Calcium Transport ATPase of Canine Cardiac Sarcoplasmic Reticulum

A COMPARISON WITH THAT OF RABBIT FAST SKELETAL MUSCLE SARCOPLASMIC RETICULUM*

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To define the mechanism responsible for the slow rate of calcium transport by cardiac sarcoplasmic reticulum, the kinetic properties of the Ca²⁺-dependent ATPase of canine cardiac microsomes were characterized and compared with those of a comparable preparation from rabbit fast skeletal muscle. A phosphoprotein intermediate (E-P), which has the stability characteristics of an acyl phosphate, is formed during ATP hydrolysis by cardiac microsomes. Ca²⁺ is required for the E-P formation, and Mg²⁺ accelerates its decomposition. The Ca²⁺ concentration required for half-maximal activation of the ATPase is 4.7 ± 0.2 μM for cardiac microsomes and 1.3 ± 0.1 μM for skeletal microsomes at pH 6.8 and 0°. The ATPase activities at saturating concentrations of ionized Ca²⁺ and pH 6.8, expressed as ATP hydrolysis per mg of protein, are 3 to 6 times lower for cardiac microsomes than for skeletal microsomes under a variety of conditions tested. The apparent Kₐₐ value for MgATP at high concentrations in the presence of saturating concentrations of ionized Ca²⁺ is 0.18 ± 0.03 mM for cardiac microsomes, which is similar to that of skeletal microsomes at pH 6.8 and 25°. The maximum velocity of ATPase activity under these conditions is 0.45 ± 0.05 pmol per mg per min for cardiac microsomes and 1.60 ± 0.05 pmol per mg per min for skeletal microsomes. The maximum steady state level of E-P for cardiac microsomes, 1.3 ± 0.1 nmol per mg, is significantly less than the value of 4.9 ± 0.2 nmol per mg for skeletal microsomes, so that the turnover number of the Ca²⁺-dependent ATPase of cardiac microsomes, calculated as the ratio of ATPase activity to the E-P level is similar to that of the skeletal ATPase. These findings indicate that the relatively slow rate of calcium transport by cardiac microsomes, when compared to that of skeletal microsomes, reflects a lower density of calcium pumping sites and lower Ca²⁺ affinity for these sites, rather than a lower turnover rate.

In the mammalian heart, full contractile activity occurs when 50 to 100 nmol of calcium per g wet weight of ventricular tissue are made available for binding to troponin, the calcium receptor protein of the contractile system (2-4). During each cardiac cycle at maximal contractility, this amount of calcium must first bind to and then be removed from the regulatory sites of troponin. Movement of this activator calcium within the myocardial cell is controlled by the sarcoplasmic reticulum (5-8), a membranous intracellular structure which surrounds the myofibrils, by the cell surface membrane (6, 9), and possibly by the mitochondria (10-12).

Preparations of cardiac microsomes that are enriched in fragmented sarcoplasmic reticulum can accumulate calcium against a concentration gradient in the presence of ATP and Mg²⁺ (13-19). Calcium accumulation by cardiac microsomes is coupled to ATP hydrolysis, which is catalyzed by a membrane-bound Ca²⁺-Mg²⁺-dependent ATPase. As in microsomes prepared from fast skeletal muscle (20, 21), a 2:1 stoichiometric relationship between the amount of calcium taken up and ATP hydrolyzed is found in fresh cardiac microsomes (22). ATP hydrolysis by cardiac sarcoplasmic reticulum has been reported to involve a phosphoprotein intermediate (23-26) and to proceed by a mechanism similar to that of the more completely characterized sarcoplasmic reticulum from fast skeletal muscle (8, 27 33). The rate of calcium transport and the concomitant Ca²⁺-dependent hydrolysis of ATP, however, are considerably lower in cardiac than in skeletal muscle microsomes (7, 8). In order to define the basis for this difference, as well as to elucidate the relationship between the mechanisms of the calcium transport by isolated cardiac sarcoplasmic reticulum and relaxation in the intact heart, the Ca²⁺-dependent ATPase reaction catalyzed by cardiac microsomes was characterized. The present findings indicate that although cardiac...
microsomes hydrolyze ATP by a mechanism that is generally similar to that of fast skeletal microsomes, significant quantitative differences exist between several kinetic properties of these calcium transport ATPases. The concentration of phosphorylation sites in the cardiac microsomes was found to be about four times lower than that for fast skeletal microsomes, and the affinity of these sites for calcium was about three to four times lower than that for fast skeletal microsomes. The turnover rate of these sites, however, did not differ significantly. The slower rate of calcium transport by cardiac microsomes, therefore, reflects primarily a lower density of calcium pumping sites that have a lower affinity of Ca$^{2+}$ than the corresponding sites in fast skeletal microsomes.

**EXPERIMENTAL PROCEDURE**

**Preparation of Microsomes—**Fragmented sarcomplasmic reticulum was prepared from dog heart (cardiac microsomes) and rabbit fast skeletal muscle (skeletal microsomes) by the procedures described previously (34).

**Assay of ATPase Activity—**The ATPase activity of microsomes was measured at 0 to 25° in a standard reaction medium containing 0.01 to 1.0 M Tris/HCl, 40 mM NaCl, 2.0 mM CaCl$_2$, 5 mM Na$_2$ATP, 5 mM 2-mercaptoethanol, 1 unit of pyruvate kinase, 0.4 mg/ml of pyruvate dehydrogenase complex, and 1.0 mg of protein per ml, 50 mM Tris/maleate buffer (pH 6.8), 0 to 6 mM MgCl$_2$, 1.1 mM to 5 mM ATP, 120 mM KC1, 5 mM Na$_3$H$_3$EDTA, 4 to 0 mM EGTA, 0.1 mM EDTA, and various concentrations of EGTA. Total volume of the reaction mixtures was 1.0 ml. Reactions were started by the addition of ATP or microsomal protein, and at appropriate intervals 1 to 2 ml of 10% trichloroacetic acid solution was added as carrier protein after termination of the reaction by trichloroacetic acid treatment. When [$\gamma$-$^3$P]ATP was used, the trichloroacetic acid solution contained 0.1 mM EDTA or calcium/EGTA buffer containing 0.15 to 0.3 mM CaCl$_2$ and various concentrations of EGTA. The final volume of the reaction mixtures was 1 ml. Reactions were started by the addition of ATP or microsomal protein, and at appropriate intervals 10 mM trichloroacetic acid was added to the reaction mixtures to terminate the reactions. When [$\gamma$-$^3$P]ATP was used, the trichloroacetic acid solution contained 0.1 mM P$_i$, and 1.0 mM ATP as carriers. After centrifugation at 1000 x g for 10 min at 2°, aliquots were taken from the supernatant and phosphate was extracted by the method of Martin and Doty (36). In some experiments 0.5 to 0.4 mg of pyruvate kinase per ml and 1.4 mM phosphate-pyruvate were included in the reaction medium to maintain constant concentrations of ATP. In these experiments, ATPase activity was measured as the amount of pyruvate liberated during the reaction, determined by the method of Reynaud et al. (38). The Ca$^{2+}$-dependent ATPase activity was estimated by subtracting the ATPase activity in the presence of 0.2 mM EGTA from that at each ionized Ca$^{2+}$ concentration.

**Assay of Radioactive Phosphate Incorporation into Microsomes—**Microsomes were phosphorylated under conditions similar to those used for the ATPase assay. After reactions were stopped by addition of 10% trichloroacetic acid containing 0.1 mM P$_i$, and 1 to 2 mM ATP as carriers, the denatured protein was centrifuged at 1000 x g for 10 min at 2°. When the concentration of microsomes in the reaction mixture was less than 0.25 mg per ml, 2.0 mg of untreated microsomes was added as carrier protein after termination of the reaction by trichloroacetic acid. The pellets were washed once with 8 ml of ice-cold 4% perchloric acid containing 20 mM P$_i$, and 9 mM ATP. Four times with 8 ml of ice-cold 4% perchloric acid containing 20 mM P$_i$, and once with ice-cold H$_2$O. The denatured protein was collected each time by centrifugation. The washed pellets were resuspended in 1 ml of a solution containing 0.1 N NaOH, 2% Na$_2$CO$_3$, and 0.1 mM P$_i$, and then heated in boiling water for 20 min. Aliquots were assayed for radioactivity in Bray's solution by liquid scintillation spectrometry and for protein by the method of Lowry et al. (37).

The Ca$^{2+}$-dependent $^3P$ incorporation was estimated by subtracting the amount of $^3P$ incorporated into microsomes in the presence of 0.2 mM EGTA from that at each ionized Ca$^{2+}$ concentration.

**Stability of Phosphoprotein after Trichloroacetic Acid Treatment—**Microsomes were denatured with trichloroacetic acid after phosphate incorporation reached a steady state. After centrifugation, pellets were washed twice with ice-cold 4% perchloric acid containing 20 mM P$_i$, and 2 mM ATP, and once with ice-cold H$_2$O. The washed pellets were suspended in various pH buffers (50 mM) at 25° for 45 min.

The incubation was then terminated by the addition of 10% trichloroacetic acid containing 0.1 mM P$_i$. After centrifugation, each pellet was washed twice with ice-cold 4% perchloric acid containing 20 mM P$_i$, and once with H$_2$O. The protein-bound radioactivity was measured as described above. To determine the effect of hydroxyamine upon the phosphoprotein, washed pellets were suspended in solutions containing 0.2 M hydroxyamine at 25° and pH 5.4, after which 10% acetone and then 5% trichloroacetic acid containing 0.1 mM P$_i$ were added as described previously (37, 38).

**Results**

**Characterization of ATPase of Cardiac Microsomes—**Because cardiac microsomes contain not only fragmented sarcoplasmic reticulum but also contaminating materials derived from other cellular structures (41-43), the possibility that the cardiac microsomal ATPase activity studied in the present report arose from mitochondrial or sarcolemmal contaminants was examined. The effects of EGTA, azide, and ouabain, which are generally assumed to inhibit the Ca$^{2+}$-dependent sarcoplasmic reticulum ATPase (20), mitochondrial ATPase (44, 45), and sarcolemmal (Na$^+$.K$^+$)-ATPase (46), respectively, were examined. The ATPase activity at 0° and 25° and $^3P$ incorporation from [$\gamma$-$^3$P]ATP into the cardiac microsomes at 0° were measured (Table I). Total ATPase activity measured in the presence of 17 $\mu$M ionized Ca$^{2+}$ was reduced 32% and 66% by 0.2 mM EGTA at 0° and 25°, respectively. Ouabain, 0.1 mM, did not affect significantly any ATPase activity measured in these experiments. Sodium azide, 5 mM, inhibited the total ATPase activity to 46% and 80% at 0° and 25°, respectively, but the net Ca$^{2+}$-dependent ATPase activity, estimated by subtraction of the amount of $^3P$, liberated in the presence of EGTA from that liberated in the presence of Ca$^{2+}$, was unaffected by 5 mM Na$_2$PO$_4$.

Approximately 1.3 nmol of phosphoprotein per mg of protein were formed at 0° in the presence of 17 $\mu$M ionized Ca$^{2+}$, compared to approximately 0.03 nmol per mg of protein formed in 0.2 mM EGTA (Table I). Neither 5 mM Na$_2$PO$_4$ nor 0.1 mM ouabain affected significantly the amount of phosphoprotein formed in these microsomal preparations.

In view of these findings, 5 mM Na$_2$PO$_4$ was always included in subsequent studies of Ca$^{2+}$-dependent ATPase activity and Ca$^{2+}$-dependent $^3P$ incorporation from [$\gamma$-$^3$P]ATP into microsomes.

**Stability of Phosphoprotein of Cardiac Microsomes after Trichloroacetic Acid Treatment—**The effects of pH and hydroxyamine on the stability of the phosphoprotein formed by cardiac microsomes was studied as described under “Experimental Procedures.” Phosphoprotein isolated by trichloroacetic acid treatment was stable at acidic pH after incubation for 45 min at 25°. Raising the pH from 7.0 to 6.0 resulted in a loss of approximately 25% of phosphoprotein, whereas increasing the pH from 6.0 to 8.0 led to decomposition of approximately 80% of the remaining phosphoprotein. Decomposition of 95% of the phosphoprotein followed incubation with 0.2 M hydroxyamine for 15 min at 25° at pH 5.4, whereas less than 1% of the

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1. The abbreviation used is: EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetracetic acid.
phosphoprotein was lost in control reaction mixtures incubated in the absence of hydroxylamine. These results suggest that the γ-phosphate of ATP is incorporated into the microsomes as acyl phosphate (47) as described previously for the phosphorylated intermediate of the Ca++-dependent ATPase of fast skeletal muscle sarcoplasmic reticulum (27-29, 48) and the (Na+-K+)-ATPase (38, 49).

**Time Courses of ATP Hydrolysis and 32P Incorporation—**

Typical experiments showing the time courses of ATP hydrolysis and 32P incorporation into cardiac and skeletal microsomes, determined at 0° in the presence of 25 μM [γ-32P]ATP, are shown in Fig. 1, A and B. The initial time courses of ATP hydrolysis by these microsomes were almost linear both in the presence and absence of Ca++. The Ca++-dependent ATPase activity, estimated by subtracting the ATPase activity in the presence of 25 μM EGTA from that in 17 μM ionized Ca++, was much slower in cardiac microsomes than in skeletal microsomes. In contrast, 32P incorporation into both types of microsomes was extremely rapid, even at 0°. Steady state phosphoprotein levels were reached within 10 s after the start of the reaction and were maintained for at least 90 s during the incubation at 0°. The level of phosphoprotein formed in the presence of 17 μM ionized Ca++, like that of ATPase activity, was lower in cardiac than in skeletal microsomes.

**Dependence of ATPase Activity and Phosphoprotein Level on Ionized Ca++ Concentration—**

The ATPase activity and phosphoprotein level in both cardiac and skeletal muscle microsomes depended on ionized Ca++ concentration. The Ca++-dependent portions of ATPase activity and phosphoprotein level at each ionized Ca++ concentration are plotted in Fig. 2. As illustrated in this figure, ATPase activities and phosphoprotein levels at 0° showed an identical Ca++ dependence for each type of microsomal preparation at ionized Ca++ concentrations below 10 μM. The Ca++ dependencies of ATPase activity and steady state phosphoprotein level of each type of microsomal preparation were also identical at 0° (data not shown). At concentrations higher than 20 to 30 μM, however, Ca++ inhibited ATP hydrolysis but did not cause phosphoprotein levels to decrease (Fig. 2). The ionized Ca++ concentration at which ATPase activation was half-maximal (Kca) was 5.0 μM for cardiac microsomes and 1.3 μM for skeletal microsomes (Fig. 2). The average values for Kca obtained at different temperatures for cardiac microsomes were three to four times higher than those for skeletal microsomes (Table II).

The rate of Ca++-dependent ATP hydrolysis by cardiac microsomes, measured at 0° in 23 μM ionized Ca++ and 25 μM [γ-32P]ATP in the absence of added MgCl2, increased slightly less than 2-fold in 1 mM MgCl2 (Fig. 3). In contrast, the steady state phosphoprotein level was almost independent of the Mg++ concentration under these conditions.

**Dependence of ATPase Activity and Phosphoprotein Level on ATP Concentration—**

In both cardiac and skeletal microsomes, increasing ATP concentration in the range between 3 to 200 μM caused marked increases in the Ca++-dependent ATPase activity and steady state phosphoprotein level measured at 0° in 17 μM ionized Ca++ (Fig. 4). The ratios of ATPase activity to phosphoprotein level for both types of microsomes were similar and independent of ATP concentration.

The MgATP dependence of the Ca++-dependent ATPase activity of cardiac microsomes was studied at 25° over a wide...
ATPase of Sarco14l Plasmic Reticulum

FIG. 2. Ca++-dependence of ATPase activity and phosphoprotein level in cardiac and skeletal microsomes. Reactions were carried out for 30 s at 0° with 0.52 mg per ml of cardiac microsomes (O, 0) or 0.32 mg per ml of skeletal microsomes (O, □) in 25 nM [γ-32P]ATP, 2 mM MgCl2, 50 mM Tris/maleate (pH 6.8), 120 mM KCl, 5 mM NaN3, and 0.2 mM EGTA or 0.15 to 3.2 mM EGTA and 0.15 mM CaCl2. For Ca++ concentrations above 50 nM, only CaCl2 was added to the reaction medium. The concentration of ionized Ca++ is shown in the abscissa, and the Ca++-dependent portions of ATPase activity (O, □) and phosphoprotein level (O, □) are plotted in the figure.

Table II

Ca++ concentrations producing half-maximal activation of ATPase activity and phosphoprotein formation

Assay conditions at 0° were similar to those described in the legend to Fig. 2. Tris/oxalate, when present, was at a concentration of 4 mM. At 10°, 0.1 mg per ml of cardiac microsomes or 0.03 mg per ml of skeletal microsomes and 50 nM [γ-32P]ATP were incubated in the reaction mixture, and the reactions were stopped after 15-s incubation. The values at 0° obtained in the absence of Tris/oxalate represent the mean ± S.E. of the number of preparations indicated in parentheses.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>KCa (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac</td>
<td>Skeletal</td>
</tr>
<tr>
<td>0°</td>
<td></td>
</tr>
<tr>
<td>- Oxalate</td>
<td>4.7 ± 0.2 (5)</td>
</tr>
<tr>
<td>+ Oxalate</td>
<td>4.1</td>
</tr>
<tr>
<td>10°</td>
<td>3.5</td>
</tr>
<tr>
<td>+ Oxalate</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Ca++, Mg++, and ATP concentrations (11 μM to 5.0 mM). A Lineweaver-Burk plot of the results of a typical experiment in which the MgATP concentration was varied between 1.1 μM and 0.3 mM in the presence of 23 μM ionized Ca++, is shown in Fig. 5. At low concentrations of MgATP (1.1 to 4 μM), the plot appeared linear, whereas curvature was clearly apparent at high MgATP concentrations. When experiments such as that depicted in Fig. 5 were carried out with the higher concentrations of MgATP (0.3 mM to 5.0 mM), the Lineweaver-Burk plot again appeared linear. The values for Km and Vmax calculated from the linear plots obtained in studies of cardiac microsomes at low and high MgATP concentrations are summarized in Table III. For comparative purposes, the ATP dependence of the Ca++-dependent ATPase activity of skeletal microsomes was also studied under similar experimental conditions. As shown in Table III, the apparent Km of cardiac microsomes at high MgATP concentrations is similar to that of skeletal microsomes, while Vmax for cardiac microsomes was about 3.5 times lower than that for skeletal microsomes.

Effect of Ca++, Mg++, and ATP on Phosphoprotein Levels—When the effects of Ca++, Mg++, and ATP on the steady state phosphoprotein level of cardiac microsomes were studied at pH 6.8 and 0°, the phosphoprotein levels were found to be influenced most strongly by Ca++. The highest concentration of phosphoprotein, 1.44 nmol per mg of protein, was obtained in 5 mM CaCl2 and 0.32 mM [γ-32P]ATP.
Phosphoprotein levels were reduced slightly by Mg$^{2+}$ at the lower concentrations of Ca$^{2+}$ and ATP, but Mg$^{2+}$ inhibition of Ca$^{2+}$-dependent phosphorylation was not apparent at high concentrations of either Ca$^{2+}$ or ATP.

The maximum level of phosphoprotein in the cardiac microsomal preparations was observed in 0.1 to 10 mM CaCl$_2$, 1 to 1.5 MgCl$_2$, and 0.5 mM [γ-32P]ATP at pH 6.8 to 0°. The average ± S.E. of five experiments was 1.8 ± 0.1 nmol per mg of protein. The corresponding value for five experiments in skeletal microsomes was 4.9 ± 0.2 nmol per mg of protein.

**Effect of Mg$^{2+}$ and ADP on Phosphoprotein Decomposition in Cardiac Microsomes**—The decomposition of the phosphoprotein formed in cardiac microsomes was studied at 0° after a steady state had been reached in the absence of added MgCl$_2$. After further phosphorylation was prevented by the addition of EGTA or EDTA, which chelated virtually all of the available Ca$^{2+}$ (Fig. 6, vertical arrow), the time courses of phosphoprotein decomposition and the accompanying P$_i$ liberation were measured.

Addition of 2 mM MgCl$_2$ along with 2 mM EGTA was followed, after a brief lag, by decomposition of the phosphoprotein; concomitantly, an almost stoichiometric amount of P$_i$ was liberated. When the phosphorylation reaction was stopped with 3 mM EDTA which chelated both Ca$^{2+}$ and Mg$^{2+}$, the phosphoprotein decomposed much more slowly, and little or no P$_i$ was liberated during the following minute. When 0.2 mM ADP was added along with 2 mM EGTA, phosphoprotein rapidly disappeared, although significant P$_i$ liberation was again absent. These results suggest that Mg$^{2+}$ may be necessary for phosphoprotein decomposition in cardiac microsomes and that ADP stimulates phosphoprotein decomposition. In the latter, as P$_i$ liberation was not observed to accompany phosphoprotein decomposition, it is reasonable to postulate that in the presence of ADP, this process is accompanied by ATP formation (30, 60).

**pH Dependence of ATPase Activity and Phosphoprotein Level**—Both the Ca$^{2+}$-dependent ATPase activity and steady state phosphoprotein level of cardiac microsomes at 0° were pH-dependent (Fig. 7). The Ca$^{2+}$-dependent ATPase activity had a relatively narrow optimum at about pH 7.8 to 8.3, whereas the curve relating steady state phosphoprotein level to pH showed a broader optimum. As a result, the ratio of ATPase activity to phosphoprotein level was pH-dependent, decreasing at levels of pH below 7.8 and above 8.3. The curve relating steady state level of phosphoprotein of skeletal microsomes to pH was similar to that of cardiac microsomes whereas the pH dependence of ATPase activity of skeletal microsomes was different from that of cardiac microsomes (Fig. 7). In skeletal microsomes ATPase activity was maximal at more acidic pH (7.4 to 7.8) than in cardiac microsomes (pH 7.8 to 8.3). The ATPase activity of skeletal microsomes at pH 6.8 was about five times higher than that of cardiac microsomes; this difference was less at more alkaline pH. As a result, the turnover numbers of the ATPase of cardiac microsomes,

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**Table III**

<table>
<thead>
<tr>
<th>Cardiac</th>
<th>Skeletal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 to 4.0 μM$^a$</td>
<td>0.3 to 5.0 μM$^a$</td>
</tr>
<tr>
<td>$K_m$ for MgATP (μM)</td>
<td>1.1</td>
</tr>
<tr>
<td>$V_{max}$ (nmol/mg/min)</td>
<td>52</td>
</tr>
<tr>
<td>1.1 to 4.0 μM$^a$</td>
<td>140 ± 33 (3)</td>
</tr>
</tbody>
</table>

$^a$ MgATP concentration.

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**Table IV**

<table>
<thead>
<tr>
<th>ATP Concentration (mM)</th>
<th>3.1 μM ATP</th>
<th>44.2 μM ATP</th>
<th>518 μM ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0° to 30°</td>
<td>0° to 30°</td>
<td>0° to 30°</td>
<td></td>
</tr>
<tr>
<td>0.4 mM EGTA</td>
<td>0.002</td>
<td>0.006</td>
<td>0.01</td>
</tr>
<tr>
<td>8.3 mM Ca$^{2+}$</td>
<td>0.71</td>
<td>0.55</td>
<td>0.43</td>
</tr>
<tr>
<td>5.0 mM Ca$^{2+}$</td>
<td>0.99</td>
<td>0.97</td>
<td>0.93</td>
</tr>
</tbody>
</table>

$^a$ MgCl$_2$ concentration (mM).
Comparison of initial Ca**+-dependent ATPase activities of cardiac and skeletal microsomes

ATPase activities were measured in 17 to 20 \( \mu \)M ionized Ca**+, and 4 mM Tris/oxalate under standard conditions. At 0°, Tris/oxalate was omitted from the reaction medium. At 0° and 10°, the incubation times were 20 s and 15 s, respectively. At 25°, the specific activity was calculated from the linear time course of P\(_i\) liberation during the first 40 s of incubation. The values at 0° and 95° represent the mean ± S.E. of the number of preparations indicated in parentheses.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>pH</th>
<th>MgATP (( \mu )M)</th>
<th>Specific activity (nmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cardiac</td>
</tr>
<tr>
<td>0°</td>
<td>6.8</td>
<td>26</td>
<td>2.12 ± 0.08 (6)</td>
</tr>
<tr>
<td>7.4</td>
<td>26</td>
<td>3.59 ± 0.28 (3)</td>
<td>14.8 ± 0.7 (3)</td>
</tr>
<tr>
<td>10</td>
<td>6.8</td>
<td>30</td>
<td>16.9</td>
</tr>
<tr>
<td>6.8</td>
<td>100</td>
<td>20.5</td>
<td>90.3</td>
</tr>
<tr>
<td>25</td>
<td>6.8</td>
<td>5000</td>
<td>450 ± 30 (5)</td>
</tr>
</tbody>
</table>

Skeletal Microsomes—Measurements of the initial Ca**+-dependent ATPase activities of both cardiac and skeletal microsomes in 50 \( \mu \)M ionized Ca**+ and 4 mM oxalate over the temperature range of 4 to 40° allowed construction of the Arrhenius plots shown in Fig. 8. These plots showed different slopes below and above 19° in both types of microsomes. The activation energies of the Ca**+-dependent ATPase of cardiac microsomes calculated from these plots were 17 and 34 kcal per mol at high and low temperatures, respectively. The corresponding values for skeletal microsomes obtained under the same experimental conditions were 16 and 33 kcal per mol, respectively.

Comparison of Ca**+-dependent ATPase Activities of Cardiac and Skeletal Microsomes—The initial Ca**+-dependent ATPase activities of cardiac microsomes at saturating concentrations of ionized Ca**+ and at neutral pH were 3 to 5 times lower than those of skeletal microsomes at different MgATP concentrations and temperatures (Table V). The ratio between the Ca**+-dependent ATPase activity of cardiac and skeletal microsomes was similar to the ratio of the number of phosphorylation sites in the two types of microsomal preparations.

**Discussion**

The present findings agree well with previous reports (23–26) that ATP hydrolysis by cardiac sarcoplasmic reticulum involves a phosphoprotein intermediate whose characteristics are generally similar to those of fast skeletal muscle sarcoplasmic reticulum (8, 27–33). The present study demonstrates, however, that several quantitative differences exist between the kinetic parameters of ATPase of cardiac and fast skeletal muscle sarcoplasmic reticulum. Some of these differences provide an explanation for previous observations that calcium transport by cardiac sarcoplasmic reticulum is much slower than that of fast skeletal sarcoplasmic reticulum (7, 8).

The Ca**+-dependent ATPase activities of cardiac sarcoplasmic reticulum at saturating concentrations of ionized Ca**+ and at neutral pH are 3 to 6 times lower than those of fast skeletal muscle sarcoplasmic reticulum (Figs. 1, 2, 4, 7, and 8, and Table V). MgATP concentration does not appear to affect this difference in ATPase activities (Fig. 4 and Tables III and V) so that a similar 4-fold difference is also observed when values for the \( V_{\text{max}} \) of both types of ATPases are compared (Table III). Temperature also does not affect this ratio of the ATPase activities of both types of sarcoplasmic reticulum (Fig. 8 and Table V).

A similar difference is also found between the number of phosphorylation sites in each type of sarcoplasmic reticulum preparation. The present estimate of the maximum level of phosphoprotein in cardiac preparations is 1.3 ± 0.1 nmol per mg of protein which is similar to those obtained previously (25, 26), while the estimate for fast skeletal muscle preparations (4.9 ± 0.2 nmol per mg of protein) is in good agreement with previously reported values in comparable preparations (8, 30, 32). As it is reasonable to assume that the number of phosphorylation sites reflects the number of active enzyme sites in each sarcoplasmic reticulum preparation (51), these results indicate that the density of active sites in cardiac preparations is about four times lower than that in the fast skeletal muscle preparation. Thus, the turnover rates of the ATPase sites in both types of sarcoplasmic reticulum appear not to differ significantly at saturating concentrations of ionized Ca**+ and at neutral pH.

It should be noted that the pH optimum for Ca**+-dependent ATPase activity to phosphoprotein level, were about 70 to 80% of those for skeletal microsomes at pH 6.8 to 7.0, whereas the turnover number for cardiac microsomes was about 100% of that for skeletal microsomes at pH 8.2. A similar difference in the pH dependence of ATPase activities of both types of microsomes was observed at 25° in 2 mM MgATP, 50 \( \mu \)M CaCl\(_2\), and 2.5 mM oxalate under standard conditions (data not shown).

**Effect of Temperature on ATPase Activities of Cardiac and Skeletal Microsomes**

The Ca**+-dependent ATPase activities of both cardiac and skeletal microsomes in 50 \( \mu \)M ionized Ca**+ and 4 mM oxalate over the temperature range of 4 to 40° allowed construction of the Arrhenius plots shown in Fig. 8. These plots showed different slopes below and above 19° in both types of microsomes. The activation energies of the Ca**+-dependent ATPase of cardiac microsomes calculated from these plots were 17 and 34 kcal per mol at high and low temperatures, respectively. The corresponding values for skeletal microsomes obtained under the same experimental conditions were 16 and 33 kcal per mol, respectively.

**Comparison of Ca**+-dependent ATPase Activities of Cardiac and Skeletal Microsomes**

The initial Ca**+-dependent ATPase activities of cardiac microsomes at saturating concentrations of ionized Ca**+ and at neutral pH were 3 to 5 times lower than those of skeletal microsomes at different MgATP concentrations and temperatures (Table V). The ratio between the Ca**+-dependent ATPase activity of cardiac and skeletal microsomes was similar to the ratio of the number of phosphorylation sites in the two types of microsomal preparations.

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The present findings agree well with previous reports (23–26) that ATP hydrolysis by cardiac sarcoplasmic reticulum involves a phosphoprotein intermediate whose characteristics are generally similar to those of fast skeletal muscle sarcoplasmic reticulum (8, 27–33). The present study demonstrates, however, that several quantitative differences exist between the kinetic parameters of ATPase of cardiac and fast skeletal muscle sarcoplasmic reticulum. Some of these differences provide an explanation for previous observations that calcium transport by cardiac sarcoplasmic reticulum is much slower than that of fast skeletal sarcoplasmic reticulum (7, 8).

The Ca**+-dependent ATPase activities of cardiac sarcoplasmic reticulum at saturating concentrations of ionized Ca**+ and at neutral pH are 3 to 6 times lower than those of fast skeletal muscle sarcoplasmic reticulum (Figs. 1, 2, 4, 7, and 8, and Table V). MgATP concentration does not appear to affect this difference in ATPase activities (Fig. 4 and Tables III and V) so that a similar 4-fold difference is also observed when values for the \( V_{\text{max}} \) of both types of ATPases are compared (Table III). Temperature also does not affect this ratio of the ATPase activities of both types of sarcoplasmic reticulum (Fig. 8 and Table V).

A similar difference is also found between the number of phosphorylation sites in each type of sarcoplasmic reticulum preparation. The present estimate of the maximum level of phosphoprotein in cardiac preparations is 1.3 ± 0.1 nmol per mg of protein which is similar to those obtained previously (25, 26), while the estimate for fast skeletal muscle preparations (4.9 ± 0.2 nmol per mg of protein) is in good agreement with previously reported values in comparable preparations (8, 30, 32). As it is reasonable to assume that the number of phosphorylation sites reflects the number of active enzyme sites in each sarcoplasmic reticulum preparation (51), these results indicate that the density of active sites in cardiac preparations is about four times lower than that in the fast skeletal muscle preparation. Thus, the turnover rates of the ATPase sites in both types of sarcoplasmic reticulum appear not to differ significantly at saturating concentrations of ionized Ca**+ and at neutral pH.

It should be noted that the pH optimum for Ca**+-dependent ATPase activity to phosphoprotein level, were about 70 to 80% of those for skeletal microsomes at pH 6.8 to 7.0, whereas the turnover number for cardiac microsomes was about 100% of that for skeletal microsomes at pH 8.2. A similar difference in the pH dependence of ATPase activities of both types of microsomes was observed at 25° in 2 mM MgATP, 50 \( \mu \)M CaCl\(_2\), and 2.5 mM oxalate under standard conditions (data not shown).
ATPase activity of cardiac sarcoplasmic reticulum is at a slightly more alkaline level than that of fast skeletal muscle sarcoplasmic reticulum, while the curve relating phospho-
protein level of cardiac sarcoplasmic reticulum to pH is similar to that of skeletal muscle sarcoplasmic reticulum (Fig. 7).

Thus, at slightly alkaline pH (~pH 8.0) where cardiac ATPase is
optimally activated (Fig. 7), the turnover rate of the ATPase
site in cardiac sarcoplasmic reticulum is even larger than that
for skeletal muscle sarcoplasmic reticulum (Fig. 7).

At low concentrations of ionized Ca** (~1.0 - 2.0 μM), the
ATPase activity of cardiac sarcoplasmic reticulum is more
than 10 times lower than that of fast skeletal muscle sarcoplas-
mic reticulum (Fig. 2). A similar difference has been noted
previously when the rates of calcium uptake by both types of
ATPase of cardiac sarcoplasmic reticulum is more
affinity of the ATPase of the cardiac sarcoplasmic reticulum
than 10 times lower than that of fast skeletal muscle sarcoplas-
mic reticulum (Fig. 2). A similar difference has been noted
previously when the rates of calcium uptake by both types of
ATPase activity of cardiac sarcoplasmic reticulum is more

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