Sarcolemmal and Sarcoplasmic Reticular ATPase Activities in the Failing Canine Heart

By Richard J. Mead, Myron B. Peterson, and Joseph D. Welty

ABSTRACT

The specific activity of both the sarcolemmal Na⁺-K⁺-activated and the sarcoplasmic reticular ATPase systems was studied in dogs with right ventricular hypertrophy and congestive heart failure induced by progressive pulmonary artery stenosis. In these enzyme preparations, mitochondrial or cross contamination of either of the cellular fractions studied did not appear to be a factor. In the sarcoplasmic reticular fraction, ouabain was without effect and succinic dehydrogenase assay showed less than 10% of the activity observed in pure mitochondrial preparations. In the sarcolemmal fraction, sodium azide did not significantly inhibit the ATPase activity, but ouabain inhibited 91% of it. The activity of the sarcolemmal ATPase system of the failing heart did not differ from that of the control; however, the activity of failing heart sarcolemmal preparations was found to be only 61% inhibited by 10⁻⁴M ouabain, and the activity of control preparations was 91% inhibited. The sarcoplasmic reticular ATPase activity from right ventricles of dogs with congestive heart failure was significantly less (P<0.001) than the respective control activity (average 15.9 compared to 28.1 µmole hr⁻¹ mg⁻¹), whereas the activity from left ventricles of failing hearts did not differ significantly from the respective control value. These findings suggest that a possible explanation for the diminished contractility of the chronically failing heart lies within the subcellular enzymatic ion-regulating systems.

KEY WORDS Na⁺-K⁺ ATPase ouabain pulmonary artery stenosis right ventricular hypertrophy congestive heart failure myocardial contractility

Recent studies strongly indicate that reduced myocardial contractility is characteristic of failing heart muscle (1, 2). The fundamental mechanisms involved are not well defined, although it is possible that abnormal intracellular ion distribution may result in an alteration in the excitation-contraction coupling process (3).

Normal muscle function is dependent on both the intracellular Ca²⁺ and Na⁺-K⁺ concentrations, which are regulated by the interactions of four major subcellular elements: mitochondria, contractile proteins, sarcoplasmic reticulum, and sarcolemmal system (4, 5). Two distinct events arising from these interactions are directly related to the excitation-contraction coupling mechanism: depolarization of the sarcolemmal system via Na⁺-K⁺ flux, and transport of Ca²⁺ by the sarcoplasmic reticulum. Both processes involve energy-dependent ion transfer that can be related to active adenosinetriphosphatase (ATPase) systems (6-12).

The ATPase activity of the sarcolemmal system has two components. The major one is Na⁺-K⁺-activated, Mg²⁺-dependent, and subject to ouabain inhibition, whereas the other is Mg²⁺-activated and unaffected by ouabain (10). The total ATPase activity associated with the sarcoplasmic reticular system also has two components: one is calcium-stimulated and the other a basal component which is measured in the absence of Ca²⁺ (13). Both

From the Department of Physiology and Pharmacology, University of South Dakota School of Medicine, Vermillion, South Dakota 57069.

This work was supported in part by U. S. Public Health Service Grant HE 00294-06 from the National Heart and Lung Institute and by a grant from the South Dakota Heart Association.

Received November 30, 1970. Accepted for publication April 19, 1971.
of these components are Mg²⁺-dependent and unaffected by ouabain.

Since the normally functioning heart requires the precise regulation of ion distribution, a lesion in either or both of the ATPase systems could be a contributing factor in congestive heart failure (7, 11, 14, 15).

The purpose of the present study is to assess the activity of both the sarcolemmal and the sarcoplasmic reticular ATPase systems isolated from common samples of failing canine heart muscle.

Methods

Healthy male and female mongrel dogs, 1–4 years of age and weighing 15–27 kg, were used in this study. Water and dry chow were provided ad libitum to all animals. Body weights remained stable in all dogs except for increases observed with the appearance of ascites.

Right ventricular hypertrophy and congestive heart failure were produced by the method of Bishop and Cole (16). All thoracic surgery was done with the animal under pentobarbital sodium anesthesia. Intracardiac pressure measurements (mean right atrial, right atrial systolic, right ventricular systolic, and right ventricular end-diastolic) were made prior to inflation, and immediately before ventricular end-diastolic pressure measurements (mean right atrial, right atrial systolic, right ventricular systolic, and right ventricular end-diastolic) were made prior to surgery, at each inflation, and immediately before the dogs were killed (two animals were killed without pressure measurements because of their critical condition). Cardiac catheterization was accomplished by percutaneous puncture of the right jugular vein. A 7.5 French radiopaque polyethylene catheter with two laterally opposed side openings was inserted into the heart under fluoroscopy and connected to a Statliam P23AA pressure transducer placed at the level of the midaxillary line. The pressure data were recorded with a Sanborn recorder (model 964).

Right ventricular size was followed by serial electrocardiography and radiography. Right ventricular hypertrophy was confirmed by direct weight measurement at necropsy (16). Right ventricular hypertrophy and congestive heart failure were considered present when the following signs appeared: peripheral venous distention, ascites, enlarged and palpable liver, dyspnea, and decreased exercise tolerance. This was confirmed by direct intracardiac pressure measurements, thoracic radiographs, electrophrograms, and diagnosis by a staff veterinarian.

The control group of four healthy dogs was arbitrarily chosen from the pool of 11 normal animals. All intracardiac pressure measurements were normal and no clinical signs of disease were seen. The seven remaining animals composed the group with congestive heart failure.

Biochemical Preparations

Animals were anesthetized with pentobarbital sodium and the hearts quickly excised. The chambers were washed with cold water, connective tissue and fat trimmed away, the right and left ventricles separated, and the tissues placed in cracked ice. All subsequent isolation procedures were carried out between 0° and 4°C.

The sarcolemmal ATPase was isolated from 25 g of cardiac tissue by the method of Matsui and Schwartz (10). The final pellet was suspended in 1 mM EDTA to a final protein concentration of approximately 1 mg/ml and the activity immediately assayed. The sarcoplasmic reticular ATPase fraction was prepared by a modification of the technique of Greaser et al. (17). One gram of ventricular tissue was minced and placed in a precooled all-glass tissue grinder. Nine volumes of homogenizing medium (100 mM KC1 and 5 mM histidine, pH 7.2) were added to the tissue and homogenization carried out in an ice bath. The resulting homogenate was centrifuged at 1,000 g for 10 minutes and the pellet discarded. The supernatant was centrifuged at 10,000 g for 20 minutes and the pellet again discarded. The supernatant was centrifuged again at 30,000 g for 90 minutes to sediment the crude sarcoplasmic reticular fraction. The pellet was suspended in 5 ml of 0.6 M KC1 and extracted for 30 minutes in the cold to render the contaminating actomyosin soluble (18, 19). This suspension was again centrifuged at 30,000 g for 30 minutes to sediment the reticular fragments. The pellet was resuspended in 5 ml of original medium and placed on a sucrose gradient (three layers of 8 ml each at 35%, 40%, and 45% sucrose, respectively). Centrifugation was carried out for 90 minutes at 63,600 g. The upper band was removed from the gradient and centrifuged at 60,000 g for 30 minutes. The pellet was suspended in 5 ml of the original medium.

The protein concentrations of all samples were determined by the procedure of Lowry et al. (20), using crystalline bovine serum albumin (Mann Research Laboratories) as a standard. Reagents were prepared with deionized, glass-distilled water.

Determination of ATPase Activity

ATPase activity was determined in both fractions using a linked-enzyme system (12) in which the oxidation of NADH was monitored at 340 μM with a Beckman DB spectrophotometer.

Circulation Research, Vol. XXIX, July 1971
FIGURE 1

Ratios of ventricular weight (g) to body weight (kg) in normal dogs and dogs with right ventricular hypertrophy (RVH) and congestive heart failure (CHF). RV = right ventricular free wall; LV = left ventricular free wall; IVC = interventricular septum; BW = ascites-free body weight. A number in parentheses is the number of dogs. Columns represent means, and standard errors are shown.

equipped with a recorder and constant temperature reaction chamber. Assays were carried out in duplicate at a temperature of 37.0 ± 0.2°C.

Sarcolemmal ATPase activity was measured in an incubation medium containing, in mM (final concentrations): 3 MgCl₂, 100 KCl, 20 KC1, 30 Tris-HCl (pH 7.2), 3 Tris-ATP, 0.5 NADH, 2.5 phosphoenolpyruvic acid, and 0.05 ml of a combined pyruvate kinase-lactic dehydrogenase suspension. The reaction was started by adding the isolated protein in concentrations of 70–120 µg (0.1 ml). Final volume of this reaction mixture was 2 ml. No ouabain was added to this system after initial studies demonstrated that it did not inhibit the ATPase activity of this preparation. Atomic absorption analysis of the assay system following incubation showed a calcium concentration of ouabain. In addition, the absence of mitochondrial contamination was confirmed by the failure of sodium azide (10⁻³ M) to inhibit the sarcolemmal ATPase activity.

The sarcoplasmic reticular ATPase activity was assayed in essentially the same manner as that of the sarcolemmal system. The incubation medium consisted of (in mM, final concentrations): 20 MgCl₂, 100 KCl, 5 histidine, 10 Tris-HCl (pH 7.2), 3 Tris-ATP, 0.5 NADH, 2.5 phosphoenolpyruvic acid, and 0.02 ml of a combined pyruvate kinase-lactic dehydrogenase suspension. The reaction was started by adding the isolated protein in concentrations of 70–120 µg (0.1 ml). Final volume of this reaction mixture was 2 ml. No ouabain was added to this system after initial studies demonstrated that it did not inhibit the ATPase activity of this preparation. Atomic absorption analysis of the assay system following incubation showed a calcium concentration of

FIGURE 2

Intracardiac blood pressure determinations taken immediately before death in normal dogs and dogs with right ventricular hypertrophy (RVH) and congestive heart failure (CHF). RA = right atrial; RV = right ventricular. A number in parentheses is the number of dogs. Columns represent means, and standard errors are shown.

ATPase activity in failing canine heart

approximately $4 \times 10^{-3}$ M. Since that concentration was applicable for the present study, no exogenous Ca$^{2+}$ was added to the assay system. No appreciable mitochondrial contamination was present in the sarcoplasmic reticular fraction, since the succinic dehydrogenase assay yielded a specific activity which was less than 10% of that obtained from a pure mitochondrial preparation (13, 21).

Results

Seven dogs developed right ventricular hypertrophy and congestive heart failure following one to three constrictions of the pulmonary artery. The complete syndrome developed approximately 50 days following the initial surgery. The ratios of heart weight to body weight measured at necropsy are presented in Figure 1. A significant difference ($P < 0.001$) between control dogs and dogs with congestive heart failure was seen when ratios of right ventricular weight to body weight were calculated, but there was no significant difference between these two groups in ratios of left ventricular plus interventricular septal weight to body weight. These findings indicate that right ventricular hypertrophy was present without left ventricular hypertrophy.

Intracardiac pressure measurements are given in Figure 2. All pressure measurements obtained from dogs with congestive heart failure were found to differ significantly ($P < 0.001$) from those of the control dogs. In all dogs with failure, right ventricular end-diastolic pressure was elevated to at least 10 mm Hg. Elevation of right atrial mean and right ventricular end-diastolic pressures correlated well with the development of ascites. In addition to the signs of congestive heart failure present in all dogs, three dogs had hydrothorax (22).

The ATPase activities of sarcoplasmic reticulum from right ventricles from animals with congestive heart failure differ significantly from their respective controls (Table 1). The enzyme activity of right ventricular preparations from dogs with failure was markedly lower than that of control preparations ($P < 0.01$) or that of left ventricular preparations from the same hearts. Although no strict quantification was carried out, the amount of sarcoplasmic reticular protein appeared to be lower in the fraction isolated from the right ventricles of dogs with failure than in other samples. Furthermore, these right ventricular samples from dogs with failure exhibited a diffuse band in the sucrose gradient.

The ATPase activities of sarcolemma from four control animals and six animals with congestive heart failure were measured (Table 1). No significant differences were observed between the two groups in either the right or left ventricle when specific activity of the total system was measured. Inasmuch as the ATPase activity of this system can be inhibited 91% by ouabain in control samples, the finding that the preparations from dogs with failure could not be inhibited more than 60% indicates the possibility of some alteration

| TABLE 1 |

ATPase Activity of Sarcolemmal and Sarcoplasmic Reticular Enzyme Systems from Dogs with Right Ventricular Hypertrophy and Congestive Heart Failure

<table>
<thead>
<tr>
<th></th>
<th>Sarcoplasmic reticular ATPase</th>
<th>Sarcolemmal ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity (umole Pi/hr/mg protein)</td>
<td>Specific activity (umole Pi/hr/mg protein)</td>
</tr>
<tr>
<td></td>
<td>RV</td>
<td>LV</td>
</tr>
<tr>
<td>Control</td>
<td>28.1 ± 3.2</td>
<td>30.7 ± 3.4</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>15.9 ± 1.5</td>
<td>25.8 ± 0.6</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

RV = right ventricle; LV = left ventricle. A number in parentheses is the number of animals. All values are means ± SE.

Circulation Research, Vol. XXIX, July 1971
in the system during failure, despite the unchanged total activity.

Discussion

The sarcolemmal, the sarcoplasmic reticular, or both ATPase systems of cardiac muscle may well be the site of dysfunction contributing to the reduced cardiac contractility observed in chronic congestive heart failure. The activity of the sarcolemmal ATPase is a combination of two ion-activated mechanisms. The major portion of the total activity of this system is due to Na+-K+ activation, i.e., that portion of the total activity which can be inhibited by ouabain, while the remaining activity can be attributed to Mg2+ activation (10,23-27).

No alteration was found in the specific activity of the Na+-K+-activated sarcolemmal ATPase isolated from failing dog hearts. However, the percent of the specific activity that could be inhibited by ouabain was significantly lower (P < 0.001) in the dogs with congestive heart failure. Therefore, by implication, the specific activity of this system must have been maintained by an increase in the activity of the Mg2+-activated component. In a study of failing human hearts (28) the specific activity of the sarcolemmal ATPase system was reported to have been decreased, but the samples analyzed were obtained from patients who had previously received digitalis. Inasmuch as cardiac glycosides are known to depress this enzyme system, any inference concerning this finding with respect to congestive heart failure is limited.

Specific total ATPase activity of the sarcoplasmic reticulum from failing right ventricles was consistently lower than either the corresponding left ventricular or control activities. Since the total activity of the sarcoplasmic reticulum is composed of a measured basal activity and a calculated specific Ca2+-stimulated activity (13), it is uncertain in this study which of these two components was responsible for the reduced total activity. However, it has been reported in a study using calf hearts (13) that the basal ATPase activity is normal and the total activity is reduced, thereby implying that the Ca2+-stimulated ATPase activity is depressed in congestive heart failure. If, as has been suggested, the level of the ATPase activity in the sarcoplasmic reticulum reflects the amount of Ca2+ uptake (14,29,30), the observed decrease in ATPase activity could reflect a reduction in the Ca2+ content of sarcoplasmic reticulum. A further reduction in the Ca2+ content could occur as a result of the inability of the "Ca2+ pump" to successfully compete with the mitochondria, which have been shown to sequester Ca2+ normally in heart failure (31). The data presented herein have implications which lend support to the findings by others in which a reduction was demonstrated in the binding and release of Ca2+ by sarcoplasmic reticulum from failing hearts (13,31-33).

The reduced ATPase activity of sarcoplasmic reticulum could account for the ultimate depression of contractility observed in failing hearts. The observed alteration in the Na+-K+-stimulated sarcolemmal system is consistent with the finding of an abnormal Na+ distribution in the failing heart muscle (34,35).

The finding of alterations in the two enzyme systems studied suggests that defective ion transport is instrumental in the development of congestive heart failure.

References

6. Fanburg, B., Fenkel, R.M., and Maironos, M.
ATPase Activity in Failing Canine Heart


Sarcolemmal and Sarcoplasmic Reticular ATPase Activities in the Falling Canine Heart
Richard J. Mead, Myron B. Peterson and Joseph D. Welty

_Circ Res._ 1971;29:14-20
doi: 10.1161/01.RES.29.1.14

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1971 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/29/1/14

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/