Reduced Cardiac Myosin Adenosinetriphosphatase Activity in Dogs with Spontaneously Occurring Heart Failure

By Robert J. Luchi, M.D., Eve Marie Kritcher, A.B., and Per T. Thyrum, Ph.D.

ABSTRACT

Cardiac myosin was isolated from the hearts of seven dogs with naturally occurring heart failure. Six of the seven dogs had heart failure secondary to acquired mitral valvular insufficiency; the seventh was believed to have a primary myocardialopathy. The characteristics of this myosin were compared to cardiac myosin from a group of normal dogs studied concurrently.

Cardiac myosin was extracted from heart muscle with a phosphate salt solution and purified by repeated fractionation with ammonium sulfate in the presence of 2M lithium chloride. Myosin from dogs with heart failure had a significantly reduced adenosinetriphosphatase activity compared to myosin from the control group. Sulfhydryl group content, shown to influence myosin enzyme activity, was unchanged in myosin from dogs with heart failure. Molecular weight of myosin was similar in both groups. Sedimentation velocity of myosin from dogs with heart failure was suggestive of a configurational change in the molecule, but this was not confirmed by measurement of the intrinsic viscosity and helical content of the protein. These studies suggested that the reduced contractile performance of the myocardium in congestive heart failure complicating a disease process is, in part at least, the result of depressed myosin adenosinetriphosphatase activity.

ADDITIONAL KEY WORDS

contractile protein cardiac failure canine heart myosin

The reduced contractile performance of the heart in congestive heart failure is characterized by a depression of both the myocardial force-velocity and length-tension curves (1-3). Study of the pathophysiology and biochemistry of failing human and animal heart muscle has not revealed the cause of this impairment in contractility. Results of studies of myocardial metabolism in heart failure have been conflicting (4-8). There is no evidence that in chronic congestive heart failure overstretching of the heart has increased sarcomere length to a point where major disengagement of the actin and myosin filaments has occurred (9). Reduction in myocardial catecholamine stores in heart failure impairs augmentation of myocardial contractility by neurohumoral means but does not explain the underlying abnormality in contraction (10). Studies of cardiac myosin in heart failure induced surgically in dogs have yielded contradictory results for the hydrodynamic characteristics of myosin in heart failure (11, 12). Myosin adenosinetriphosphatase (ATPase) activity was measured in only one study and was not found to be altered in the heart failure state (11). Yet, Alpert and
Gordon (13) found reduced myofibrillar ATPase activity at autopsy in hearts of patients that had died with heart failure.

Myosin ATPase undoubtedly plays an important role in the contraction process (14). In the studies of cardiac myosin mentioned above, the myosin was prepared by the basic Szent-Györgyi method, a method which has been shown to result in an inhomogeneous myosin solution with a low ATPase activity (15). The lithium chloride-ammonium sulfate method of preparing cardiac myosin eliminates the inhomogeneity, including an inhibitor of myosin ATPase activity; the result is a purer preparation of myosin with a significantly higher ATPase activity than myosin prepared by the Szent-Györgyi method (15, 16).

The purpose of this investigation was to apply the lithium chloride-ammonium sulfate method of preparing myosin to the study of the molecular characteristics of cardiac myosin obtained from animals with heart failure. Alteration in cardiac myosin enzyme activity, if present, should be more easily detectable by this method of preparing myosin. We chose as our experimental model the type of heart failure that develops "spontaneously" (as opposed to surgically induced) in dogs.

Methods

Cardiac myosin was obtained from seven dogs with naturally occurring heart failure. The signs of heart failure included dyspnea, cough, distended neck veins, ascites, hepatic congestion, dependent edema of the extremities, pulmonary rales, cardiac enlargement, gallop sounds, and cardiac murmurs. Atrial fibrillation and signs of left ventricular hypertrophy were frequently noted on the electrocardiograms. Six dogs were considered by clinical criteria to have mitral insufficiency and one, a primary cardiomyopathy. Cardiac catheterization was carried out in two dogs. Mitral insufficiency was confirmed by cineangiography, cardiac output was reduced, and right and left ventricular end diastolic pressures were elevated in both dogs. In the remaining four dogs, the clinical findings of heart failure (confirmed by an experienced veterinarian) left no doubt concerning the presence of abnormal ventricular performance. Fifteen dogs were studied concurrently as normal controls. Both the control dogs and the dogs with congestive heart failure were anesthetized by sodium pentobarbital, 20 mg/kg iv. The hearts were excised while still beating within 1 minute after induction of anesthesia. They were immediately placed in ice water and were not disturbed for a period of 1 hour. After the lower half of each ventricle was removed for extraction of cardiac myosin, the hearts of dogs with congestive heart failure were subjected to pathological examination. Mitral insufficiency of the type associated with advancing age in dogs was found in six. There was no valve lesion in the seventh; its myocardium showed focal scarring by light microscopy.

Cardiac myosin was purified by the lithium chloride-ammonium sulfate fractionation technique as described previously (15).

Sulfhydryl groups were determined by reacting myosin with p-chloromercuribenzoate (PCMB). The change in optical density at 255 m\(\text{u}\) was measured in a Zeiss PMQ II spectrophotometer. PCMB, two times recrystallized, was dissolved in 0.4M KCl-borate. These solutions were made up before each experiment and their concentrations determined at 232 m\(\text{u}\) using 1.69 \(\times\) 10\(^4\) as the molar absorbancy index. The reaction mixture consisted of 2 ml of PCMB and 1 ml of myosin, containing approximately 1 mg. The blank consisted of 2 ml 0.4M KCl-borate and 1 ml myosin.

Adenosinetriphosphatase activity was determined in a reaction mixture containing 0.2M tris-0.025M maleic acid buffer, pH 6.5, as outlined previously (15, 16). The ATP concentration was \(1.6 \times 10^{-5}\)M, the calcium concentration \(1 \times 10^{-2}\)M, and the myosin concentration in the order of \(10^{-5}\)M. The incubation temperature was 30°C. Adenosinetriphosphatase activity is expressed as moles phosphate liberated per milligram of protein in 1 minute (moles P/mg protein • min). Duplicate determinations of ATPase activity were done on each sample of myosin. Approximately 8% of the ATP was hydrolyzed during the 5-minute incubation period, and the rate of reaction was essentially linear over this time interval. Protein concentrations were measured by the micro-Kjeldahl technique assuming that nitrogen accounts for 16% of the weight of cardiac myosin.

Ultracentrifugal analyses of cardiac myosin were performed under the conditions described previously (15). Sedimentation coefficients were determined by measuring the sedimentation rate at each protein concentration and extrapolating the results to infinite dilution. Molecular weight determinations were done by the Archibald approach to equilibrium method using the Schlieren optical system at a speed of 7,977 rpm. Molecular weight determinations were made both from calculations at the meniscus and at the

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VISCOSITY determinations were made at 15.00 ± 0.01°C in a capillary viscometer with a shear gradient of 395 sec⁻¹.

The percent helical content of the myosin molecule was estimated from optical rotatory dispersion data. Measurements were made on a Durrum-Jasco spectropolarimeter, model ORD/UV-5, using a xenon arc light source, a cell path length of 0.05 dm, and a symmetrical angle of 0.5°. Measurements were made through the wavelengths 200 to 300 μm at 23°C. Myosin was measured at a concentration of 0.05% in 0.4M KCl, pH 6.8. The optical rotatory dispersion data were evaluated in terms of the magnitude of the rotation trough at 233 μm, as expressed by Simmons et al. (17) in the following formula:

\[
[R'] = \left( \frac{\alpha_M}{LC} \right) \left( \frac{3}{n^2 + 2} \right),
\]

where \([R']\) is the specific residue rotation, \(\alpha_M\) is the observed rotation in angular degrees at wavelength \(\lambda\), \(L\) is the cell length in decimeters, \(C\) is the concentration of the solute in g/100 ml, \(M\) is the mean amino acid residue weight (assumed value 115), and \(n\) is the index of refraction of the solvent. The \(n\) value was taken from the compilations of Timmermans (18) and evaluated as 1.395 at 233 μm. The percent helix was calculated by linear interpolations (19), assuming that the specific residue rotation at 233 μm of a fully coiled helix is −16,200° and a randomly coiled helix is −1700°.

The principle of least squares was used in the construction of all graphs.

Results

ATPase Activity

The ATPase activity of myosin obtained from control dogs and dogs with heart failure is presented in Table 1. That from normal hearts is 18% greater; the difference is statistically significant (\(P < 0.001\)). This difference in ATPase activity was also apparent when myosin ATPase was measured in an incubation mixture containing 0.5M KCl-0.05M tris buffer (pH 8.0) and 5 × 10⁻²M calcium chloride. The incubation temperature was 25°C. The mean ATPase activity for myosin obtained from five normal dogs was 4.60 ± 0.31 × 10⁻⁷ moles P/mg protein·min. The mean ATPase value for myosin obtained from five dogs with heart failure was 3.60 ± 0.28 × 10⁻⁷ moles P/mg protein·min (\(P < 0.001\)). Calcium activation (maximum activation 1 × 10⁻²M) and pH optima (6.5 and 8.5) of myosin ATPase were the same for myosin whether obtained from normal dogs or dogs with heart failure. The affinity between ATP and the enzyme is indicated by the reciprocal of the Michaelis constant (\(K_m\)), derived from a Lineweaver-Burk plot. The \(K_m\) for the interaction between the cardiac myosin enzyme obtained from normal dogs and ATP is 3.83 × 10⁻⁵ (average of five determinations). Myosin obtained from dogs with heart failure gave a \(K_m\) for the myosin-ATP interaction of 7.51 × 10⁻⁵ (average of two determinations, 6.93 × 10⁻⁵ and 8.08 × 10⁻⁵). These values were not considered to be different from one another.

Optical Rotatory Dispersion

Optical rotatory dispersion curves for normal myosin and myosin from dogs with congestive heart failure were identical. Esti-
mation of the percent helix was made from measurements of the amplitude of the negative Cotton effect at 233 mµ. The mean values (Table 1) were not significantly different.

Sulfhydryl Group Determination

Myosin obtained from normal dogs had a sulfhydryl (SH) content per mole of myosin that was not significantly different in the two groups of dogs studied (Table 1). Recalcula-
tion of the SH-groups per 100 g of protein or per mole of protein when the same molecular weight is used also gave values for SH-content which were not significantly different from one another.

Viscosity

The graph of reduced viscosity as a function
The molecular weight of normal myosin (left) and myosin from dogs with congestive heart failure (right) as a function of protein concentration. The ordinate in both graphs is the reciprocal of the apparent molecular weight determined at any given protein concentration. The molecular weights at infinite dilution are given in Table 1.

of protein concentration is shown in Figure 1. The intrinsic viscosity by extrapolation for normal myosin and for myosin from dogs with heart failure did not differ significantly from one another (Table 1).

Sedimentation Velocity and Molecular Weight

Figure 2 shows the corrected sedimentation coefficient plotted as a function of protein concentration. The sedimentation coefficients at infinite dilution for myosin from normal dogs were less than for myosin prepared from dogs with heart failure. The higher mean slope and intercept value of myosin from dogs with heart failure is statistically significant \( P < 0.002 \) although the scattering of points is greater.

Molecular weight determinations are plotted in Figure 3 as the reciprocal of the apparent molecular weight on the ordinate and protein concentration on the abscissa. The molecular weight of myosin from normal dogs was not significantly different from that of myosin from dogs with heart failure (Table 1).

Discussion

The dogs with heart failure differed from the controls in two other respects: all had received digitalis and all were in the age group of 8 to 12 years. The following studies suggest that these differences were not determining factors in the results obtained. In two control dogs a full digitalizing dose of digoxin was given intravenously before they were killed. The cardiac myosin ATPase activity was not altered by the digoxin given in vivo, nor did digoxin or digitalis added to cardiac myosin solution in vitro result in a change in the observed ATPase activity. The ages of the dogs in the control group ranged from approximately 4 months to more than 9 years of age. In a separate group of control dogs, we were able to demonstrate that cardiac myosin ATPase activity did not vary as a function of age (Table 2). Thus, the difference in ATPase activity between the control group and the group with heart failure does not appear to be related to drug therapy or to a comparison between a young control group and a group of old dogs with heart failure. The difference in enzyme activity appears to be related to the heart failure state per se.

Three other factors should be mentioned in considering the interpretation of our observation of reduced cardiac myosin ATPase activity. First, we have not excluded the possibility that an ATPase inhibitor is present in our final myosin preparation in spite of the purification procedures. Second, our results could be explained on the basis that myosin from dogs with heart failure gives a falsely high result for protein concentration and, therefore, a falsely low enzyme specific
TABLE 2

Effect of Age on Normal Canine Cardiac Myosin ATPase Activity

<table>
<thead>
<tr>
<th>Age</th>
<th>No. dogs</th>
<th>Myosin ATPase activity (10⁻² moles P/mg protein • min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 mo.</td>
<td>2</td>
<td>8.86</td>
</tr>
<tr>
<td>4 mo.</td>
<td>3</td>
<td>8.83</td>
</tr>
<tr>
<td>1 yr.</td>
<td>5</td>
<td>8.88</td>
</tr>
<tr>
<td>2 yr.</td>
<td>3</td>
<td>8.86</td>
</tr>
<tr>
<td>5 yr.</td>
<td>3</td>
<td>8.80</td>
</tr>
<tr>
<td>6 yr.</td>
<td>4</td>
<td>8.82</td>
</tr>
<tr>
<td>8 yr.</td>
<td>2</td>
<td>8.87</td>
</tr>
<tr>
<td>9 yr.</td>
<td>3</td>
<td>8.85</td>
</tr>
<tr>
<td>10 yr.</td>
<td>2</td>
<td>8.81</td>
</tr>
</tbody>
</table>

activity. We do not know of any factors that might alter the microKjeldahl determination of myosin from dogs with heart failure in this fashion. Third, other possible differences between myosin from dogs with heart failure and control myosin have not been explored, viz., ATPase activities of these two kinds of myosin as a function of added actin, elution profiles from DEAE-cellulose, and acrylamide gel electrophoretograms after disrupting hydrogen bonding in 10M urea.

In the one other study of cardiac myosin ATPase activity in heart failure, Olson et al. (11) found no difference between the control group of dogs and the group with heart failure. There are several factors that might explain the difference between their study and ours. The myosin solution studied by Olson et al. (11) had enzyme activities approximately one-third to one-half of those reported in this study, presumably because of an inhibitor of cardiac myosin ATPase activity that was not removed by the Szent-Györgyi method of myosin preparation (16). The standard deviations of the ATPase activity of both the control myosin and myosin from dogs with heart failure were quite large. Thus, the presence of the myosin ATPase inhibitor resulting in low and variable enzyme activity with a considerable scatter of results may have masked a significant difference in enzyme activity of the myosin obtained from control dogs and dogs with heart failure. Finally, the etiology of the heart failure (surgically induced versus “spontaneous” or disease-induced may be important in terms of whether an alteration in dog cardiac myosin ATPase activity is found. The ATPase activity of cardiac myosin prepared by the lithium chloride-ammonium sulfate technique was not determined in dogs with surgically induced heart failure; nor was cardiac myosin obtained from dogs with spontaneous heart failure and prepared by the basic Szent-Györgyi method subjected to enzyme analysis. Until these determinations are made, the status of cardiac myosin enzyme activity in heart failure of any etiology cannot be considered settled once and for all.

The magnitude of the abnormality in cardiac myosin heart failure in dogs probably has been underestimated by our study. No attempt was made to quantify the amount of myosin present in relation to other components of the contractile apparatus, and it remains possible that a disproportionate reduction in cardiac myosin may exist in heart failure. More importantly, the selectivity of the lithium chloride-ammonium sulfate technique for preparing cardiac myosin is such that more grossly distorted myosin molecules were undoubtedly discarded during the purification procedure. In dogs with heart failure, the increase in protein content in the fractions collected at 38% and 90% saturation with ammonium sulfate (purified cardiac myosin precipitates at 45% saturation) supports this view.

The study that perhaps bears the closest relationship to ours is that of Alpert and Gordon (13). They found reduced myofibrillar ATPase activity in hearts of humans that
had died with congestive heart failure secondary to hypertensive cardiovascular disease. The reduction in myofibrillar ATPase activity was of the same order of magnitude as described in this report. More recently, Chandler et al. (20) reported reduced myofibrillar ATPase activity in cats with heart failure accompanying surgically induced pulmonic stenosis. These studies offer circumstantial evidence of reduced myosin ATPase activity in heart failure and in that sense support our data. It would appear, therefore, that reduction in cardiac myosin ATPase activity must be considered an important factor in the abnormal myocardial contractile performance associated with the heart failure state.

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References

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