Hormonal Control of Cardiac Myosin Adenosine Triphosphatase in the Rat

By Michael J. Rovetto, Ake C. Hjalmarson, Howard E. Morgan, Michael J. Barrett, and Richard A. Goldstein

ABSTRACT

Cardiac function was impaired in hearts from hypophysectomized rats. Corresponding to the decrease in performance, myosin from the hearts of these rats exhibited a decreased Ca$^{2+}$-activated ATPase activity. Myosin ATPase activated by K$^+$ or NH$_4^+$ was not different in any of the preparations studied. The decreased Ca$^{2+}$-activated ATPase was observed in cardiac myosin but not in red or white skeletal muscle myosin. Treatment with thyroxine, but not with growth hormone, restored cardiac myosin ATPase and performance to normal. Hypothyroidism produced a decrease in cardiac myosin ATPase activity comparable to that seen after hypophysectomy, suggesting that the absence of thyroid hormone was responsible for the alterations. However, hyperthyroidism did not increase ATPase activity above normal values. Adrenalectomy failed to alter myosin ATPase. Anion exchange chromatography of the myosins and addition of various concentrations of Na$^+$, Ca$^{2+}$, and Mg$^{2+}$ did not abolish the differences in myosin ATPase. The results strongly suggest that the lower myosin ATPase activity in the absence of thyroid hormone was the result of a change in the myosin molecule per se and that the lower enzymatic activity was involved in the lower cardiac performance in these rats.

KEY WORDS  cardiac function thyroid hormone hypothyroidism hyperthyroidism adrenal insufficiency skeletal muscle myosin

Several recent studies have shown an alteration in the enzymatic activity of myosin from hearts of animals in abnormal hormonal states. A decrease was found in ATPase activities of myofibrils, actomyosin, and myosin from adrenalectomized cats (1) that correlated with cardiac function (2). An increase in myosin ATPase activity was noted in hyperthyroid guinea pigs (3). In a preliminary report of the present work, we reported that decreased ventricular function and decreased ATPase activity of myosin were found in hearts of hypophysectomized rats (4).

The present work was undertaken (1) to further characterize the alteration in cardiac function and myosin ATPase in hypophysectomized rats, (2) to identify the hormone involved in this abnormal state, and (3) to try various assay conditions and modifications of myosin which might normalize the apparent low specific enzymatic activity of cardiac myosin from hypophysectomized animals.

Methods

ANIMAL TECHNIQUES

Normal, hypophysectomized (hypox) or adrenalectomized (adrenex) male Sprague-Dawley rats were used. The adrenex rats were given 0.9% saline to drink but otherwise only routine care and feeding was given to the rats.

Thyroxine, growth hormone, and propylthiouracil were administered subcutaneously daily at the levels noted in the figures and tables. Control animals received the solvent that was used for each of the drugs. After 7–15 days of drug injections or 7–15 days after operation the animals were anesthetized with pentobarbital sodium and the heart, gastrocnemius, soleus, and diaphragm muscles were quickly excised and placed in ice-cold 0.9% saline. The hearts were either perfused as working heart preparations (5), homogenized
for isolation of myosin, or frozen for storage over liquid nitrogen. The skeletal muscles were either homogenized for isolation of myosin or frozen for later preparation of the protein.

**CARDIAC FUNCTION DETERMINATION**

The maximum rate of pressure development (dP/dt) and peak left ventricular pressure were measured at left atrial pressures from 0 to 25 cm H2O, at 5-cm steps during perfusion as working heart preparations (5). Left atrial pressure was adjusted by varying the height of the left atrial bubble trap above the heart. The pressure head against which the hearts pumped was maintained at 60 mm Hg. Left ventricular pressure and dP/dt were recorded via a Statham pressure transducer connected to a 20-gauge needle that was placed into the left ventricle through the apex. Maximum rate of pressure development and peak left ventricular pressure were obtained with Tektronix type 3A6 operational and type 3A9 differential amplifiers, respectively. The outputs from the amplifiers were recorded on a Tektronix type RMS64 storage oscilloscope. Cardiac output was calculated by summing the coronary flow and the volume of fluid pumped by the heart against the hydrostatic pressure head of 60 mm Hg.

**MYOSIN ISOLATION**

Myosin was prepared by the differential centrifugation method of Weeds and Hartley (6). Frozen tissue that had been pulverized at liquid nitrogen temperatures in a percussion mortar or fresh tissue that had been cut into small pieces was homogenized in Dounce homogenizers in 3 volumes of a solution containing 0.10M KH2PO4, 0.05M K2HPO4, 0.3M KCl, pH 6.5 at 0°C. Samples contained 3–10 hearts, 3–5 gastrocnemius muscles or combined diaphragm and soleus muscles from 10–20 rats. No differences could be detected in either yield or ATPase activity of myosins from normal and experimental animals.

The remainder of the isolation procedure was the same as that described by Weeds and Hartley (8) except that the step using batch treatment with DEAE Sephadex for removal of nucleoproteins employed DEAE-A25 rather than DEAE-A50 Sephadex, and that the final precipitation by dilution employed 1mM sodium-free ethylenedi-aminotetraacetic acid (EDTA), pH 6.5, rather than disodium EDTA. The pH of all buffers was adjusted with either Trisbase or acetic acid, and the ATP that was used in the assays was the Tris salt. These steps minimized contamination of samples with sodium.

The final myosin solution in 0.5M KCl was diluted to about 1 mg of protein/ml before assay of ATPase activity. Protein concentration was determined by the absorbance at 280 and 260 nm (7), the Lowry method (8) using bovine serum albumin as a standard, or by absorbance in 0.5M NaOH at 291 and 350 nm (9). All three methods were standardized against micro-Kjeldahl determinations, assuming 16% nitrogen content. The absorbance at 280 and 260 nm was routinely used because of the rapidity with which determinations could be made. With this method, the absorbance ratio (280/260 nm) of myosin was between 1.15 and 1.35. No significant differences in the ratio were found between myosins from normal and experimental animals.

**DETERMINATION OF ATPASE ACTIVITY**

Assays were run for 5 minutes at pH 7.5 and 25°C. The rate of ATP hydrolysis was found to be linear over this period. The reaction was initiated by addition of myosin (0.2 ml) to a reaction mixture (1.8 ml) to give a final protein concentration of 0.1 mg/ml. The reaction was stopped by adding 15% trichloroacetic acid (1 ml), and the precipitated protein was removed by centrifugation. ATP hydrolysis was followed by measuring the appearance of inorganic phosphate in the supernatant fraction, using the procedure of Harris and Popat (10). The routine reaction mixtures contained 50 mM Tris and 5 mM Tris-ATP. In addition, Ca2+-activated ATPase was assayed in the presence of 0.1mM KCl and 10 mM CaCl2. Reaction mixtures for assay of monovalent cation-activated ATPase contained either 1mM NH4Cl or 1mM KCl and 5 mM EDTA. Variations in these assay mixtures are reported with the table and figures. For simplicity in presentation of results and discussion, ATPase activities measured in Ca2+-containing solutions or with EDTA in the presence of NH4+ or K+ will be referred to as Ca2+-ATPase, NH4+-ATPase, and K+-ATPase, respectively.

**COLUMN CHROMATOGRAPHY OF MYOSIN**

Cardiac myosin (50–150 mg) isolated as described above was purified by chromatography on DEAE-A50 Sephadex using the method of Richards et al. (11) or on a phosphocellulose column followed by a DEAE cellulose column (12). The columns were 2.5 x 25–35 cm. Myosin that was chromatographed on DEAE-A50 Sephadex was dialyzed overnight against a phosphate buffer, pH 7.5 (0.15mM K2HPO4, 0.01mM EDTA) and diluted to 2–3 mg protein/ml. The myosin was applied to a column equilibrated with the same buffer. A linear gradient from 0 to 0.5mM KCl was used to elute the myosin from the column. The column effluent was collected at 20-minute intervals (5–6 ml) and the 280-nm absorbance was determined on each fraction. A major peak beginning after 250 ml of eluent had passed through the column contained the myosin. This peak was pooled and exhaustively dialyzed.
MYOSIN ADENOSINE TRIPHOSPHATASE

against 0.04M KCl, 4 mM Tris, pH 6.5. The resulting precipitate was washed twice with fresh dialysis buffer to remove traces of phosphate. The precipitate was dissolved in 0.5M KCl and the ATPase activity was determined as above. About 80% of the protein placed on the column was recovered in the eluate, with 50–70% of the protein in the myosin fraction. No significant differences in recovery of myosin from hearts of normal and hypophysectomized rats was found during the chromatographic procedure.

Chromatography on phosphocellulose and DEAE cellulose was accomplished by precipitating myosin by dilution to 0.04M KCl. The precipitate was dissolved in an equal volume of 0.8M KCl, 0.08M Tris, pH 7.8. The solution was diluted to 5–8 mg protein/ml by adding 0.4M KCl, 0.04M Tris, pH 7.8 and placed on a phosphocellulose column equilibrated with the same buffer. The major peak of protein eluted from the column was immediately placed on a DEAE cellulose column equilibrated with the same buffer. The major peak of protein from this column was pooled, adjusted to pH 7.0, and fractionated with (NH₄)₂SO₄. The fraction precipitating between 32 and 50% saturation was redissolved and dialyzed against 0.04M KCl, 4 mM Tris, pH 6.5. The protein was collected by centrifugation, washed twice with dialysis buffer, dissolved in 0.5M KCl, and assayed as above. The amount of myosin recovered from these columns was less predictable, and the yield was lower than from the DEAE Sephadex columns.

**Results**

**CARDIAC PERFORMANCE IN ALTERED HORMONAL STATES**

The effects of hypophysectomy and of the treatment of hypophysectomized rats with growth hormone and thyroxine on performance of the isolated rat heart perfused with a left atrial filling pressure of 20 cm H₂O are shown in Table 1. Since hypophysectomy reduced heart size and prevented the animal from growing, comparisons of performance were made between normal and hypophysectomized rats matched both for body and heart weight. When comparisons of cardiac function were made between animals of the same body size (Table 1, Group I normal and Group I hypox and Group II normal and Group II hypox), lower peak intraventricular pressure and dP/dt were found in the hearts from hypox animals. In addition, there was a larger cardiac output in the normal II than in hypox II, which was probably due to larger heart...
size in the normal animals. Cardiac atrophy occurred in these hypox animals, as indicated by the lower ratio of heart to body weight. When comparisons were made between hearts of the same size (Group I normal and Group II hypox), a decrease in peak pressure and dP/dt in the hypox preparations was still observed.

Injections of hypox animals with growth hormone (100 \( \mu g/\text{day} \)) or thyroxine (5 \( \mu g/\text{day} \)) for 7 days maintained heart weight (Group I hypox and Group II normal). Although thyroxine (5 \( \mu g/\text{day} \)) failed to maintain body weight in the hypox animals, it restored cardiac performance to normal. On the other hand, growth hormone failed to increase performance to normal levels as judged from development of peak ventricular pressure and dP/dt. Thus, dP/dt and development of peak pressure were decreased in hypox animals. Thyroxine, but not growth hormone, was capable of maintaining these parameters at normal levels. Significant differences in development of peak ventricular pressure and dP/dt in hearts of hypox and normal rats also were observed at 15 cm H\(_2\)O left atrial filling pressure but not at 10 cm H\(_2\)O or below.

### Table 2

**ATPase Activity of Cardiac Myosin in Normal and Altered Hormonal States**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>n</th>
<th>( \text{NH}_4^+ ) ATPase (( \mu \text{moles Pi/mg min}^{-1} ))</th>
<th>( K^+ ) ATPase (( \mu \text{moles Pi/mg min}^{-1} ))</th>
<th>( Ca^{2+} ) ATPase (( \mu \text{moles Pi/mg min}^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>None</td>
<td>3</td>
<td>3.37 ± 0.13</td>
<td>0.61 ± 0.06</td>
<td>0.97 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>GH, 100 ( \mu g/\text{day} )</td>
<td>3</td>
<td>3.54 ± 0.21</td>
<td>0.69 ± 0.12</td>
<td>1.02 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>( \text{Te}_1 ), 5 ( \mu g/day )</td>
<td>3</td>
<td>3.87 ± 0.36</td>
<td>0.63 ± 0.04</td>
<td>1.05 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>GH + ( \text{Te}_1 ), 5 &amp; 100 ( \mu g/\text{day} )</td>
<td>3</td>
<td>3.92 ± 0.32</td>
<td>0.66 ± 0.06</td>
<td>1.08 ± 0.04</td>
</tr>
<tr>
<td>Hypox</td>
<td>None</td>
<td>3</td>
<td>3.25 ± 0.08</td>
<td>0.66 ± 0.08</td>
<td>0.69 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>GH, 100 ( \mu g/\text{day} )</td>
<td>3</td>
<td>3.15 ± 0.13</td>
<td>0.69 ± 0.06</td>
<td>0.69 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>( \text{Te}_1 ), 5 ( \mu g/day )</td>
<td>3</td>
<td>3.18 ± 0.19</td>
<td>0.68 ± 0.02</td>
<td>0.98 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>GH + ( \text{Te}_1 ), 5 &amp; 100 ( \mu g/\text{day} )</td>
<td>3</td>
<td>3.30 ± 0.23</td>
<td>0.66 ± 0.04</td>
<td>1.01 ± 0.06</td>
</tr>
<tr>
<td>Normal</td>
<td>None</td>
<td>24</td>
<td>2.61 ± 0.08</td>
<td>0.75 ± 0.02</td>
<td>0.89 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>PTU, 20 ( \text{mg/day} )</td>
<td>13</td>
<td>2.22 ± 0.06</td>
<td>0.70 ± 0.03</td>
<td>0.70 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>PTU, 40 ( \text{mg/day} )</td>
<td>8</td>
<td>2.52 ± 0.05</td>
<td>0.83 ± 0.03†</td>
<td>0.48 ± 0.04§</td>
</tr>
<tr>
<td>Adrenex</td>
<td>None</td>
<td>8</td>
<td>2.55 ± 0.06</td>
<td>0.77 ± 0.03§</td>
<td>0.87 ± 0.02</td>
</tr>
<tr>
<td>Normal</td>
<td>( \text{Te}_1 ), 100 ( \mu g/\text{day} )</td>
<td>4</td>
<td>2.56 ± 0.12</td>
<td>0.63 ± 0.04†</td>
<td>0.97 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>( \text{Te}_1 ), 600 ( \mu g/\text{day} )</td>
<td>9</td>
<td>2.39 ± 0.06</td>
<td>0.62 ± 0.02†</td>
<td>0.89 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE. n = number of separate myosin preparations assayed; Adrenex = adrenalectomy; PTU = propylthiouracil; other abbreviations as in Table 1.

* = Combined mean of controls for all groups in the lower part of the table; † P < 0.05 vs. normal control; ‡ P < 0.01 vs. normal control; § P < 0.001 vs. normal control; ¶ P < 0.001 vs. hypox + \( \text{Te}_1 \) or hypox + \( \text{Te}_1 \) and GH.
The Ca$^{2+}$-ATPase of myosin from hearts of hypox rats was only 50-70% of the activity of normal preparations (Table 2). Neither the NH$_4^+$-ATPase nor K$^+$-ATPase exhibited any change in activity with hypophysectomy or in any of the other hormonal states shown in this table. Growth hormone was ineffective in maintaining Ca$^{2+}$-ATPase at normal levels, as it had been in maintaining normal cardiac performance. In contrast, thyroxine alone or with growth hormone completely prevented the decrease in the Ca$^{2+}$-ATPase of myosin when given to hypox animals. When thyroxine, growth hormone, or both were given to normal animals, the activity of neither divalent nor monovalent cation-activated ATPase was altered. Although there is a tendency for the combination of thyroxine and growth hormone to increase the Ca$^{2+}$-ATPase above normal values, the significance of the change was questionable, since large doses of thyroxine failed to increase this activity (Table 2).

To distinguish between the effects of deficiency of adrenal and thyroid hormones, cardiac myosin was isolated from adrenalectomized and propylthiouracil-treated rats. Adrenalectomy failed to alter either the monovalent or divalent cation-activated ATPase. Propylthiouracil treatment, on the other hand, induced a dose-related decrease in the Ca$^{2+}$-ATPase similar (40 mg/day) in magnitude to the reduction with hypophysectomy. Thus the lack of thyroxine, but not of glucocorticoids, appeared to be responsible for the change in enzymatic activity. However, thyroxine at very high doses failed to enhance the activity of either the monovalent or divalent cation-activated ATPase. In other experiments, treatment with thyroxine (100 μg/day) for 30 days failed to alter the ATPase activity. However, these large doses did induce cardiac hypertrophy.

**EFFECT OF Ca$^{2+}$, K$^+$, AND NH$_4^+$ ON CARDIAC MYOSIN ATPASE**

The ATPase activity of myosin activated by a range of concentrations of monovalent and divalent cations is shown in Figure 1. The Ca$^{2+}$-ATPase activity of myosin from hypox...
The enzymatic activity was determined as described in Methods. Sodium chloride was added at the concentration on the abscissa. CaCl₂ (10 mM) and KCl (1m) were added as the activating ions. Each value is the mean of three myosin preparations and the vertical lines show the SE.
Although sodium inhibited both Ca\(^{2+}\)-ATPase of cardiac myosin from normal and hypox rats, the activity of the hypox rat myosin was significantly different at all sodium concentrations. Inhibition of K\(^{+}\)-ATPase by sodium was more marked than the inhibition of Ca\(^{2+}\)-ATPase, but no differences in the degree of inhibition were found with any of the myosins assayed.

Sensitivity of myosin from normal, hypox, or thyroxine-treated hypox rats to inhibition by Mg\(^{2+}\) was also similar (Fig. 3). Although Mg\(^{2+}\) was a strong inhibitor of both ATPases, it failed to abolish the differences in Ca\(^{2+}\)-ATPase between normal and hypox preparations. The K\(^{+}\)-ATPase was more susceptible to Mg\(^{2+}\) inhibition than was Ca\(^{2+}\)-ATPase. The half maximal inhibitions occurred at about 30 and 10 \(\mu\)M Mg\(^{2+}\) for the Ca\(^{2+}\)- and K\(^{+}\)-ATPases, respectively.

**EFFECT OF SULFHYDRYL REAGENTS ON CARDIAC MYOSIN ATPASE**

Preferential binding of heavy metal ions to the sulfhydryl groups of cardiac myosin of hypothyroid rats could account for the differences in Ca\(^{2+}\)-ATPase. If this were the case, treatment of myosin with n-ethylmaleimide or dithiothreitol would be expected to abolish the difference.

Skeletal muscle myosin has been shown to be activated by treatment with n-ethylmaleimide (13). This effect appeared to involve modification of sulfhydryl (SH) groups near the enzymatic site of myosin. In the present experiments, myosin was incubated for 40 minutes at 0°C in the presence of varying concentrations of n-ethylmaleimide. The activity of the enzyme thus treated was assayed at 37°C in a reaction mixture that contained 0.1M KCl, 10 mM CaCl\(_2\), 5 mM Tris-ATP and 50 mM Tris, pH 7.5. There was no significant alteration in Ca\(^{2+}\)-ATPase when 1, 2, or 4 moles of n-ethylmaleimide per 10⁶ g myosin were added. The largest increases were from 1.08 to 1.11 and 0.72 to 0.75 \(\mu\)moles P\(_{i}\)/mg min\(^{-1}\) for myosin from normal and hypox rats, respectively. Increasing the KCl concentration in the assay mixture to 0.6M did not modify

**FIGURE 3**

*Effect of Mg\(^{2+}\) concentration on Ca\(^{2+}\)- and K\(^{+}\)-ATPase of cardiac myosin. The assay solutions for Ca\(^{2+}\)-ATPase were those given in Methods plus the Mg\(^{2+}\) concentration shown on the abscissa. The solutions for assay of K\(^{+}\)-ATPase contained 50 mM Tris, 1m KCl, 5 mM Tris-ATP, and EDTA or Mg\(^{2+}\) concentration shown on the abscissa. Each point represents the mean of three myosin preparations and the vertical lines show the se.*

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the effect of n-ethylmaleimide; at 25°C, only inhibition occurred with any concentration used. Addition of dithiothreitol (5 mM) produced only a small activation of Ca\(^{2+}\)-ATPase and did not modify the difference between cardiac myosins from normal and hypox animals.

**COLUMN CHROMATOGRAPHY OF CARDIAC MYOSIN**

The reduction in Ca\(^{2+}\)-ATPase of cardiac myosin from hypox rats may have been caused by the presence of some inhibitory protein that contaminated the myosin. To check this possibility, myosin was isolated in the usual manner and was then chromatographed on two systems. The first system employed a column of phosphocellulose followed by one of DEAE cellulose. Since the recoveries from this system were variable, a second chromatography procedure utilizing DEAE-A50 Sephadex also was employed.

The results of the two methods of purification were very similar (Table 3). With both methods of purification, the difference between Ca\(^{2+}\)-ATPase of cardiac myosin of normal and hypox rats still existed. There were no differences in either the K\(^{+}\)- or NH\(_4\)\(^{+}\)-ATPase. The enzymatic activities tended to increase following chromatography, but Ca\(^{2+}\)-ATPase showed the largest percent change.

The K\(^{+}\)-ATPase of hypox myosin increased sufficiently to become significantly higher than the normal value following DEAE Sephadex chromatography. The significance of this is not understood. The NH\(_4\)\(^{+}\)-ATPase of myosin prior to chromatography was significantly lower in both hypox groups as compared to normal values. This difference disappeared following column fractionation. Differences in NH\(_4\)\(^{+}\)-ATPase of normal and hypox myosin have not been consistently observed during the course of these studies.

These experiments indicated that the difference in Ca\(^{2+}\)-ATPase between cardiac myosin of normal and hypox rats was an intrinsic property of the myosin molecule and not due to the presence of contaminating ions or proteins. The final series of experiments was undertaken to determine whether a similar difference was present in skeletal muscle myosin.

**SKELETAL MUSCLE MYOSIN ATPASE**

Myosin isolated from skeletal muscle failed to exhibit any alterations in either monovalent or divalent cation-activated ATPase activity following hypophysectomy. This was true at all concentrations of activator ion (Fig. 4). The monovalent and divalent cation ATPases were higher in the predominantly white

<p>| Table 3: Column Treatment of Myosin from Normal and Hypophysectomized Rat Hearts |
|-----------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>NH(_4)(^{+})</th>
<th>K(^{+})</th>
<th>Ca(^{2+})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phosphocellulose</td>
<td>DEAE Cellulose Chromatography</td>
<td>DEAE A50 Sephadex Chromatography</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before (4)</td>
<td>2.62 ± 0.04</td>
<td>0.73 ± 0.03</td>
<td>0.90 ± 0.04</td>
</tr>
<tr>
<td>After (3)</td>
<td>2.53 ± 0.20</td>
<td>0.87 ± 0.02</td>
<td>1.25 ± 0.08</td>
</tr>
<tr>
<td>Hypox</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before (4)</td>
<td>2.23 ± 0.09</td>
<td>0.77 ± 0.04</td>
<td>0.44 ± 0.02()</td>
</tr>
<tr>
<td>After (4)</td>
<td>2.39 ± 0.06</td>
<td>0.92 ± 0.08</td>
<td>0.86 ± 0.03()</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before (4)</td>
<td>2.64 ± 0.09</td>
<td>0.68 ± 0.02</td>
<td>0.94 ± 0.03</td>
</tr>
<tr>
<td>After (4)</td>
<td>2.54 ± 0.11</td>
<td>0.86 ± 0.06</td>
<td>1.24 ± 0.09</td>
</tr>
<tr>
<td>Hypox</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before (5)</td>
<td>2.25 ± 0.11</td>
<td>0.80 ± 0.04()</td>
<td>0.38 ± 0.02()</td>
</tr>
<tr>
<td>After (5)</td>
<td>2.36 ± 0.13</td>
<td>1.16 ± 0.09()</td>
<td>0.70 ± 0.04()</td>
</tr>
</tbody>
</table>

Values are means ± se. Number of myosin samples assayed is in parentheses.

*\(P < 0.05\); †\(P < 0.02\); ‡\(P < 0.005\); §\(P < 0.001\) compared to control values.
(gastrocnemius) than in the mixture of two predominantly red muscles (soleus + diaphragm). In contrast to cardiac myosin, the K⁺-ATPase of gastrocnemius myosin was higher than Ca²⁺-ATPase. It is also interesting to note that Ca²⁺-ATPase of cardiac myosin was higher at all Ca²⁺ concentrations than gastrocnemius myosin (Figs. 1 and 4). The reverse was true for the K⁺- and NH₄⁺-ATPases.

The degree of activation by increasing concentrations of activator ions was considerably different in skeletal muscle compared to cardiac muscle (Figs. 1 and 4). Increasing Ca²⁺ concentration from 0 to 10 mM only slightly activated ATPase of skeletal muscle myosin, whereas a tenfold or greater activation was achieved in cardiac myosin. The high NH₄⁺ concentrations that gave maximal activation in cardiac myosin inhibited both types of skeletal muscle myosin. On the other hand, the shape of K⁺-ATPase activation curves was similar for all three muscle types. K⁺-ATPase differed only in the total activity at any given K⁺ concentrations.

**Discussion**

Decreased cardiac function in hypophysectomized rats has been described in whole animals (14) and heart-lung preparations (15). Also, decreased muscle performance was evident in isolated papillary muscle from hypophysectomized rats, as shown by decreased maximum tension development and rate of tension development (16). Treatment with thyroid hormone, but not with growth hormone, prevented these changes. The present work indicated that a positive relation existed between the reduced cardiac performance and the Ca²⁺-ATPase of myosin. A similar correlation has been reported in adrenalectomized cats (2). In these later reports (2, 15) and the present experiments, impairment of mechanical performance was apparent only at high left atrial filling pressures. Although Ca²⁺-ATPase and mechanical performance were directly related in various hormonal deficiencies, it should be noted that actin is the physiological activator of myosin ATPase in intact muscle. Measurements of actomyosin ATPase were not per-

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**Figure 4**

*Effect of concentrations of activating ion on ATPase of skeletal muscle myosin. The assay conditions were as described in Methods and Figure 1. Three myosin preparations were assayed at each point, and ±x is indicated.*

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formed in the present experiments, but reductions in this activity were reported earlier in adrenalectomized cats (1). The major derangement in hypox rats that accounted for decreased performance appeared to involve a reduction in thyroid function. Thyroxine, but not growth hormone, administered to these animals prevented both the decline in cardiac function and Ca\(^{2+}\)-ATPase of myosin. When thyroid function was reduced by injection of propylthiouracil, Ca\(^{2+}\)-ATPase decreased to the same extent as with hypophysectomy. This result reinforced the suggestion that the major hormonal deficiency responsible for changes in cardiac function in hypox animals was in thyroid hormone. The contribution of corticosteroids to this defect appeared to be of lesser importance as judged from studies of cardiac myosin from adrenalectomized rats. The failure to note a change in Ca\(^{2+}\)-ATPase in cardiac myosin of adrenalectomized rats was in contrast to results obtained with adrenalectomized cats (1, 2). The difference may reflect the well-known tolerance of saline-maintained rats to adrenalectomy.

Injection of large doses of thyroxine did not increase the Ca\(^{2+}\)-ATPase of cardiac myosin. These results are in contrast to those of Thyrum et al. (3), who reported that large doses of thyroxine (0.25 mg/kg day\(^{-1}\)) increased Ca\(^{2+}\)-ATPase of cardiac myosin in guinea pigs. Buccino et al. (17) noted an increased contractility of papillary muscles from hyperthyroid cats (1 mg thyroxine/kg day\(^{-1}\)) and decreased contractility of these muscles from hypothyroid cats. In the present experiments, the lack of effect of toxic doses of thyroxine (2 mg/kg day\(^{-1}\)) on cardiac myosin suggested that thyroxine played a permissive role in affecting activity of the protein or, alternatively, that low doses produced the maximal change in activity. The cardiac hypertrophy that followed injection of high doses of thyroxine indicated that it affected protein turnover either directly or as a result of the increased cardiac work.

Cardiac myosin obtained from many species has a lower Ca\(^{2+}\)-ATPase activity than does white skeletal muscle (18). However, the present work demonstrated that the Ca\(^{2+}\)-ATPase of rat cardiac myosin was about 1.3 times the activity of myosin prepared from the predominantly white-fibered gastrocnemius. When myosin that was prepared from predominantly white skeletal muscle of rabbits was assayed with the same reagents used for assay of rat cardiac myosin, the rabbit skeletal muscle myosin had about the same maximal activity of Ca\(^{2+}\)-ATPase as myosin from the gastrocnemius of the rat (0.55-0.65 \(\mu\)moles Pi/mg min\(^{-1}\)).

The relation between ATPase activity and concentrations of Ca\(^{2+}\) suggested that the concentration of calcium required for half maximal activation of cardiac myosin from hypox rats was unchanged but that the maximal Ca\(^{2+}\)-ATPase was reduced. Similarly, Ca\(^{2+}\)-ATPase was not abnormally sensitive to inhibition by either Mg\(^{2+}\) or Na\(^{+}\). Since the difference in Ca\(^{2+}\)-ATPase between cardiac myosins of normal and hypox rats persisted over a wide range of concentration of these inhibitory ions, the difference in Ca\(^{2+}\)-ATPase would not appear to have resulted from the binding of greater amounts of these two ions to the myosin isolated from hypophysectomized rats. Additional evidence that Mg\(^{2+}\) contamination did not account for the difference in Ca\(^{2+}\)-ATPase was the magnitude of activation with monovalent cations. Activation by these ions was inhibited by Mg\(^{2+}\). This inhibition could be prevented by addition of EDTA (19, 20). If Mg\(^{2+}\) had been present in greater amounts in the myosin preparations from hypox rats, addition of EDTA would have been expected to result in greater K\(^{+}\)-ATPase. However, the increase in activity resulting from 1 and 5 mM EDTA was similar in myosin from normal and hypox rats.

Variations in the concentrations of Mg\(^{2+}\), Na\(^{+}\), K\(^{+}\), or NH\(_{4}\)\(^{+}\) failed to reveal any differences between the monovalent cation-activated ATPase of cardiac myosin from normal and hypophysectomized rats. These results suggested that the site on the enzyme responsible for calcium binding was distinct from the binding site for monovalent cations.
Only the site involved in calcium binding appeared to be modified in hormonal deficiency.

It has been suggested that the monovalent cation and Ca\(^{2+}\)-activated myosin ATPase involved different sulphydryl groups (13). If the SH groups involved in the Ca\(^{2+}\)-ATPase were modified in hormonal deficiency or during isolation of myosin to produce the difference in Ca\(^{2+}\)-ATPase between cardiac myosin of normal and hypophysectomized rats, reaction of these groups with sulphydryl-blocking agents might be expected to remove any difference in Ca\(^{2+}\)-ATPase. However, n-ethylmaleimide failed to significantly enhance the Ca\(^{2+}\)-ATPase of cardiac myosin from either normal or hypox rats when assayed at 0.1M KCl. At 0.6M KCl, both activities were slightly increased, but the difference in ATPase activity still persisted. These results suggest that modification of SH groups was probably not involved in the differences seen in hormonal deficiency. These results also indicated that rat cardiac myosin was less sensitive to n-ethylmaleimide than rabbit skeletal muscle myosin (13, 21).

The difference in Ca\(^{2+}\)-ATPase of cardiac myosin from normal and hypox rats might have resulted from a protein bound to the myosin. These contaminants might inhibit the ATPase activity of hypox myosin more strongly than normal myosin. Starr and Offer (22) have shown that all but a small fraction of the contaminating proteins could be removed by chromatography on DEAE-Sephadex. The difference between the Ca\(^{2+}\)-ATPase of cardiac myosin of normal or hypox rats was not altered by chromatography on either DEAE-Sephadex or phosphocellulose and DEAE-cellulose. These results further indicated that the difference in the ATPase activity of myosin from hypox and normal rats could not be explained on the basis of selective inhibition of myosin from hypox rats by contaminants in the preparation but was due to a change in the myosin per se. The increase in Ca\(^{2+}\)-, K\(^+\)-, and NH\(_4\)\(^+\)-ATPase activities following chromatography may have been due to removal of inhibitory proteins or of myosin that had been inactivated. Removal of inactive myosin would have been expected to increase the activity of both monovalent and divalent cation-activated ATPase to the same extent. The increase in Ca\(^{2+}\)-ATPase was proportionally greater, however, than either K\(^+\)- or NH\(_4\)\(^+\)-ATPase.

The altered ATPase activities of myosin of animals with heart failure (23-25), hormonal deficiency, a period of exercise (26), or cross-innervation of skeletal muscle (27) may result from similar changes in the myosin molecule. Myosin components which might change under these circumstances include both the light and heavy chains. Myosin light chains are distinctive for different types of muscle (28-31) and may function to modify enzyme activity (32-34). It has recently been demonstrated that by exchanging light chains of red and white muscle myosin the specific activities of the reconstituted myosins were similar to the specific activity of the myosin from which the subunits arose (35). In other studies (36), the ATPase activity and the quantity of one of the light chains were found to be reduced in skeletal muscle from vitamin E dystrophic rabbits. Heavy chains of myosin may be altered either by methylation of lysine or histidine residues or by synthesis of new chains. Myosin from white skeletal muscle has been found to contain 3-methylhistidine, but this amino acid was absent from myosin of either red skeletal muscle or heart (37, 38). However, cardiac muscle contains both mono- and trimethyllysine (37). Although it has not been demonstrated that methylation of basic amino acids in the purified protein can modify myosin ATPase activity, the variation in amounts of methylated amino acids between myosins of different muscle types suggests this possibility. Since methylation of amino acids appears to occur following peptide synthesis (39), content of methylated amino acids could be due to a change in the rate of methylation or demethylation. Lobley et al. (36) have found that the amount of 3-methylhistidine was decreased in myosin of skeletal muscle from vitamin E dystrophic rabbits and resembled myosins from adult red
muscle and neonatal skeletal muscle. The hormonal effect on ATPase activity would not appear to involve synthesis of new heavy chains with altered properties. This suggestion is based on the fact that the turnover time for rat heart myosin is about 21 days (40), whereas the hormonal effect was well established after 6–7 days. Studies are presently in progress to investigate possible changes in light and heavy chains.

In contrast to the results with cardiac muscle, neither the monovalent nor divalent cation-activated ATPase of skeletal muscle myosin was altered in hypophysectomized rats, suggesting that the hormone effect may be specific for the heart. The results are similar to those found in adrenalectomized cats in respect to the white-fibered gastrocnemius but are different from those reported in the red-fibered soleus (2). A decrease in Ca²⁺-ATPase of myosin from cat soleus was similar in magnitude to that found in the heart. The apparent discrepancy between the present results in rats and the earlier studies in cats may be due to the species difference or to the use of a combination of diaphragm muscle and soleus to prepare the myosin from red-fibered muscle. A decrease in the Ca²⁺-ATPase of soleus myosin of hypophysectomized rats may have been obscured by the larger mass of diaphragm muscle. These muscles were combined in the present experiments to obtain sufficient tissue for the preparation of myosin.

References


