Mechanism by which Cyclic Adenosine 3':5'-Monophosphate-Dependent Protein Kinase Stimulates Calcium Transport in Cardiac Sarcoplasmic Reticulum

MICHAEL J. HICKS, MUNEKAZU SHIGEKAWA, AND ARNOLD M. KATZ

SUMMARY We examined the mechanism by which cyclic AMP-dependent protein kinase (PK) stimulates the calcium pump of canine cardiac sarcoplasmic reticulum vesicles. The Ca\(^{2+}\) dependence of calcium uptake rates by 30 µg/ml canine cardiac sarcoplasmic reticulum was measured at 25°C in 120 mM KCl, 40 mM histidine buffer (pH 6.8), 5 mM MgATP, and an ATP-regenerating system (75 µg/ml pyruvate kinase + 5 mM phosphoenolpyruvate) with 50 mM phosphate as calcium-precipitating anion. Preincubation with PK, 100 µg/ml, plus 1 µM cyclic AMP for 10 minutes increased calcium uptake rates from 2- to 3-fold at Ca\(^{2+}\) concentrations between 0.25 and 2.0 µM. This stimulation was associated with a decrease in the Ca\(^{2+}\) concentration needed to produce 50% maximal calcium uptake velocity from 2.38 ± 0.11 to 1.07 ± 0.10 µM (n = 7, P < 0.001). The Ca\(^{2+}\) dependence of calcium uptake in nonphosphorylated cardiac sarcoplasmic reticulum vesicles exhibited positive cooperativity, whereas cooperativity was not evident in the corresponding preparations from "fast" rabbit skeletal muscle. The estimated Hill coefficient for control vesicles was 1.77 ± 0.15; this value decreased significantly to 1.24 ± 0.08 (P < 0.01) after preincubation with PK. After exposure to PK, therefore, cardiac sarcoplasmic reticulum exhibited the low Ca\(^{2+}\) cooperativity seen with fast skeletal sarcoplasmic reticulum. Phosphorylation of cardiac sarcoplasmic reticulum by PK thus appears to increase the apparent Ca\(^{2+}\)-sensitivity of the calcium pump while decreasing positive cooperativity between the two Ca\(^{2+}\)-binding sites of the calcium pump. Circ Res 44: 384-391, 1979

CANINE CARDIAC sarcoplasmic reticulum vesicles have a slower rate of calcium transport than comparable preparations obtained from rabbit fast skeletal muscle (Weber, 1966; Martonosi, 1972; Repke and Katz, 1972). This slower transport rate is associated with a lower ATPase activity, and has been shown to reflect a lower density of calcium pumping sites and a lower affinity of these sites for calcium, but not a lower turnover rate per site (Shigekawa et al., 1976). The rate of calcium transport by cardiac sarcoplasmic reticulum vesicles has been shown to be increased when a component of the vesicles is phosphorylated by both endogenous and exogenous cyclic AMP-dependent protein kinases (PK) (Tada et al., 1974; La Raia and Morkin, 1974; Katz et al., 1975). Initial rates of both Ca\(^{2+}\)-dependent ATPase activity and calcium uptake are increased two to three times following incubation of cardiac sarcoplasmic reticulum vesicles with PK and cyclic AMP without a change in the stoichiometry of 2 mol of calcium transported per mole ATP hydrolyzed (Tada et al., 1974). This stimulation of calcium transport is associated with the phosphorylation of phospholamban, a 22,000-dalton component of the sarcoplasmic reticulum vesicles which is separable from the Ca\(^{2+}\)-dependent ATPase protein (mol wt 100,000) on polyacrylamide gel electrophoresis (La Raia and Morkin, 1974; Tada et al., 1975; Kirchberger and Chu, 1976; Kirchberger and Raffo, 1977).

A previous study of the mechanism by which phosphorylation of the cardiac sarcoplasmic reticulum stimulated calcium uptake velocity suggested that the observed stimulation of calcium transport reflected an increase in the Ca\(^{2+}\) sensitivity of the calcium pump (Tada et al., 1974). However, the use of 5 mM oxalate as a Ca\(^{2+}\)-precipitating anion in these studies prevented detailed kinetic analyses of the Ca\(^{2+}\) dependence of the calcium uptake process (Repke and Katz, 1972; Li et al., 1974). The present studies, carried out with 50 mM phosphate as Ca\(^{2+}\)-precipitating anion, permitted such a kinetic analysis of the mechanism by which calcium transport in the cardiac sarcoplasmic reticulum is stimulated by the action of cyclic AMP-dependent PK. Enhanced calcium transport was found to be due largely to an increased apparent Ca\(^{2+}\) sensitivity of the calcium pump, accompanied by a decrease in
cooperativity between the two Ca\textsuperscript{2+}-binding sites on the calcium pump.

**Methods**

**Preparation of Sarcoplasmic Reticulum Vesicles**

Cardiac sarcoplasmic reticulum vesicles were prepared from the hearts of dogs anesthetized with pentobarbital (30 mg/kg, iv), or from the white-appearing leg muscle of rabbits according to the method of Harigaya and Schwartz (1969), with minor modifications. Immediately after excision, the muscle was placed in deionized, distilled water at 4°C; all further manipulations were at this temperature. Ventricles were freed of most nonmyocardial tissue, cut into approximately 1-g pieces, and combined with five volumes of 0.1 M NaHCO\textsubscript{3} at pH 6.8. This mixture was homogenized at top speed in a Waring blender for a total of 40 seconds, in pulses of 5-10 seconds each. The resultant slurry was centrifuged at 5000 g for 10 minutes, and the supernatant extract was filtered once through four-layer gauze mesh and centrifuged at 5000 g for 20 minutes. The gauze mesh filtration was repeated and the supernatant extract was centrifuged at 27,000 g for 30 minutes. The pellets were resuspended in 0.6 M KCl, 20 mM Tris-HCl (pH 6.8) and homogenized gently in a Dounce glass homogenizer. This suspension was centrifuged at 27,000 g for 30 minutes, and the final pellets were resuspended by gentle homogenization in a loosely fitting glass-Teflon homogenizer containing a small volume of 0.4 M sucrose, 20 mM Tris-HCl at pH 6.8. Protein concentration was determined by the biuret method with bovine serum albumin as standard. This procedure yielded 0.4-0.6 mg of protein per gram wet weight of cardiac muscle, and 0.8-1.2 mg of protein per gram wet weight of skeletal muscle. Samples containing approximately 25 mg of protein/ml were quick-frozen in liquid nitrogen and stored at −70°C for up to 4 weeks. Storage caused only minor loss of calcium uptake activity, as compared to the fresh state.

**Preparation of Calcium Buffers**

Analytical grade anhydrous CaCO\textsubscript{3}, oven-dried to ensure its anhydrous state, was used in the preparation of Ca\textsuperscript{2+}-EGTA solutions which, when diluted 1:10 by addition to reaction mixtures, would provide known calculated Ca\textsuperscript{2+} concentrations according to the equations of Katz et al. (1970), with the exception that the dissociation constant used for the Ca\textsuperscript{2+}-EGTA complexed at pH 6.8 was 10\textsuperscript{-5.8} M\textsuperscript{-1}, half that given by Schwarzenbach (1960). A small amount of carrier-free \textsuperscript{44}CaCl\textsubscript{2} was added to each buffer to give sufficient radioactivity for measurement during the calcium uptake studies. The total concentration of calcium in the reaction mixtures was 100 μM, which is 30-50 times the level of contaminating calcium (Katz and Repke, 1967).

**Assay of Calcium Uptake**

The sarcoplasmic reticulum vesicles were incubated at 25°C in a standard mixture containing 0.12 M KCl, 0.04 M histidine buffer (pH 6.8), 0.05 M Tris-phosphate, 5 mM MgATP, and an ATP-regenerating system consisting of 5 mM phosphoenolpyruvate and 75 μg of pyruvate kinase/ml. Cardiac sarcoplasmic reticulum vesicles were studied at a protein concentration of 30 μg/ml, and skeletal sarcoplasmic reticulum at 6 μg/ml. Reaction mixtures were equilibrated at 25°C for 5 minutes, after which the vesicles were incubated for 10 minutes with or without added cyclic AMP-dependent PK (see below). Calcium uptake reactions were then initiated by the addition of the calcium buffer. Calcium uptake was terminated at predetermined intervals by filtration of a small volume (0.2-0.3 ml) of the reaction mixture through a 0.45-μm Millipore filter. A 50-μl sample of the filtrate was then added to 2 ml of Biofluor (New England Nuclear) and analyzed for radioactivity in a liquid scintillation counter. The amount of calcium taken up by the vesicles was determined from the difference between radioactivity in the unfiltered reaction mixture and in the filtrate.

Calcium uptake velocities were calculated at each free calcium concentration from five measurements of calcium content during the initial phase of the reaction, when the time course of calcium uptake versus time was found to be linear (Fig. 1). During this period, the duration of which was varied according to the reaction velocity, the total amount of calcium taken up ranged between 0.3 and 0.6 μmol per mg of sarcoplasmic reticulum protein, or approximately 10-20% of the total calcium present in the reaction mixture. Values for calcium content were plotted against time after initiation of the calcium uptake reaction and were subjected to linear regression analysis. The slope of the line of best fit, expressed as μmol calcium/mg protein per min, was taken as the calcium uptake velocity. Only data giving a correlation to linearity ≥ 0.9, from a minimum of four calcium content values, were considered acceptable in these studies.

**Phosphorylation of Cardiac Sarcoplasmic Reticulum by Cyclic AMP-Dependent PK**

PK from bovine myocardial tissue (Sigma Chemical Co.; phosphorylating activity, 2 pmol units/μg) was dissolved in and dialyzed overnight at 4°C against several changes of 5 mM K\textsubscript{2}HPO\textsubscript{4} (pH 6.8), and stored at 4°C at a concentration of 7.2 mg/ml. Monosodium cyclic AMP was dissolved in 5 mM K\textsubscript{2}HPO (pH 6.8) and stored at 1.8 × 10\textsuperscript{-4} at −15°C. Phosphorylation of cardiac sarcoplasmic reticulum was carried out at 25°C in the Ca\textsuperscript{2+}-free reaction mixture for calcium uptake after addition of PK and cyclic AMP to final concentrations of 100 μg/ml and 1.0 μM, respectively.

Preliminary experiments were carried out with
the commercial PK to define the preincubation time and enzyme concentration needed to achieve maximal stimulation of calcium uptake velocity (Table 1). To minimize preincubation time in the present studies, the high PK concentration of 100 μg/ml was chosen for a 10-minute phosphorylation reaction prior to initiation of the calcium uptake reaction.

Materials

\(^{45}\text{CaCl}_2\) (~20 Ci/g Ca) was obtained from ICN Chemical and Radioisotope Division. Sodium cyclic AMP, pyruvate kinase (as lyophilized powder from rabbit muscle), and ethylene glycol-bis (β-aminoethyl ether)-\(\text{N}\)\(\text{N}\)\(\text{-}\)tetraacetic acid (EGTA) were obtained from Sigma Chemical Co.). Disodium ATP and phosphoenolpyruvate monopotassium were obtained from Boehringer Mannheim. Disodium ATP was freed of metal ions by cation exchange chromatography on Dowex 50 and neutralized with Tris and MgCl\(_2\) (Tada et al., 1974). Water was deionized and distilled from glass prior to use.

Results

Comparison of the Ca\(^{2+}\) Dependence of Calcium Uptake in Skeletal and Cardiac Sarcoplasmic Reticulum

The Ca\(^{2+}\) dependence of calcium uptake velocities of cardiac and skeletal sarcoplasmic reticulum were compared at Ca\(^{2+}\) concentrations ranging between 0.2 and 30 μM. As reported previously, calcium uptake was inhibited at Ca\(^{2+}\) concentrations greater than approximately 3–6 μM (Hasselbach, 1964; Weber et al., 1966). Substrate concentration at one-half maximal observed velocity (K\(_{Ca}\)) was taken as an index of the Ca\(^{2+}\) sensitivity of the calcium uptake reaction. Figure 2 depicts the results.
TABLE 1  Time and Protein Kinase Concentration-Dependence of Stimulation of Calcium Uptake

<table>
<thead>
<tr>
<th>Protein kinase concentration (μg/ml)</th>
<th>Calcium uptake velocity (μmol/mg per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 min*</td>
</tr>
<tr>
<td>Cyclic AMP absent</td>
<td>0</td>
</tr>
<tr>
<td>Cyclic AMP present</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.055</td>
</tr>
<tr>
<td>20</td>
<td>0.070</td>
</tr>
<tr>
<td>50</td>
<td>0.094</td>
</tr>
<tr>
<td>100</td>
<td>0.113</td>
</tr>
</tbody>
</table>

Reactions were carried out with cardiac sarcoplasmic reticulum as described in Methods at a Ca²⁺ concentration of 1.0 μM

* Duration of phosphorylation reaction.

Effects of Phosphorylation of Cardiac Sarcoplasmic Reticulum

Comparison of the Ca²⁺ dependence of calcium uptake velocity in cardiac sarcoplasmic reticulum before and after phosphorylation by exogenous cyclic AMP-dependent PK demonstrated differences similar to those seen between cardiac and skeletal sarcoplasmic reticulum. When the data from a single experiment were plotted on linear coordinates, the control curve for nonphosphorylated sarcoplasmic reticulum was seen to be sigmoid, whereas the curve defining the Ca²⁺ dependence of calcium uptake velocity of the phosphorylated sarcoplasmic reticulum exhibited a hyperbolic contour.

A total of seven such paired simultaneous studies were performed and the data normalized by setting calcium uptake velocities for nonphosphorylated cardiac sarcoplasmic reticulum, measured at 10 μM Ca²⁺, to 100%. All other calcium uptake rates, both for phosphorylated and nonphosphorylated vesicles, in each of the seven studies were expressed as a percent of this rate. The mean and standard error of the mean were then computed for each value.

A semilogarithmic plot of the Ca²⁺ dependence of calcium uptake velocities of the pooled, normalized data demonstrated that the Kc for nonphosphorylated sarcoplasmic reticulum was approximately 2.4 μM, whereas that for phosphorylated sarcoplasmic reticulum was approximately 1.1 μM (Fig. 3). When these data were plotted in double reciprocal form, a marked difference in the degree of upward concavity between phosphorylated and nonphosphorylated sarcoplasmic reticulum was apparent (Fig. 4).

The curves in Figure 4 were linearized by computing the value for n that gave the highest correlation coefficient for a linear relationship between 1/v and 1/[Ca²⁺]ⁿ. The exponential n (which provides an estimate of the Hill coefficient) that gave the closest approximation to linearity was determined by computing correlation coefficients for a series of least mean squares linear regression analyses. From the pooled data in Figures 3 and 4, n was found to be 1.81 for the nonphosphorylated cardiac sarcoplasmic reticulum and 1.27 for the phosphorylated vesicles.

Statistical evaluation of the apparent fall in Kc and the Hill coefficient after phosphorylation of the cardiac sarcoplasmic reticulum vesicles was carried out by analyzing the individual values for Kc and the Hill coefficient in each of the seven experiments (Table 2). The mean values (± SEM) for Kc were

![Figure 2: Comparison of Ca²⁺ dependence of calcium uptake rates of rabbit fast skeletal (○) and canine cardiac (●) sarcoplasmic reticulum. Calcium uptake rates, determined as described in Methods, are plotted against the calculated ionized calcium concentration on a logarithmic scale. In the inset, these data are plotted in double reciprocal form.](http://circres.ahajournals.org/issue/11/3/387/CYCLIC-AMP-AND-CARDIAC-SARCOPLASMIC-RETICULUM-Hicks-et-al.-387.png)
Comparison of the Ca\(^{2+}\) dependence of calcium uptake rates by phosphorylated (○) and nonphosphorylated (●) canine cardiac sarcoplasmic reticulum. These data represent the mean ± SEM of seven experiments after normalization of the data as described in text. The error bars from all points except "control" at 10 μM Ca\(^{2+}\) represent ± SEM calculated from the normalized data; the error bar for "control" at 10 μM Ca\(^{2+}\) represents the SEM of the actual calcium uptake rates for the control vesicles at that level of Ca\(^{2+}\).

Discussion

The mechanical responses of cardiac muscle to agents that increase intracellular cyclic AMP levels include: (1) enhanced tension development, (2) increased rate of tension development, and (3) increased rate of relaxation (Katz et al., 1975). The last of these effects can be attributed to an increase in the rate of dissociation of Ca\(^{2+}\) from the troponin complex, which in turn may be due to an increased rate of calcium transport into the sarcoplasmic reticulum, a decreased Ca\(^{2+}\) affinity of the troponin complex, or both.

Membranous vesicles representing a purified preparation of fragmented sarcoplasmic reticulum provide a useful means of examining the calcium transport properties of the sarcoplasmic reticulum in vitro. Active calcium transport by sarcoplasmic reticulum vesicles has been found to be mediated by a Ca\(^{2+}\)-dependent ATPase that transports 2 mol of Ca\(^{2+}\) per mol ATP hydrolyzed in both skeletal and cardiac sarcoplasmic reticulum (Tada et al., 1978). Early attempts to define the mechanism by which catecholamines mediate the increased rate of cardiac relaxation yielded conflicting results (Hess et al., 1968; Entman et al., 1969; Shinebourne and White, 1970), which came to be understood better only after the discovery that intracellular effects of cyclic AMP were, in many instances, mediated by cyclic AMP-dependent PK. These PK, after activation by cyclic AMP, catalyze the phosphorylation of various intracellular proteins (Walsh et al., 1968; Miyamoto et al., 1969), including the cardiac sarcoplasmic reticulum (La Raia and Morkin, 1973; Tada et al., 1975; Kirchberger and Chu, 1976; Kirchberger and Raffo, 1977; Wray et al., 1973; Kirchberger et al., 1974; Wray and Gray, 1977). It is now generally accepted that calcium uptake by cardiac sarcoplasmic reticulum vesicles is increased by as much as 2- to 3-fold after preincubation with cyclic AMP and cyclic AMP-dependent PK (La Raia and Morkin, 1973; Katz et al., 1975; Tada et al., 1975; Will et al., 1976).

The present findings indicate that phosphorylation of the cardiac sarcoplasmic reticulum by a cyclic AMP-dependent PK increases the apparent Ca\(^{2+}\) sensitivity of the calcium pump (Fig. 3, Table 2). This interpretation is in accord with earlier findings of Tada et al. (1974), who studied oxalate-supported calcium uptake. In the present study, the use of 50 mM phosphate as calcium-precipitating anion allowed the Ca\(^{2+}\) activation of the calcium pump to be analyzed kinetically, although an inhib-
The present data suggest that a high degree of positive cooperativity exists between the two Ca\(^{2+}\)-binding sites of the calcium pump ATPase protein in nonphosphorylated cardiac sarcoplasmic reticulum vesicles (Fig. 2 and 3, Table 2). In contrast, calcium transport by sarcoplasmic reticulum vesicles from fast skeletal muscle shows no evidence for significant cooperativity under these conditions (Fig. 2). Phosphorylation of the cardiac sarcoplasmic reticulum vesicles by cyclic AMP-dependent PK markedly reduces the positive cooperativity that characterizes the activation of the cardiac calcium pump by Ca\(^{2+}\) (Figs. 3 and 4, Table 2), and so causes the Ca\(^{2+}\) activation of cardiac sarcoplasmic reticulum to resemble that in skeletal muscle sarcoplasmic reticulum (Fig. 2).

Findings similar to those shown in Figure 3 have recently been reported by Wray and Gray (1977), who examined the effects of Ca\(^{2+}\) on the stimulation of the cardiac transport ATPase of cardiac sarcoplasmic reticulum in the presence and absence of cyclic AMP. Wray and Gray interpreted their findings to suggest that a Ca\(^{2+}\)-dependent PK mediated the effects of cyclic AMP on phosphorylation of the vesicles, but these results differ from the findings of Kirchberger et al. (1974). An alternative explanation for the data of Wray and Gray (1977) would be that the increase in Ca\(^{2+}\) sensitivity caused by cyclic AMP resulted from a change in the Ca\(^{2+}\) dependence of the cardiac pump ATPase that was related to a Ca\(^{2+}\)-independent phosphorylation of phospholamban. A possible action of a Ca\(^{2+}\)-dependent PK, such as was suggested by Wray and Gray (1977), is unlikely to contribute to the present results, because the vesicles were phosphorylated with the cyclic AMP-dependent PK in a complete reaction mixture lacking only the Ca-EGTA buffer. Under these conditions, small amounts of contaminating calcium would be transported into the vesicles by the calcium pump. Furthermore, this phosphorylation reaction previously has been shown to be dependent on cyclic AMP (Kirchberger et al., 1974). The Ca\(^{2+}\)-dependent stimulation of calcium uptake velocity noted after addition of the Ca-EGTA buffers is not readily attributable to phosphorylation of the vesicles by a Ca\(^{2+}\)-dependent PK, because such a reaction would be expected to proceed slowly at the low concentrations of vesicles, whereas the measurements of calcium uptake remained linear and did not increase with time (Fig. 1B).

The present findings, which indicate that phosphorylation of phospholamban reduces a high degree of cooperativity between the two Ca\(^{2+}\)-binding sites of the cardiac pump of cardiac sarcoplasmic reticulum vesicles, could be explained if phosphorylated phospholamban reduced cooperative properties during calcium uptake by the cardiac calcium pump. Alternatively, phospholamban in its non-phosphorylated state could confer a high degree of cooperativity upon the Ca\(^{2+}\)-binding sites of the cardiac calcium pump during these calcium uptake reactions. The latter interpretation is favored by the apparent absence of a protein similar to phospholamban in fast skeletal sarcoplasmic reticulum (Kirchberger and Tada, 1976), and by the finding that the Ca\(^{2+}\) dependencies of the purified cardiac pump ATPase from cardiac and skeletal sarcoplasmic reticulum are similar (Levitsky et al., 1976).

A tentative model that may explain the effects of phospholamban phosphorylation on the cardiac sarcoplasmic reticulum is illustrated in Figure 5. Although evidence for this model is incomplete, it is in accord with our current understanding of the effects of cyclic AMP-dependent PK on the cardiac sarcoplasmic reticulum. The upper diagram depicts phospholamban in its dephospho-form interacting with the cardiac pump ATPase in a manner that confers positive cooperativity upon the Ca\(^{2+}\)-binding sites of the latter. After phosphorylation of phospholamban, the interaction between phospholamban and the calcium pump protein is diminished.

**Table 2** Values for K\(_\text{ca}\) and n Values of Individual Paired Experiments with Control and Phosphorylated Cardiac Sarcoplasmic Reticulum (SR) Vesicles

<table>
<thead>
<tr>
<th>Date of preparation of SR</th>
<th>Date of study</th>
<th>K(_\text{ca}) (M)</th>
<th>n</th>
<th>Control</th>
<th>K(_\text{ca}) (M)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/3/78</td>
<td>2/1/78</td>
<td>2.8</td>
<td>2.2</td>
<td>1.6</td>
<td>1.9</td>
<td>1.3</td>
</tr>
<tr>
<td>2/1/78</td>
<td>2/3/78</td>
<td>2.2</td>
<td>2.2</td>
<td>1.4</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>2/23/78</td>
<td>3/6/78</td>
<td>1.8</td>
<td>2.2</td>
<td>1.8</td>
<td>2.3</td>
<td>1.0</td>
</tr>
<tr>
<td>3/9/78</td>
<td></td>
<td>1.8</td>
<td></td>
<td>1.4</td>
<td>1.4</td>
<td></td>
</tr>
</tbody>
</table>

Mean (± SEM) 2.38 ± 0.21† 1.77 ± 0.15† 1.07 ± 0.10† 1.24 ± 0.08‡

* Fresh SR vesicles
† t = 7.635, P < 0.001
‡ t = 5.082, P < 0.01.
FIGURE 5 Possible mechanism by which phospholamban phosphorylation stimulates the calcium pump of the cardiac sarcoplasmic reticulum. Upper: Phospholamban in the dephospho-form interacts with the calcium pump ATPase protein in the membrane (shaded) so as to confer positive cooperativity between the two Ca$^{2+}$-binding sites on the latter. Lower: Phosphorylation of phospholamban reduces its interaction with the calcium pump ATPase protein so that each Ca$^{2+}$-binding site on the latter becomes able to interact independently with Ca$^{2+}$. The resulting decrease in cooperativity between these two Ca$^{2+}$-binding sites increases the apparent Ca$^{2+}$ sensitivity of the calcium pump and accelerates calcium uptake rate at low Ca$^{2+}$ concentrations. The symbol P stands for phosphate.

and cooperativity is reduced, as depicted in the lower diagram. Abolition of the putative interaction between these two Ca$^{2+}$-binding sites, by allowing greater Ca$^{2+}$ sensitivity of the pump protein at low Ca$^{2+}$ concentrations, can account for the observed ability of the cyclic AMP-dependent PK to stimulate the calcium pump of the cardiac sarcoplasmic reticulum. More direct evidence for this proposed mechanism may follow from efforts to purify phospholamban and incorporate both its phospho- and dephospho-forms into reconstituted sarcoplasmic reticulum vesicles.

Note Added in Proof

Kinetic studies of the effects of phospholamban phosphorylation on the reaction mechanism of the calcium pump of the cardiac sarcoplasmic reticulum have recently been reported by Tada et al. (1979) (Tada M, Ohmori F, Yamada M, and Abe H; Mechanism of the stimulation of Ca$^{2+}$-dependent ATPase of cardiac sarcoplasmic reticulum by adenosine 3':5'-monophosphate-dependent protein kinase. Role of the 22,000 dalton protein. J Biol Chem (in press).) These investigators found that phospholamban phosphorylation increased the turnover rate of the calcium pump ATPase as the result of accelerated decomposition of the phosphorylated enzyme intermediate. This effect was greater when measurements were carried out at high (10 to 100 μM) as compared to low (1 to 5 μM) ATP concentrations. These findings, along with those described in the present report, indicate that phospholamban affects multiple biochemical characteristics of the calcium pump of the cardiac sarcoplasmic reticulum.

References

Kirchberger MA, Chu G: Correlation between protein kinase mediated stimulation of calcium transport by cardiac sarcoplasmic reticulum and phosphorylation of a 22,000 dalton protein. Biochim Biophys Acta 419: 559-562, 1976
Kirchberger MA, Tada M: Effects of adenosine 3',5'-monophosphate-dependent protein kinase on sarcoplasmic reticulum isolated from cardiac and slow and fast contracting skeletal muscles. J Biol Chem 251: 725-729, 1976
Levitsky DO, Aliev MK, Kuzmin AL, Levchenko TS, Smirnov VN, Chazov El: Isolation of calcium pump system and purification of calcium ion-dependent ATPase from heart muscle. Biochim Biophys Acta 443: 468-484, 1976
Repke DI, Katz AM: Calcium binding and calcium uptake by cardiac microsomes: A kinetic analysis. J Mol Cell Cardiol 4: 401-416, 1972
Shinebourne E, White R: Cyclic AMP and calcium uptake of the sarcoplasmic reticulum in relation to increased rate of relaxation under the influence of catecholamines. Cardiovasc Res 4: 194-200, 1970
Tada M, Kirchberger MA, Katz AM: Phosphorylation of a 22,000-dalton component of the cardiac sarcoplasmic reticulum by adenosine 3',5'-monophosphate-dependent protein kinase. J Biol Chem 250: 2640-2647, 1975
Tada M, Yamamoto T, Tonomura Y: Molecular mechanism of active calcium transport by sarcoplasmic reticulum. Physiol Rev 58: 1-79, 1978
Widmark H, Blanck J, Smetten G, Wollenberger MA: A quench-flow kinetic investigation of calcium-ion accumulation by isolated cardiac sarcoplasmic reticulum. Dependence of initial velocity on free calcium ion concentration and influence of preincubation with a protein kinase, MgATP and cyclic AMP. Biochim Biophys Acta 449: 295-303, 1976
Wray HL, Gray RH, Olson RA: Cyclic adenosine 3',5'-monophosphate-stimulated protein kinase and a substrate associated with cardiac sarcoplasmic reticulum. J Biol Chem 248: 1496-1498, 1973
Wray HL, Gray RH: Cyclic AMP stimulation of membrane phosphorylation and Ca++-activated, Mg++-dependent ATPase in cardiac sarcoplasmic reticulum. Biochim Biophys Acta 461: 441-459, 1977
Mechanism by which cyclic adenosine 3′:5′-monophosphate-dependent protein kinase stimulates calcium transport in cardiac sarcoplasmic reticulum.

M J Hicks, M Shigekawa and A M Katz

Circ Res. 1979;44:384-391
doi: 10.1161/01.RES.44.3.384

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/44/3/384.citation

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/