Depressed Na-K-ATPase Activity in the Failing Rabbit Heart

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SUMMARY

We studied the changes in the membrane ATPase from hypertrophied rabbit myocardium, and demonstrated a correlation between heart failure and ionic transport mechanism.

In 17 animals cardiac hypertrophy was produced by constriction of the ascending aorta. A modification of the method of Tashima was used for preparation of membrane vesicles from the myocardium of the left ventricle. This preparation was carried out 10 days after the operation. Mg- and Na-K-ATPases of the membrane ATPases were measured separately by their different responses to ouabain and cations.

(1) Ventricular weight and heart weight were significantly greater in the experimental group than in the control group. However, the myocardial water content was almost constant in these groups.

(2) The specific activity of Na-K-ATPase in myocardial vesicles was relatively high compared with skeletal muscle and corresponded to about one fourth of its total ATPase activity. This Na-K-ATPase activity was inversely correlated with the left ventricular weight (p<0.02). The difference in Na-K-ATPase activity between heart failure cases and controls was significant at the 5% level. On the other hand, no significant difference in Mg-ATPase activity between the failure and the control group was demonstrated.

These results supported the fact that, during the development of heart failure, the myocardium lost potassium. From these findings, it was postulated that the heart in failure would be characterized by a diminished ability to manipulate active ion transport in response to hemodynamic stress due to the decrease in Na-K-ATPase activity.

Additional Indexing Words:
Aortic constriction Cardiac hypertrophy Heart failure Na-K-ATPase

The mechanism of the active transport system for cations has interested many investigators. Recently it has become apparent that membrane ATPase, especially Na-K-ATPase, has a part in active cation transport across cell membrane and plays an important role in the control of tissue electrolyte...
balance.\textsuperscript{1)–3)} This enzyme system was first demonstrated by Skou in crab nerve,\textsuperscript{1)} and also has been shown to have relatively high activity in heart muscle.\textsuperscript{3)–5)}

While considerable emphasis recently has been given to the calcium ion, many investigators have suggested that changes in the intracellular content and/or distribution of certain other cations also play an important role in modulating myocardial contractility.\textsuperscript{6)–13)} Sarnoff and associates have shown that the increase in myocardial contractility induced by changing afterload was associated with a net loss of myocardial potassium.\textsuperscript{8),13),14)} Iseri and associates reported that the myocardial potassium content was significantly decreased in the failing heart.\textsuperscript{15)–17)}

These observations have focused attention on the possibility that the changes in myocardial ionic balance resulting from membrane ATPase alteration may be responsible for the development of heart failure. Prompted by this idea, we have studied the properties of the ATPase system of cardiac sarcotubular vesicles and demonstrated a relationship between heart failure and the ionic transport mechanism represented by Na-K-ATPase activity.

**Materials and Methods**

*Animal experiments*

Left ventricular hypertrophy and heart failure were induced by aortic constriction. Young male rabbits weighing 2.0 to 2.5 Kg. were anesthetized with sodium pentobarbital (30 mg./Kg., i.v.). After resecting a sternal portion of the left first rib, the ascending aorta was exposed without opening the pleural cavity. In the experimental group (n=17), aortic constriction was produced by applying a circumferential silver clip of 3.7 mm. internal diameter proximal to the origin of the brachiocephalic vessels. A clip of this size led to the development of left ventricular hypertrophy in all animals and induced congestive heart failure in about a half of the experimental group, as evidenced by increased heart weight, hydrothorax, pulmonary edema, and congestion of the lungs, kidneys, liver and spleen. In the control group (n=12), sham operation was performed and consisted of an identical operation except that no constricting clip was placed around the ascending aorta. All experimental animals were killed and examined 10 days after the operation. Blood pressure in the central ear artery was measured at that time by an indirect method.\textsuperscript{18)}

*Preparation of myocardial vesicles*

Myocardial vesicles were prepared essentially as described by Tashima.\textsuperscript{5)} All preparative procedures were performed at 4°C. The rabbits were killed by a blow on the head. The chest cavity was quickly opened, and the heart was removed, blotted, weighed and immersed in ice-cold 0.25 M. sucrose. The left ventricle, including the ventricular septum, was excised, weighed and dissected free of fat, blood vessels and epicardium. The tissue (2 to 4 Gm.) was minced in a Waring
blender with 9 v. of 0.32 M. sucrose solution containing 5 mM. EDTA at pH 7.0 adjusted with 1 M. Tris and homogenized with a Teflon homogenizer. The homogenate was centrifuged for 15 min. at 1,000 g and the sediment discarded. The supernatant was spun at 13,000 g for 15 min. and the resulting supernatant was again centrifuged for 60 min. at 32,000 g. The supernatant fluid was decanted; the pellet was washed twice with the same medium by centrifugation and suspended in 7 to 9 ml. of 0.25 M. sucrose containing 5 mM. EDTA at pH 7.0 adjusted by 1 M. Tris. The protein concentration of the suspension for enzyme assay was 0.5–0.8 mg./ml. The preparation thus obtained appeared to consist exclusively of vesicles with single and double membranes in electron microscope pictures. In order to demonstrate the characteristics of myocardial vesicles, the vesicles of brain and skeletal muscle prepared by the identical procedure were studied.

**Biochemical determinations**

The dry weight of a portion of the left ventricle was determined following lyophilization for 48 hours by which time the weight remained constant. Mg- and Na-K-ATPase activities of the vesicles were measured by the method of Nakao et al.19 within 2 hours after preparation. The reaction mixture containing, in a final volume of 1 ml., 20 μM. of cysteine, 3 μM. of ATP, 5 μM. of magnesium ion, 40 μM. of Tris buffer at pH 7.5 and 0.08 mg. granule protein of the vesicle preparation, was incubated for 30 min. at 37°C. After incubation, the reaction was stopped by addition of 2 ml. of 10% trichloroacetic acid. Inorganic phosphate (Pi) was determined by the method of Fiske and Subbarow.20 Protein was determined by the method of Lowry et al.,21 using crystalline bovine albumin as a standard. The enzyme activity was expressed as μM. Pi liberated / mg. / protein / hr. The apparent increase in total ATPase activity upon addition of 140 μM. NaCl and 14 μM. KCl to the reaction mixture was underestimated as the activity of Na-K-ATPase due to contamination by Na- inhibited activity of Mg-ATPase. Therefore, Na-K-ATPase activity was estimated as the difference between the activities in the presence of Mg+Na+K and of Mg+Na in the reaction mixture. The characteristics of the ATPase system of the myocardial vesicles prepared in this study were demonstrated by the effects of ouabain, azide and oligomycin.

**Table I. Changes in Blood Pressure and Heart Weight**

<table>
<thead>
<tr>
<th>Heart Weight (Gm.)</th>
<th>LV Weight (Gm.)</th>
<th>LV Dry Weight LV Wet Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Pressure (mm. Hg)</td>
<td>Control (N=12)</td>
<td>41±3</td>
</tr>
<tr>
<td></td>
<td>Hypertrophy (N=9)</td>
<td>63±7</td>
</tr>
<tr>
<td></td>
<td>Heart Failure (N=8)</td>
<td>41±4</td>
</tr>
</tbody>
</table>

(Result: Means±S.E.M)

**RESULTS**

Heart weight

Cardiac weight and blood pressure of animals are summarized in Table I. These data were obtained from the experimental animals subjected to the
identical surgical procedure and examined 10 days after operation. On 8 of 17 experimental animals, postmortem examination showed bilateral pleural and pericardial effusion, edematous lungs and congested viscera. These postmortem findings indicated that congestive heart failure was induced in these animals. Thus we classified experimental animals as the uncomplicated left ventricular hypertrophy group (hypertrophy group) and the heart failure group (failure group). In the failure group, there was a 85% increase in heart weight and a 60% increase in left ventricular weight (Table I), but blood pressure fell to the level of 40 mmHg due to aortic constriction. In the hypertrophy group, heart weight and left ventricular weight were also increased by 60% and 39% above the control group respectively, and blood pressure fell to the level of 60 mmHg. All the observed changes were significant with P values 0.001. However, the left ventricular dry-wet weight ratio was almost constant in these 3 groups. This indicated that the increase in ventricular weight primarily corresponded to the increase in its protein.

**Characteristics of ATPase from myocardial vesicles**

The effects of sodium and ouabain on membrane ATPase activity are shown in Fig. 1. Na-K-ATPase was remarkably activated with Na⁺, but

![Graphs showing ATPase activity](image)
slightly inhibited by high concentration of Na+. The concentration of Na+ at half maximal stimulation of Na-K-ATPase was at 8.5 mM. Na-K-ATPase activity was also remarkably influenced by the low concentration of ouabain and was inhibited completely by 10^{-4}M. ouabain. A 50% inhibition with this Na-K-ATPase was reached at an ouabain concentration of 1.8 \times 10^{-7}M. On the other hand, when the myocardial vesicles were assayed for their ATPase activity with increasing concentration of Na+ in addition to Mg^{2+} but without K+, progressive depression of the activity was observed. This means that Mg-ATPase activity of the vesicles was inhibited proportionally to the concentration of Na+. The degree of inhibition of Mg-ATPase at a Na+ concentration of 140 mM was about one fourth of the activity without Na+ added. As contrasted with the marked inhibition of Na-K-ATPase by ouabain, Mg-ATPase activity was not inhibited even by 10^{-3}M. ouabain. From their different responses to ouabain and Na+, it was revealed that Mg- and Na-K-ATPases observed in our study were biochemically separated. Mg-ATPase activity was inhibited by azide and oligomycin (Fig. 2). The inhibitions by 10^{-4}M. azide and oligomycin were 47% and 28% respectively. However, Mg-ATPase activity was not influenced by 10^{-4}M. dinitrophenol. These myocardial ATPase activities are summarized compared with the ATPase activities in skeletal muscle and brain in Table II. Na-K-ATPase activity corresponded to about one fourth of total activity in the myocardium. Since the specific activity of
Table II. Effects of Ions on Membrane ATPase Activity
(The reaction mixture contained 40 mM Tris-HCl buffer (pH 7.5), 3 mM ATP,
5 mM MgCl₂, 0.5 mM EDTA, and 0.1 ml enzyme in a total
volume of 1 ml. NaCl (140 mM), and KCl (14 mM)
were added as indicated. The specific activity
was expressed as μ mole Pi/mg. protein/hr.)

<table>
<thead>
<tr>
<th></th>
<th>Mg</th>
<th>Mg+Na</th>
<th>Mg+Na+K</th>
<th>(Mg+Na+K) - (Mg+Na)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Muscle (N=12)</td>
<td>41.2±1.1</td>
<td>31.8±0.7</td>
<td>41.4±1.1</td>
<td>9.6±0.6</td>
</tr>
<tr>
<td>Skeletal Muscle (N=7)</td>
<td>27.2±0.9</td>
<td>20.0±0.6</td>
<td>20.8±0.6</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>Brain (N=7)</td>
<td>20.1±0.6</td>
<td>17.1±0.6</td>
<td>41.3±1.8</td>
<td>24.2±133</td>
</tr>
</tbody>
</table>

(Mean±S.E.M)

Na-K-ATPase was markedly high in the myocardium compared with that in skeletal muscle, the composition of the membrane ATPase system in the myocardium was similar to that in the brain rather than in the skeletal muscle.

ATPases in hypertrophied and failing heart

The changes in myocardial vesicular ATPase activity following cardiac hypertrophy and failure are summarized in Figs. 3 and 4. Na-K-ATPase activity of myocardial vesicles was inversely correlated with the left ventricular

![Fig. 3. Relation between Na-K-ATPase activity and left ventricular weight after aortic constriction.](image-url)
weight (Fig. 3). In the failure group Na-K-ATPase activity was $6.3 \pm 0.8 \mu\text{MPi/mg.hr.} \text{ (S.E.M.)}$. This decrease in Na-K-ATPase activity corresponded to 35% of Na-K-ATPase activity in the control group being $9.7 \pm 0.6 \mu\text{M.Pi/mg.hr.}$ This difference in Na-K-ATPase activity between the failure group and the control was significant at the 5% level. On the other hand, the activity of Mg-ATPase was not changed significantly in the failure group as contrasted with the marked reduction of Na-K-ATPase activity (Fig. 4). The properties of the myocardial membrane ATPase system were preserved even in the failure group. In the failure group, Mg-ATPase activity was inhibited by Na+, azide and oligomycin and Na-K-ATPase activity was stimulated by Na+ and K+ and inhibited by ouabain to the same degree as observed in the control group.

**DISCUSSION**

We studied the properties of myocardial membrane ATPase and observed the changes in its specific activity following cardiac hypertrophy and failure. Mg- and Na-K-ATPase were measured by their different responses to ouabain and Na+.

Na-K-ATPase in our preparations was completely inhibited by a small amount of ouabain ($10^{-5}\text{M.}$). This result showed that our preparative procedures would work reasonably well for heart muscle. The specific activity of Na-K-ATPase in heart muscle was substantially higher than in skeletal muscle.

Recently Stam has demonstrated Na-K-ATPase location in myocardial sarcolemma and Rostgaard has found it in the entire T system. As the
transverse tubular system is larger and far more extensive than in skeletal muscle, it would be expected that our preparations from the heart would contain a greater amount of T system fragments than similar preparations obtained from skeletal muscle. Therefore, it was reasonable that heart preparations showed a high activity of Na-K-ATPase.

This high activity of Na-K-ATPase observed in myocardial preparation indicates that ionic fluxes across the membrane are maintained at a high level in the myocardium. There is considerable evidence that Na-K-ATPase is involved in the active transport of Na\(^+\) and K\(^+\) across the cell membrane, although the metabolic role of membrane Mg-ATPase is unknown at present.\(^{1,3}\)

On the other hand, there has recently been an increasing accumulation of evidence in support of the idea that intracellular sodium and potassium ion might be responsible for altering the myocardial contractility, although considerable emphasis has been given to the calcium ion. Langer suggested from his study in the arterially perfused papillary muscle of the dog that alterations in sodium exchange form a primary determinant of calcium movement and that a lag in the sodium pump was indirectly responsible for the changes in tension seen in the positive rate staircase.\(^{11}\) Many other investigators suggested that changes in myocardial performance is modulated with respect to both acute and chronic adaptation.\(^{6-14}\) Sarnoff and Gilmore reported that, when left ventricular outflow resistance was increased, myocardial contractility increased and potassium was lost from the heart.\(^{8,13}\) From their observations the net potassium ion loss from the heart seemed to be at least a biochemical symptom of the altered state of the myocardium. However, these reports did not demonstrate how long the heart can continue to lose potassium when the load is increased, and the specific mechanism whereby these changes in myocardial ionic balance are induced has not been elucidated.

In this investigation we studied the changes in the membrane Na-K-ATPase system which may be responsible for change in the active cation transport induced by heart failure and various physiological and pharmacological manipulation. In our study cardiac hypertrophy and failure was experimentally produced by constriction of ascending aorta. By applying a circumferential silver clip of 3.7 mm. internal diameter proximal to the origin of the brachiocephalic vessels, left ventricular hypertrophy was produced in all animals with the greatest increase being observed in animal with heart failure. Since atria and right ventricle, especially left atrium, were also markedly dilated and hypertrophied, the increase in its total heart weight was greater than that in the left ventricular weight. The left ventricular dry-wet weight ratio was almost constant in the experimental animals. This indicated the increase in ventricular weight corresponded primarily to the increase in protein content
and not to the increase in water content.

The changes in myocardial vesicular ATPase activity after aortic constriction are summarized in Figs. 3 and 4. Na-K-ATPase activity of myocardial vesicles was inversely correlated with the left ventricular weight at the level of 0.02, although Mg-ATPase activity was maintained constant. In the failure group, ventricular weight was markedly increased and Na-K-ATPase activity was decreased inversely. This decrease observed in Na-K-ATPase activity from the failing heart was statistically significant \( p < 0.05 \). On the other hand, the activity of Mg-ATPase was not changed significantly in the failure group as contrasted with the marked reduction of Na-K-ATPase. This observation that Mg-ATPase activity of our preparation was the same in both failing and control animals supported the reliability of the above findings since Mg-ATPase served as a control for any preparative procedures which might alter the yield of enzyme and thus effect of Na-K-ATPase activity. Further, it was also contradicted that the reduction of Na-K-ATPase might reflect dilution of enzyme activity because of a generalized increase in myocardial protein. Therefore, the available data strongly suggested that the reduction of Na-K-ATPase activity in the failure group would be a primary response for some biochemical alterations on the myocardial transverse tubular system. Recently Mead and his associates have reported that the activity of the sarcolemmal ATPase system of the failing heart did not differ from that of the control; however, the proportion of the specific activity that could be inhibited by ouabain was significantly lower in the failing heart.\(^{25}\) This report supported our results that Na-K-ATPase activity in the failing heart was reduced. On the other hand, Gibson and Harris have reported that microsomal Na-K-ATPase activity in the heart was unaffected by hypertrophy induced by experimental pulmonary hypertension in rats.\(^{26}\) As shown in Fig. 4, our result also revealed that the difference in Na-K-ATPase activity between the hypertrophy and the control group was not statistically significant.

As mentioned above, many investigators reported that, when myocardial cells were losing K\(^+\) and presumably gaining Na\(^+\), cardiac contractility was increased. Dransfeld and associates suggested from their study on the positive inotropic effect of ouabain that the inhibition of Na-K-ATPase would result in an increase in intracellular free Ca\(^{++}\) and a resulting augmentation of cardiac performance following a high intracellular Na\(^+\)/K\(^+\) ratio after the excitation of the membrane.\(^{27}\) From their observations it was certain that myocardial ionic balance might play a role in the control of myocardial contractility. However, if there were a limiting level of myocardial active ion transport system, the myocardial ionic balance would be disturbed following longstanding and excessive hemodynamic stress, and myocardial contractility would be respon-
sively depressed. In fact, it was reported by some investigators that the myocardial potassium content was significantly decreased in the failing heart.\textsuperscript{15)\textendash 17)} Therefore, our observation, demonstrating the significant reduction of Na-K-ATPase activity in the failing heart, supported the view that the heart in failure was characterized by a diminished ability to manipulate active ion transport in response to hemodynamic stress.

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**References**