crystalline bovine serum albumin as a standard. This method was standardized against a micro-Kjeldahl determination.

**TREATMENT OF MYOSIN WITH SULFHYDRYL REAGENTS**

Myosin (4 mg/ml) was incubated at 0°C for 2 hours in a solution containing 0.6M KCl and 10 mM Tris at pH 7.5 with varying concentrations of N-ethylmaleimide. Excess N-ethylmaleimide was removed by dialysis against 0.6M KCl and 10 mM Tris at pH 7.5. In the case of modification by 5, 5'-dithio-bis(2-nitro)benzoic acid (DTNB), myosin (4 mg/ml) was incubated with 0.2 mM DTNB in a solution containing 0.6M KCl and 10 mM Tris at pH 7.5 for 20 minutes at 0°C.

**POLYACRYLAMIDE GEL ELECTROPHORESIS OF MYOSIN**

Polyacrylamide gel electrophoresis was run in the presence of sodium dodecyl sulfate according to the method of Weber and Osborn (12). The gels contained 10% acrylamide and 0.1% sodium dodecyl sulfate. The electrophoresis was carried out at 7 mA/gel for 220 minutes. The gels were stained with 0.25% Coomassie brilliant blue. To obtain the composition of myosin components, the gels were scanned densitometrically with a Gelford gel scanner according to the method described by Lowey and Risby (13).

**AMINO ACID ANALYSIS OF MYOSIN SUBUNITS**

Individual subunits of cardiac myosin were prepared as previously reported (14). The heavy and light chains were dissociated by guanidine denaturation. Fractionation of individual light chains was performed by preparative polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The gel contained 10% acrylamide, 0.12% bisacrylamide, 50 mM sodium phosphate buffer (pH 7.0), and 0.1% sodium dodecyl sulfate. The two species of cardiac light chains were eluted from the gel as two well-separated peaks, each peak yielding essentially a single band on analytical acrylamide gel electrophoresis. Pooled peak fractions were dialyzed and lyophilized. Separated heavy chains and lyophilized light chains were washed three times with 10% trichloroacetic acid, twice with acetone, and then once with ether. Samples weighing about 2.0 mg were hydrolyzed in 6M HCl for 24 hours at 110°C in evacuated glass tubes. Amino acid analyses were performed on an amino acid analyzer (15) by Dr. M. Eizengr in the Boston Biomedical Research Institute and by the AAA Laboratory, Washington. Cysteine was determined as cysteic acid after performic acid oxidation (16). Tryptophan determination was based on base hydrolysis (17).

**RESULTS**

**EFFECT OF THYROID ACTIVITY ON HEART WEIGHT**

The combined weights of the right and left ventricles were examined in a group of normal animals and compared with weights obtained in a group of hyper- or hypothyroid animals. Administration of thyroxine stimulated growth of the heart; heart weight reached 151% and 162% of that for the controls in the thyroxine-treated rats and rabbits, respectively (P < 0.01 vs. euthyroid). In contrast, thyroidectomy decreased growth of the heart. The heart weights of these rats and rabbits were 80% and 86%, respectively, of that for the controls (P < 0.05 vs. euthyroid).

**ATPase ACTIVITIES OF CARDIAC MYOSIN AT THREE LEVELS OF THYROID ACTIVITY IN RATS AND RABBITS**

The ATPase activity of cardiac myosin from animals in various states of thyroid activity is shown in Table 1. A high level of Ca²⁺-activated ATPase activity was observed in cardiac myosin from normal rats; the level was approximately four times that found in the euthyroid rabbits. However, K⁺-EDTA-activated ATPase activity was about the same in both control groups. After thyroxine treatment, the Ca²⁺-activated ATPase activity of cardiac myosin was markedly increased in the rabbit, but there was no change in the activity in KCl-EDTA. This increased Ca²⁺-activated ATPase activity was about three times that of euthyroid controls. However, in the rat, treatment with thyroxine failed to enhance further the activity of Ca²⁺-activated ATPase. Conversely, thyroidectomy in the rat decreased cardiac myosin ATPase activity.
TABLE 1

ATPase Activities of Cardiac Myosin at Three Levels of Thyroid Activity

<table>
<thead>
<tr>
<th></th>
<th>Myosin ATPase activity (µmoles Pi/mg min⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td>K⁺-EDTA</td>
<td>Ca²⁺</td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperthyroid (4)</td>
<td>0.54 ± 0.04</td>
<td>1.20 ± 0.08</td>
</tr>
<tr>
<td>Euthyroid (10)</td>
<td>0.56 ± 0.03</td>
<td>1.16 ± 0.04</td>
</tr>
<tr>
<td>Hypothyroid (4)</td>
<td>0.57 ± 0.05</td>
<td>0.41 ± 0.04*</td>
</tr>
<tr>
<td>Rabbit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperthyroid (3)</td>
<td>0.60 ± 0.05</td>
<td>0.87 ± 0.07</td>
</tr>
<tr>
<td>Euthyroid (6)</td>
<td>0.62 ± 0.03</td>
<td>0.29 ± 0.02†</td>
</tr>
<tr>
<td>Hypothyroid (3)</td>
<td>0.61 ± 0.04</td>
<td>0.24 ± 0.04†</td>
</tr>
</tbody>
</table>

Values are means ±SE. The number of hearts in each group is given in parentheses.

*P < 0.001 vs. euthyroid or hyperthyroid rat.
†P < 0.001 vs. hyperthyroid rabbit.

myosin ATPase activity was observed: the value was approximately 60% greater than normal. After 7, 10, 15, and 21 days of thyroxine treatment, myosin ATPase activity was markedly increased to 200, 255, 290, and 300% of control values, respectively. These results suggest that the hormonal effect on myosin ATPase activity may be well established after 2 weeks of treatment.

EFFECT OF SULFHYDRL REAGENTS ON CARDIAC MYOSIN ATPase

In view of the evidence that sulfhydryl groups play an important role in many enzymes, we examined the effect of sulfhydryl reagents on myosin ATPase activity in different thyroid states. According to previous reports (18-20), treatment with a low concentration of N-ethylmaleimide blocks mainly a class of sulfhydryl groups which reacts rapidly. At higher concentrations, a second class of sulfhydryl groups in myosin also reacts with the sulfhydryl reagent. We therefore treated cardiac myosin from rats and rabbits with various concentrations of N-ethylmaleimide. The effects on ATPase activity are shown in Figure 1. We have reported previously (8) that treatment with N-ethylmaleimide reveals a difference between rat and rabbit cardiac myosin. In rabbit cardiac myosin, both K⁺-EDTA-activated and Ca²⁺-activated ATPase activities were inhibited by increasing the N-ethylmaleimide concentration. On the other hand, at high ionic strength (0.6M KCl), Ca²⁺-activated ATPase was increased from 0.08 ± 0.01 to 0.36 ± 0.02 µmoles Pi/mg min⁻¹ (P < 0.02) in the modified myosin with lower concentrations of N-ethylmaleimide. In contrast, rat cardiac myosin was not activated by N-ethylmaleimide (0.59 ± 0.02 to 0.54 ± 0.04 µmoles Pi/mg min⁻¹), and, with increasing concentrations of

TIME COURSE OF INCREASED MYOSIN ATPASE ACTIVITY DUE TO THYROXINE TREATMENT

Ca²⁺-activated ATPase activity of cardiac myosin was measured in rabbits given thyroxine daily for 3, 7, 10, 15, and 21 days. After 3 days of thyroxine administration, a significant increase in
the reagent, the Ca\textsuperscript{2+}-activated ATPase at high ionic strength was inhibited.

After thyroxine treatment, rabbit cardiac myosin changed its enzymatic properties to the rat cardiac myosin type in terms of the N-ethylmaleimide effect. The modified myosin was not activated by N-ethylmaleimide (0.44 ± 0.03 to 0.39 ± 0.03 μmoles Pi/mg min\(^{-1}\)) although euthyroid myosin was activated as previously described. Administration of toxic doses of thyroxine failed to alter the enzymatic properties of rat cardiac myosin. Conversely, thyroidectomy changed the enzymatic properties in the rat. With decreasing Ca\textsuperscript{2+}-activated ATPase activity, the activating effect of N-ethylmaleimide appeared. Ca\textsuperscript{2+}-activated ATPase at high ionic strength was increased from 0.11 ± 0.02 to 0.49 ± 0.02 μmoles Pi/mg min\(^{-1}\) (P < 0.01) in the modified myosin. No change was observed in rabbit cardiac myosin after thyroidectomy. Thus, the difference in the N-ethylmaleimide effect on cardiac myosin ATPase between rats and rabbits was observed only in the euthyroid state. Both rat and rabbit cardiac myosins showed one pattern similar to the euthyroid rat type in the hyperthyroid state, and another pattern similar to the euthyroid rabbit type in the hyperthyroid state.

Temperature dependence of ATPase activity of rat and rabbit cardiac myosin at three levels of thyroid activity. The assay solutions contained 0.05M Tris, 5 mM ATP, 10 mM CaCl\(_2\), and 0.025M KCl (pH 7.5). Apparent enthalpy of activation was calculated from the formula \(\Delta H^\circ = -R\ln Vd / (1/T) - RT\), where \(V = \) ATPase activity, \(R = \) molar gas constant, and \(T = \) absolute temperature.

Another sulfhydryl reagent, DTNB was examined because this reagent preferentially reacts with the rapidly reacting sulfhydryl groups in the myosin molecule (21, 22). Cardiac myosin from euthyroid and hyperthyroid rabbits was treated with 0.2 mM DTNB. Ca\textsuperscript{2+}-activated ATPase activity at high ionic strength was activated from 0.086 ± 0.007 to 0.174 ± 0.010 μmoles Pi/mg min\(^{-1}\) (P < 0.05) for euthyroid rabbit myosin but was not changed significantly for hyperthyroid rabbit myosin, as was the case with N-ethylmaleimide (0.44 ± 0.03 to 0.40 ± 0.02 μmoles Pi/mg min\(^{-1}\)).

Effects of temperature and alkaline pH on cardiac myosin ATPase in different thyroid states

Temperature dependence was different for rat and rabbit cardiac myosin ATPases (Fig. 2). Apparent enthalpies of activation at 25°C evaluated from the data in Figure 2 were 5.8 and 7.1 kcal/mole in the presence of 10 mM CaCl\(_2\) for rat and rabbit cardiac myosin ATPases, respectively, in the euthyroid state. After thyroxine treatment, rabbit cardiac myosin became less temperature-dependent compared with euthyroid myosin; the apparent enthalpy of activation was 5.9 kcal/mole, which was approximately the same as the value for rat cardiac myosin. No change was observed in the case of rat cardiac myosin with thyroxine treatment. On the other hand, thyroidectomy increased the apparent enthalpy of activation of rat cardiac myosin from 5.8 kcal/mole to 7.2 kcal/mole, a value similar to that for euthyroid rabbits; the value for rabbit cardiac myosin was not affected by thyroidectomy.

Inactivation at alkaline pH of myosin ATPase activity in the absence of ATP was also different for rat and rabbit cardiac myosin (Fig. 3). We have shown that the lability of ATPase at alkaline pH is greater in the presence of K\(^+\)-EDTA than it is in the presence of Ca\textsuperscript{2+} in both cases (8). In the present study, therefore, the effect of alkaline pH on cardiac myosin ATPase in the presence of EDTA was examined. The ATPase activity of rat cardiac myosin was less affected than that of rabbit myosin by exposure to pH 9.0. In the hyperthyroid state, both myosins were of the less labile type. Conversely, in the hypothyroid state, both myosins were more readily inactivated at alkaline pH. Thus, both rat and rabbit cardiac myosins showed a pattern similar to the rat type in the hyperthyroid state and one similar to the rabbit type in the hypothyroid state, as observed in the N-ethylmaleimide experiments.
FIGURE 3
Effect of incubation at pH 9.0 on ATPase activity of rat and rabbit cardiac myosin at three levels of thyroid activity. Myosin (4 mg/ml) was added to a reaction mixture containing 1 mM EDTA, 0.6 M KCl, and 0.1 M Tris-glycine at pH 9.0. At times indicated on the abscissa, 5 mM ATP was added; after 5 minutes, the ATPase reaction was stopped by the addition of trichloroacetic acid (TCA). Values for zero incubation at pH 9.0 were determined by adding myosin to the assay system containing ATP and terminating the reaction with trichloroacetic acid after 5 minutes. The enzymatic activity was constant during the 5-minute incubation, since the presence of ATP prevented inactivation.

GEL ELECTROPHORESIS OF CARDIAC MYOSIN
Figure 4A and B shows the electrophoretic patterns of myosin from euthyroid and hypothyroid rat hearts, respectively. Figure 4C shows euthyroid rabbit cardiac myosin. The strong band at the top of the gel corresponds to the heavy chains of cardiac myosin. The light chains appear as two bands near the middle of the gel. These electrophoretograms show no significant contamination of myosin with actin, tropomyosin, or other protein. By using sodium dodecyl sulfate–acylamide gel electrophoresis, the molecular weight and the proportion of the two cardiac light chains were compared at three different thyroid states in rats and rabbits. Comparison of the electrophoretic mobilities with those of proteins of known molecular weight gave values of 27,000 and 20,000 for the two light chains of both rat and rabbit cardiac myosins. These values were not changed in different thyroid states. The ratio of the two light chains was also determined in rat and rabbit cardiac myosins at three different levels of thyroid activity. The concentration of protein in the light chain bands on sodium dodecyl sulfate–acylamide gels was measured densitometrically. The concentration ratio of the upper light chain to the lower one was about 5:1:49 for both rat and rabbit cardiac myosins at any thyroid state. Although we could get only a relative amount of each light chain with this method, the electrophoretograms failed to demonstrate any change in the composition of the cardiac light chains after thyroid modification.

FIGURE 4

Circulation Research, Vol. 36, January 1975
Amino Acid Composition of Cardiac Myosin Heavy Chain and Light Chains from Euthyroid and Hypothyroid Rat

**THYROID EFFECT ON CARDIAC MYOSIN**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Heavy chain</th>
<th>Light chain 1</th>
<th>Light chain 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>176 ± 3 (185)</td>
<td>25 ± 1 (23)</td>
<td>16 ± 1 (16)</td>
</tr>
<tr>
<td>Histidine</td>
<td>31 ± 1 (31)</td>
<td>2 ± 1 (2)</td>
<td>1 ± 1 (1)</td>
</tr>
<tr>
<td>Arginine</td>
<td>101 ± 2 (106)</td>
<td>11 ± 1 (11)</td>
<td>8 ± 1 (8)</td>
</tr>
<tr>
<td>Cysteic acid</td>
<td>16 ± 1 (15)</td>
<td>4 ± 1 (4)</td>
<td>&lt;0.3 (&lt;0.2)</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>170 ± 2 (165)</td>
<td>22 ± 1 (21)</td>
<td>18 ± 2 (17)</td>
</tr>
<tr>
<td>Threonine</td>
<td>75 ± 1 (76)</td>
<td>12 ± 1 (13)</td>
<td>9 ± 1 (9)</td>
</tr>
<tr>
<td>Serine</td>
<td>70 ± 1 (70)</td>
<td>7 ± 1 (8)</td>
<td>5 ± 1 (6)</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>316 ± 4 (305)</td>
<td>40 ± 2 (41)</td>
<td>32 ± 2 (34)</td>
</tr>
<tr>
<td>Proline</td>
<td>52 ± 2 (59)</td>
<td>14 ± 2 (14)</td>
<td>7 ± 1 (6)</td>
</tr>
<tr>
<td>Glycine</td>
<td>81 ± 1 (80)</td>
<td>15 ± 1 (16)</td>
<td>12 ± 1 (12)</td>
</tr>
<tr>
<td>Alanine</td>
<td>152 ± 2 (149)</td>
<td>27 ± 1 (22)</td>
<td>16 ± 1 (17)</td>
</tr>
<tr>
<td>Valine</td>
<td>90 ± 1 (87)</td>
<td>12 ± 1 (11)</td>
<td>9 ± 1 (9)</td>
</tr>
<tr>
<td>Methionine</td>
<td>40 ± 1 (41)</td>
<td>7 ± 1 (8)</td>
<td>7 ± 1 (6)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>83 ± 1 (78)</td>
<td>9 ± 2 (10)</td>
<td>9 ± 1 (9)</td>
</tr>
<tr>
<td>Leucine</td>
<td>180 ± 2 (172)</td>
<td>17 ± 1 (16)</td>
<td>11 ± 1 (12)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>36 ± 1 (35)</td>
<td>3 ± 1 (4)</td>
<td>2 ± 1 (2)</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>54 ± 1 (55)</td>
<td>11 ± 1 (10)</td>
<td>11 ± 1 (9)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>16 ± 1 (17)</td>
<td>&lt;0.2 (&lt;0.2)</td>
<td>&lt;0.2 (&lt;0.2)</td>
</tr>
</tbody>
</table>

Values are means ± SD for three different preparations from the euthyroid rat heart. Values of preparations from the hypothyroid rats, given in parentheses. The molecular weight of each subunit was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (200 × 10^3, 27 × 10^3, and 20 × 10^3 for heavy chain, light chain 1, and light chain 2, respectively).

from animals with different thyroid activities. K^+-EDTA-activated and Ca^2+-activated ATPase activities were 1.70 ± 0.02 μmoles Pi/mg min^-1 and 0.68 ± 0.02 μmoles Pi/mg min^-1 for rat skeletal myosin and 1.83 ± 0.04 μmoles Pi/mg min^-1 and 0.71 ± 0.02 μmoles Pi/mg min^-1 for rabbit skeletal myosin. Neither thyroxine treatment nor thyroidectomy altered the ATPase activities of rat and rabbit skeletal myosin. Furthermore, the activating effect of N-ethylmaleimide on Ca^2+-activated ATPase activity at high ionic strength was demonstrated in both rat and rabbit skeletal myosin at any level of thyroid state.

**Discussion**

The present study demonstrates differences between rat and rabbit cardiac myosins in various thyroid states. The high level of the Ca^2+-activated ATPase activity of cardiac myosin from hyperthyroid animals has been recently reported (1–3), but the difference in the enzymatic properties of cardiac myosin, especially with respect to the response to sulphydryl reagents, has not been previously defined. In the hyperthyroid state, rabbit myosin showed a high level of ATPase activity. The presence of activators in cardiac myosin from hyperthyroid animals is unlikely because of the lack of change in ATPase activities of cardiac myosins after purification of the enzymes by column chromatography. The gel electrophoresis of preparations also showed no significant contamination of myosin with actin, troponyosin, or other proteins. Thus, the increased ATPase activity of cardiac myosin from thyroxine-treated animals suggests an intrinsic change in the myosin molecule. The extent of activation of ATPase activity by thyroxine treatment appeared to depend on the level of the activity of myosin ATPase in the euthyroid state, since the thyroxine effect was not observed on rat cardiac myosin showing high activity of Ca^2+-activated ATPase in the euthyroid state.

The experiments dealing with the effect of N-ethylmaleimide on myosin ATPase brought out a difference between cardiac myosins from animals in different thyroid states (Fig. 1). According to Kielley and Bradley (18) and Sekine et al. (19), myosin treated with low levels of N-ethylmaleimide possesses the characteristics associated with the blocking of the rapidly reacting sulphydryl groups, although reaction with a number of the other sulphydryl groups occurs. Masking of this class of sulphydryl groups causes an activation of Ca^2+-activated ATPase at high ionic strength. Rabbit cardiac myosin treated with low concentrations of N-ethylmaleimide showed this pattern. However, in the cardiac myosin from hyperthyroid rabbits, the activation of Ca^2+-activated ATPase activity at high ionic strength was not observed. The difference in the effect of N-ethylmaleimide on cardiac myosin from euthyroid and hyperthyroid rabbits is not due to a different reaction rate. The experiments using [1-14C]N-ethylmaleimide showed the same amount of radioactive uptake by euthyroid and hyperthyroid rabbit cardiac myosin in the range of N-ethylmaleimide examined. Cardiac myosins from both euthyroid and hyperthyroid rats did not show activation of ATPase activity by N-ethylmaleimide. After thyroidectomy, however, rat cardiac myosin showed a pattern of activity similar to euthyroid rabbit cardiac myosin with lower activity of ATPase and activation by N-ethylmaleimide, but no effect was observed in rabbit cardiac myosin due to thyroidectomy.

Activation energy of ATPase and lability at alkaline pH were also different for rat and rabbit cardiac myosin and depended on thyroid state (Figs. 2 and 3). After thyroxine treatment, rabbit cardiac myosin showed enzymatic properties similar to those of rat cardiac myosin: it was less sensitive to alkaline pH and less temperature
dependent. Conversely, thyroidectomy changed rat cardiac myosin ATPase to the rabbit type: it was more sensitive to alkaline pH and temperature. These results strongly suggest, together with the observations from the N-ethylmaleimide experiments, that thyroxine treatment may have produced a structural change in rabbit myosin involving some sulfhydryl groups at or near the active site of myosin inducing the enzymatic properties characterized by high Ca"+-activated ATPase activity, no activation by N-ethylmaleimide, and less sensitivity to alkaline pH and temperature. Rat cardiac myosin appeared to be very sensitive to thyroid hormone, and the structural change in the cardiac myosin molecule may be maintained by a normal level of thyroid hormone, since even under euthyroid conditions the cardiac myosin already showed the altered pattern of enzymatic properties observed in hyperthyroid rabbits. In the thyroid-deficient rat, the myosin was converted to resemble euthyroid rabbit cardiac myosin. According to the report of Abrams and Larsen (23), the concentration and metabolism of thyroid hormones in rat serum were not different from those of other animals.

No change in the molecular weight or in the proportion of the cardiac light chains was observed in hyperthyroid or hypothyroid animals. Furthermore, amino acid analysis of the subunits of cardiac myosin failed to demonstrate any significant difference between normal and hypothyroid rats, although a marked difference in myosin ATPase activity was observed. Therefore, it is unlikely that there are major differences in the gross size or components of the myosin molecule in different thyroid states. Thyrum et al. (1) have reported that the helical content and the amino acid composition of the myosin isolated from hyperthyroid guinea pig hearts are different from those of myosin isolated from euthyroid animals. However, the molecular weight of myosin is so great that it is difficult to compare directly the amino acid composition of the whole myosin molecule, since a small change in such a great number of amino acid residues is difficult to assess.

Thyroxine might directly act on cardiac myosin to alter the enzymatic properties, but the absence of an effect of thyroxine on myosin ATPase activity after in vitro incubation contradicts this possibility. Also, the time course of increased ATPase activity tends to negate this mechanism. Daily administration of thyroxine gradually increased myosin ATPase activity, and the hormonal effect was well established only after 2 weeks, corresponding to the turnover rate of cardiac myosin (24-26). These changes were specific for the heart, since no change was observed in skeletal myosin. Thus, we conclude that administration of thyroxine to the rabbit stimulates the synthesis of new cardiac myosin with altered enzymatic properties, although it has not been determined whether the structural change is present in heavy chains or light chains, and the synthesis of this type of cardiac myosin is maintained by the normal level of thyroid hormone in the rat.

Acknowledgment

We wish to thank Mrs. Joanne Stevens for her technical assistance and Dr. M. Elzinga, Boston Biomedical Research Institute, for the amino acid analyses.

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Circulation Research, Vol. 36, January 1975
THYROID EFFECT ON CARDIAC MYOSIN

Effect of the thyroid state on the enzymatic characteristics of cardiac myosin. A difference in behavior of rat and rabbit cardiac myosin.
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Circ Res. 1975;36:208-215
doi: 10.1161/01.RES.36.1.208

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