Comparative Studies of Atrial and Ventricular Myosin from Normal, Thyrotoxic, and Thyroidectomized Rabbits

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SUMMARY. Changes in enzymatic and structural properties of ventricular myosin in thyrotoxic rabbit hearts have been investigated extensively. However, there is little information regarding the effect of thyroid hormone on the atrial myosin. In this study, we have compared enzymatic and structural changes of ventricular and atrial myosin from euthyroid, hypothyroid, and hyperthyroid rabbits. In euthyroid rabbits, Ca++- and actin-activated ATPase activities of atrial myosin were 2-fold greater than those of the ventricular myosin. The Ca++- and actin-activated ATPase activities of atrial myosin from hypothyroid and hyperthyroid rabbits were identical with the values for atrial myosin from euthyroid rabbits. The same ATPase activities of ventricular myosin decreased in hypothyroid hearts but increased in hyperthyroid rabbits. The K+ (EDTA)-ATPase activities of all myosins were the same, irrespective of the thyroid status of the animal. Pyrophosphate-polyacrylamide gel electrophoresis patterns showed two isoenzymes (designated as A1 and A3) of atrial myosin in euthyroid hearts. The same electrophoretic patterns also showed in atrial myosin from hypothyroid and hyperthyroid hearts. The ventricular myosin from euthyroid hearts also exhibited two isoenzymes (designated as V1 and V3) but each with slower electrophoretic mobilities than the corresponding atrial myosin. In hypothyroid hearts, only V3 isoenzyme was seen, whereas, in hyperthyroid hearts, only V1 isoenzyme was seen. These results suggest that thyroid hormone controls ventricular myosin ATPase activity by controlling synthesis of a specific ventricular isoenzyme, whereas thyroid hormone does not affect atrial myosin ATPase, possibly due to its inability to control atrial myosin synthesis. (Circ Res 52: 131-136, 1983)

IN recent years, considerable attention has been paid to alterations of cardiac myosin by thyroid hormone (Morkin, 1979). Myocardial contractility and myosin ATPase activities of ventricular myosin are increased in hyperthyroid hearts (Buchino et al., 1967; Yazaki and Raben, 1975; Banerjee et al., 1976; Banerjee et al., 1977; Alpert et al., 1979), whereas the same activities are decreased in hypothyroidism (Buchino et al., 1967; Hoh et al., 1978; Pope et al., 1980). These alterations are believed to result from thyroid hormone modulating relative proportion of preexisting ventricular myosin isoenzymes (Hoh et al., 1978; Clark et al., 1982; Martin et al., 1982; Litten et al., 1982).

There is little information regarding the effects of thyroid hormone on the myosin from the atrium. Atrial myosin has been shown to possess higher Ca++- and actin-activated ATPase activities compared with myosin from ventricular muscle (Long et al., 1977; Flink et al., 1978; Syrovoy et al., 1979; Yazaki et al., 1979). Isoelectric focusing of light chains (Price et al., 1980) and CNBr peptide mapping of heavy chains (Flink et al., 1978) have shown differences between ventricular and atrial myosin. Since functional and immunological properties of atrial muscle differ significantly from the ventricular muscles (Sartore et al., 1978), the effect of thyroid hormone on the control of expression of specific myosin may differ in the atrial muscle.

In this report, we have carried out detailed enzymatic and structural characterization of ventricular and atrial myosin from hyperthyroid and thyroidectomized rabbits. The results demonstrate that the effects of thyroid hormone on the control of myosin expression in the two muscles differ significantly.

Methods

Animal Experiments

All rabbits used in these studies were male New Zealand white weighing 1.5-3.0 kg. We induced thyrotoxicosis in four rabbits weighing about 1.5-2.0 kg by injecting l-thyroxine for 18 days, as described previously (Banerjee et al., 1976). Total thyroidectomy was performed on two other rabbits weighing 2.5-3.0 kg, by the supplier, Endocrine Surgical Services. Then those rabbits were housed in our facility for 14 days. Uninjected rabbits from the same litters were used as controls. Before the animals were killed, blood samples were obtained from each, for determination of serum thyroxine. Serum thyroxine levels were determined by radioimmunoassay at commercial facilities (Upjohn).
Preparation of Proteins

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Inorganic phosphate \((P_i)\) liberated in the reactions was measured by the method of Fiske and Subbarow (1925). SDS gel electrophoresis was carried out by the methods of Weber and Osborn (1966). Protein concentrations were determined by using absorbance of \(E_{280}\) 0.56 for myosin (Small et al., 1961) and 11.49 for F-actin (Eisenberg and Morkin, 1977). The reaction medium contained 0.05 M KG, KG was increased to 0.5 M. Actin-activated ATPase activity was assayed at 30°C as described previously (Banerjee and Morkin, 1977). The reaction medium contained 0.05 M Tris-HCl, pH 7.5, 0.05 M KCl, 0.005 M Na4P2O7, pH 8.8, 10% glycerol, and 2 μM cysteine. Staining and destaining of gels were performed as described previously (Hoh et al., 1978).

ATPase Assays

The Ca**+-ATPase activity was measured as described previously (Banerjee et al., 1976) at 30°C in a medium containing 0.05 M Tris-HCl, pH 7.5; 0.05 M KCl; 0.005 M ATP; and 0.001 M CaCl2. To determine the K* (EDTA) ATPase activity, CaCl2 was replaced by 0.001 M EDTA and KCl was increased to 0.5 M. Actin-activated ATPase activity was assayed at 30°C as described previously (Banerjee and Morkin, 1977). The reaction medium contained 0.05 M KCl, 0.015 M Tris-HCl, pH 7.6, 0.002 M ATP, and 0.004 M MgCl2. The myosin concentration was 200 μg/ml. Actin concentrations were varied from 0.2 to 1.2 mg/ml.

Analytical Procedures

Inorganic phosphate \((P_i)\) liberated in the reactions was measured by the method of Fiske and Subbarow (1925). SDS gel electrophoresis was carried out by the methods of Weber and Osborn (1966). Protein concentrations were determined by using absorbance of \(E_{280}\) 0.56 for myosin (Small et al., 1961) and 11.49 for F-actin (Eisenberg and Morkin, 1977), as well as by biuret technique using bovine serum albumin as standard. Statistical differences between mean values were evaluated by an unpaired t-test. Densitometric scanning of pyrophosphate gel bands were performed at 550 nm in Gilford Model 250 Spectrophotometer using 2401 S linear transport.

Results

In all, seven control, six thyrotoxic, and five thyroidectomized groups of rabbits were used in the present study. The serum thyroxine average was 5.0 ± 0.3 (mean ± SD) μg/100 ml in control, 15.5 ± 1.2 μg/100 ml in thyroxine-treated, and 1.0 ± 0.5 μg/100 ml in thyroidectomized rabbits. Body weight of thyroidectomized rabbits increased very minimally during the 18-day period following surgery, whereas the control rabbits gained weight normally. Thyroxine-treated rabbits ceased to grow in body weight during the 18-day period. The ratio of heart weight to body weight was 1.8 ± 0.2 mg/g, 1.2 ± 0.3 mg/g, and 3.3 ± 0.3 mg/g for control, thyroidectomized, and thyrotoxic rabbits, respectively. The purity of the myosin preparation was checked by SDS gel electrophoresis patterns which showed a heavy chain and two light chain bands in normal and thyrotoxic myosin, as previously described (Banerjee et al., 1976). The ratio of O.D. 280/O.D. 260 for the ventricular myosin was between 1.5 to 1.7 and that of atrial myosin was 1.6 to 1.7.

Ca**- and K* (EDTA)-ATPase Activities

Table 1 shows Ca**+- and K* (EDTA)-ATPase activities of atrial and ventricular myosin from control, thyrotoxic, and thyroidectomized rabbits. In agreement with reports published by several groups (Yazaki and Raben, 1975; Banerjee et al., 1976; Alpert et al., 1979, Litten et al., 1982), the Ca**+-ATPase activity of thyrotoxic myosin is 2-fold greater than that of the control, but the same ATPase activity of myosin from thyroidectomized rabbits is about 28% (P < 0.01) lower than that from the controls. However, the K* (EDTA)-ATPase activities are the same in all groups of animals. Thus, Ca**+-ATPase activities of ventricular myosin differ significantly, depending on the thyroid status of the animal, whereas the K* (EDTA)-ATPase remains the same.

It is seen, from Table 1, that Ca**+-ATPase activities of atrial myosin from control animals are significantly greater than those of ventricular myosin, which confirms similar observations reported in other animal species (Flink et al., 1978; Syrovy et al., 1979; Yazaki et al., 1979; Srivastava and Wikman-Coffelt, 1982). However, unlike ventricular myosin, the Ca**- present study. The serum thyroxine average was 5.0 ± 0.3 (mean ± SD) μg/100 ml in control, 15.5 ± 1.2 μg/100 ml in thyroxine-treated, and 1.0 ± 0.5 μg/100 ml in thyroidectomized rabbits. Body weight of thyroidectomized rabbits increased very minimally during the 18-day period following surgery, whereas the control rabbits gained weight normally. Thyroxine-treated rabbits ceased to grow in body weight during the 18-day period. The ratio of heart weight to body weight was 1.8 ± 0.2 mg/g, 1.2 ± 0.3 mg/g, and 3.3 ± 0.3 mg/g for control, thyroidectomized, and thyrotoxic rabbits, respectively. The purity of the myosin preparation was checked by SDS gel electrophoresis patterns which showed a heavy chain and two light chain bands in normal and thyrotoxic myosin, as previously described (Banerjee et al., 1976). The ratio of O.D. 280/O.D. 260 for the ventricular myosin was between 1.5 to 1.7 and that of atrial myosin was 1.6 to 1.7.

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ATPase activity of atrial myosin remains unaltered by different thyroid states. It is interesting to note that—regardless of the thyroid status—the Ca\(^{++}\)-ATPase activity of atrial myosin is identical to that found for thyrotoxic ventricular myosin. The slightly lower values of K\(^{+}\) (EDTA)-ATPase activities of atrial myosin compared with those of ventricular myosin are not statistically significant (P > 0.2).

**Actin-Activated Mg\(^{++}\)-ATPase**

Figures 1 and 2 show Lineweaver-Burk plots of actin-activated ATPase activities of ventricular and atrial myosin, respectively, from thyrotoxic and thyroidectomized rabbits. Kinetic parameters, V\(_{\text{max}}\) (actin-activated ATPase activities of myosin at infinite actin concentration), and K\(_{\text{m}}\) (apparent affinity of actin with the myosin-ATP complex) obtained from the data of Figures 1 and 2 are given in Table 2. Values of V\(_{\text{max}}\) and K\(_{\text{m}}\) of ventricular and atrial myosin obtained in the same manner for four different sets of experiments were the same within the experimental error.

It can be seen that the physiologically important ATPase activity of thyrotoxic myosin is over 2-fold greater than that of thyroidectomized rabbit, which is about 30% lower (P < 0.01) than that of the control rabbit. However, the actin-activated ATPase activities of atrial myosin in all groups remain the same. Actin-activated ATPase activities of atrial myosin from normal and thyroidectomized rabbits are about 50% greater (P < 0.02) than those of the corresponding ventricular myosin; however, the same ATPase activity of thyrotoxic atrial myosin is about 30% lower (P < 0.01) than that of thyrotoxic ventricular myosin. Thus, although Ca\(^{++}\)- and K\(^{+}\) (EDTA)-ATPase activities of atrial myosin are identical to those of thyrotoxic ventricular myosin (described in the preceding paragraph) the actin-activated ATPase activities of these two myosins differ significantly.

**Pyrophosphate-Polyacrylamide Gel Electrophoresis**

Figures 3 and 4 show densitometric scan of the pyrophosphate-polyacrylamide gel electrophoretic patterns of rabbit ventricular and atrial myosin. Both

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**Figure 1.** Double reciprocal plots of actin-activated ATPase activities of ventricular myosin vs. actin concentration. The data were fitted by means of a least-squares program. Assay conditions are described in Methods. ● = myosin from thyrotoxic rabbit hearts; ○ = myosin from thyroidectomized rabbit hearts.

**Figure 2.** Double reciprocal plots of actin-activated ATPase activities of atrial myosin vs. actin concentration. The data were fitted by means of a least-squares program. Assay conditions are described in Methods. ▲ = myosin from thyrotoxic rabbit hearts; △ = myosin from thyroidectomized rabbit hearts.

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**Table 2**

<table>
<thead>
<tr>
<th>Source of myosin</th>
<th>Mg(^{++})-ATPase ((\text{µmol P}_i/\text{mg per min}))</th>
<th>V(_{\text{max}}) ((\text{µmol P}_i/\text{mg per min}))</th>
<th>K(_{\text{m}}) ((\text{µM}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal—atria</td>
<td>0.03 ± 0.01</td>
<td>0.34 ± 0.05</td>
<td>18.0 ± 2.8</td>
</tr>
<tr>
<td>Thyrotoxic—atria</td>
<td>0.03 ± 0.008</td>
<td>0.33 ± 0.02</td>
<td>16.5 ± 1.8</td>
</tr>
<tr>
<td>Thyroidectomized—atria</td>
<td>0.03 ± 0.01</td>
<td>0.33 ± 0.02</td>
<td>16.5 ± 1.8</td>
</tr>
<tr>
<td>Normal—ventricles</td>
<td>0.01 ± 0.005</td>
<td>0.25 ± 0.02</td>
<td>28.0 ± 4.0</td>
</tr>
<tr>
<td>Thyrotoxic—ventricles</td>
<td>0.03 ± 0.01</td>
<td>0.50 ± 0.04</td>
<td>25.0 ± 2.0</td>
</tr>
<tr>
<td>Thyroidectomized—ventricles</td>
<td>0.01 ± 0.005</td>
<td>0.20 ± 0.02</td>
<td>22.5 ± 3.3</td>
</tr>
</tbody>
</table>

Assays were performed as described in Methods.
atrial and ventricular myosin from normal rabbits display two isoenzyme bands designated as Vi and V₃ for ventricular myosin and A₁ and A₃ for atrial myosin. The relative proportions of the ventricular isoenzymes are 80 ± 5% for V₃ and 20 ± 5% for Vi; those of atrial isoenzymes are 45 ± 5% and 55 ± 5% for A₃ and A₁. In agreement with previous reports (Hoh et al., 1978; Clark et al., 1982; Martin et al., 1982; Litten et al., 1982), the thyroid state modulates relative proportion of ventricular isoenzymes leading to Vi isoenzyme in hyperthyroidism or V₃ isoenzyme in hypothyroidism. In contrast, there is no significant change in the isoenzyme patterns of atrial myosin in thyrotoxic and thyroidectomized rabbits.

It is noted that the pyrophosphate-polyacrylamide gel electrophoretic bands from atrial myosin are much less sharply defined than those from ventricular myosin. Addition of various proteolytic inhibitors in the myosin preparation did not improve the sharpness or the resolution of these bands. Although the reasons for this are not clear, they may be related to structural similarities between the atrial isoenzymes.

Discussion

The main observations presented in this paper demonstrate that rabbit atrial myosin differs significantly from the ventricular myosin in its response to alterations by thyroid hormone. Unlike ventricular myosin, atrial myosin from rabbit hearts does not
undergo alterations in Ca++- and actin-activated Mg++-ATPase activities. In this respect, atrial myosin resembles white skeletal myosin, which also remains unaffected by thyroid status (Rovetto et al., 1972). The actin-activated Mg++-ATPase activities of myosin correlate with speed of contraction of skeletal (Barany, 1967) and cardiac (Carey et al., 1979) muscle. Thus, the results suggest that the velocity of contraction of atrial muscle is not changed by the level of thyroid hormone.

Another interesting observation made in this study is that Ca++-ATPase activities of atrial myosin are identical with those of thyrotoxic ventricular myosin, but the physiologically important actin-activated Mg++-ATPase activities of atrial and thyrotoxic ventricular myosin differ significantly. Since the Ca++-ATPase activities are governed by the identity of heavy chains (Wagner and Weeds, 1977), the same Ca++-ATPase activities of atrial and thyrotoxic ventricular myosin seem to support the hypothesis that heavy chains of atrial myosin from euthyroid rabbit hearts are structurally similar to those of thyrotoxic ventricular myosin (Libera and Sartore, 1981). On the other hand, differences between the actin-activated ATPase activities of these myosins suggest structural variances in the actin-binding domain (Mornet et al., 1981) between these myosins. Structural similarities between heavy chains of atrial and thyrotoxic ventricular myosin based on one-dimensional peptide mapping (Libera and Sartore, 1981), however, should be viewed with caution. It has been shown that such structural differences between heavy chains of white skeletal myosin and thyrotoxic myosin could be demonstrated only by two-dimensional peptide mapping techniques (Flink et al., 1979). Although heterogeneity of light chains of atrial and thyrotoxic ventricular myosin cannot be ruled out, recent evidence (Wagner and Giniger, 1981; Sivaramakrishnan and Burke, 1982) suggests that light chains are not essential for actin-activated Mg++-ATPase activity in vitro.

The atrial myosin from thyrotoxic as well as thyroidecomitised rabbits shows the same two pre-existing isoenzymes, suggesting that thyroid hormone neither stimulates nor deactivates preferential synthesis of the atrial isoenzymes. This is in contrast to the observed effect in ventricular myosin in which V1 isoenzyme is preferentially stimulated in hyperthyroidism but V2 isoenzyme is preferentially stimulated in hypothyroidism. The mechanisms for these differential effects of thyroid hormone on the ventricular and atrial myosin are not clear. Several inferences can be drawn: (1) the thyroid hormone effect may proceed via different pathways, depending on the nature of the target tissues; (2) thyroid hormone may affect specific genes present in the ventricles; and (3) factor(s) of initiating isoenzyme redistribution may be related to functional demands on the heart induced by thyroid hormone.

A comment should be made regarding the actin-activation of the Mg++-ATPase activities of ventricular and atrial myosin. This activity, measured in vitro by the methods described here and by others, is much less than that determined in intact muscles (Barany and Burt, 1979). Recently, Mornet et al. (1981) attached actin covalently to S1 subfragment and showed that actin activates the Mg++-ATPase activities by thousand-fold rather than by the 10- to 20-fold activation observed here. The significant difference in the degree of activation may result from the covalent actin-S1 derivative-mimicking assembly in vitro of the interacting actin-head complex found in the native cell.

In conclusion, the results presented here demonstrate that thyroid hormone does not appear to modulate the ATPase activities and isoenzyme redistribution of atrial myosin, whereas ventricular myosin ATPase activities and isoenzyme patterns are closely regulated by thyroid hormone. Investigations to clarify the structural basis for this differential response of atrial and ventricular myosin to thyroxine have to be pursued in great detail to elucidate the inability of thyroid hormone to regulate the atrial myosin isoenzymes.

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References


Barany M (1967) ATPase activity of myosin correlated with speed of muscle shortening. J Gen Physiol 50: 197–216


Flink IL, Rader JH, Banerjee SK, Morkin E (1978) Atrial and
ventricular cardiac myosins contain different heavy chain species. FEBS Lett 94: 125-130
Flink IL, Rader JH, Morkin E (1979) Thyroid hormone stimulates synthesis of a cardiac myosin isozyme. J Biol Chem 254: 3105-3110
Syrovy I, Delcayre C, Swynghedauw B (1979) Comparison of ATPase activity and light chain subunits in myosin from left to right ventricles and atria in seven mammalian species. J Mol Cell Cardiol 11: 1129-1135

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