The Stimulation of Calcium Transport in Cardiac Sarcoplasmic Reticulum by Adenosine 3':5'-Monophosphate-dependent Protein Kinase*

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SUMMARY

The effects of an adenosine 3':5'-monophosphate (cyclic AMP)-dependent protein kinase on Ca²⁺-activated adenosine triphosphatase (ATPase) activity and calcium uptake of canine cardiac microsomes were examined. The cardiac microsomes, which represent an enriched preparation of fragmented sarcoplasmic reticulum, were preincubated with varying concentrations of cyclic AMP-dependent protein kinase or cyclic AMP or both in the presence of Mg²⁺ and ATP. Ca²⁺-activated ATPase activity and calcium uptake were determined in the presence of oxalate and various concentrations of Ca²⁺. Ionized Ca²⁺ concentrations were maintained with Ca²⁺ buffers containing CaCl₂ and ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid. The initial rates of both Ca²⁺-activated ATPase and calcium uptake were increased 2 to 3 times following 10-min preincubation with protein kinase and 1 μM cyclic AMP. Pretreatment with protein kinase in the absence of cyclic AMP caused a lesser degree of stimulation, whereas pretreatment with cyclic AMP alone had no detectable effect. Stimulation of the Ca²⁺-activated ATPase and calcium uptake by protein kinase was dependent on cyclic AMP concentration: maximal stimulation was seen at approximately 10⁻⁶ M with apparent half-maximal stimulation at approximately 10⁻⁷ M. Marked stimulation of both Ca²⁺-activated ATPase activity and calcium uptake by cyclic AMP and protein kinase was seen at a Ca²⁺ concentration of approximately 1 μM. The stoichiometric coupling of Ca²⁺-activated ATPase and calcium uptake was maintained at 2 moles of calcium taken up per mole of ATP hydrolyzed, following stimulation by protein kinase and cyclic AMP. The stimulatory effects on Ca²⁺-activated ATPase and calcium uptake of pretreatment with protein kinase and cyclic AMP could be shown after microsomes were washed with buffered 50 mM KCl. These findings indicate that protein kinase can increase the rate of calcium transport by the cardiac sarcoplasmic reticulum without altering the efficiency of the calcium pump. This effect may account for the abbreviation of systole that is caused by agents, like epinephrine, which increase cyclic AMP production, and resulting alterations in the distribution of Ca²⁺ within the myocardial cell may be responsible, at least in part, for the augmentation of myocardial contractility.

The energy-dependent transport of Ca²⁺ across the membranes of the sarcoplasmic reticulum plays an important role in excitation-contraction coupling of muscle. In skeletal muscle (2-4), the accumulation of Ca²⁺ by the sarcoplasmic reticulum, which is coupled with the hydrolysis of ATP (5-7), is associated with relaxation, whereas contraction is believed to be initiated by the release of Ca²⁺ from the sarcoplasmic reticulum. An acyl phosphoprotein intermediate (8-10) of ATPase has been shown to play a key role in the calcium transport process. In the myocardium, the contraction-relaxation cycle is somewhat more complex than that of skeletal muscle in that transcellular movements of Ca²⁺ also may participate in the control of myocardial function (11, 12). Although calcium transport by the cardiac sarcoplasmic reticulum is slower than that of skeletal sarcoplasmic reticulum, substantial evidence indicates that both calcium transport systems are basically similar (13-16).

We have previously reported that calcium transport by the cardiac microsomes, which consist largely of fragmented sarcoplasmic reticulum, can be influenced by a cardiac adenosine 3':5'-monophosphate-dependent protein kinase, which enhances calcium uptake by cardiac microsomes (17). This functional alteration is associated with the formation of a phosphoprotein that is catalyzed by exogenous (18) and endogenous (18-20) cyclic AMP-dependent protein kinase. As was shown in the

* This work was supported by the New York Heart Association, Contract NHL-72-2973-M from the National Heart and Lung Institute, Research Grants HL-13159 and HL-13191 (to A. M. K.) and HL-15764 (to M. A. K.) from the United States Public Health Service, and a gift from the Jack Martin Fund. A preliminary report of this paper was presented at the 57th Annual Meeting of the Federation of American Societies for Experimental Biology.

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ATPase and calcium uptake of pretreatment with protein kinase and cyclic AMP could be shown after microsomes were washed with buffered 50 mM KCl. These findings indicate that protein kinase can increase the rate of calcium transport by the cardiac sarcoplasmic reticulum without altering the efficiency of the calcium pump. This effect may account for the abbreviation of systole that is caused by agents, like epinephrine, which increase cyclic AMP production, and resulting alterations in the distribution of Ca²⁺ within the myocardial cell may be responsible, at least in part, for the augmentation of myocardial contractility.

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of calcium transported for each mole of ATP hydrolyzed.

**EXPERIMENTAL PROCEDURE**

### Materials

Canine cardiac microsomes, which consist largely of fragmented sarcoplasmic reticulum, and bovine cardiac protein kinase were prepared by the procedures described previously (13, 21), modified slightly (17, 18). Microsomes were stored on ice and used within 2 days after preparation, unless otherwise indicated. [γ-32P]ATP (5 to 10 mCi per μ mole) and [γ-32P]ATP (2 to 3 mCi per μ mole) were purchased from New England Nuclear Co., Boston, Mass. Disodum ATP and sodium cyclic AMP were obtained from Sigma Chemical Co., St. Louis, Mo. Disodium ATP was freed of metal ions by cation exchange chromatography on Dowex 50 and neutralized with Tris and MgCl₂ as described earlier (22). EGTA was obtained from LaMont Laboratories, Dallas, Texas. Calcium-EGTA buffers contained, in final concentration, 25 or 125 mM CaCl₂ and various concentrations of EGTA. The equations used to calculate Ca²⁺ concentration were those of Katz et al. (23). All other compounds were of analytical grade.

### Methods

#### ATPase Assay

The ATPase activity of canine cardiac microsomes was determined at 25°C in standard reaction mixtures containing 40 mM histidine buffer (pH 6.8), 5 mM MgCl₂, 5 mM β, β′-iminodipropionitrile (pH 6.8), 25 nmol of ATP, 25 μg of mitochondrial protein in a volume of 0.3 to 0.6 ml. Reaction mixtures were equilibrated at 25°C for 5 min, after which reactions were started by the addition of microsomal protein, unless otherwise stated. To determine the “basic” (Ca²⁺-independent) ATPase activity, reactions were carried out in the presence of 0.5 mM EGTA instead of calcium-EGTA buffer. At various times intervals after the start of the reactions, 100-μl aliquots were added to tubes (5 × 50 mm) containing 25 μl of 25% (w/v) trichloroacetic acid and 125 μl of carrier P₁. After centrifugation (1000 g, 10 min at 4°C), the amount of 32P in the supernatant was determined by the isobutanol-extraction procedure described by Siegel and Albert (24) with slight modifications. All samples were assayed in duplicate unless otherwise stated. Ca²⁺-activated ATPase activity (“extra” ATP hydrolysis (25)) was estimated by subtracting the “basic” ATPase activity from the rate of P₁ liberation at each Ca²⁺ concentration (“total” ATPase activity). Because mitochondrial ATPase activity, which represents a minor contaminant in these microsomal preparations (13), could interfere with the measurement of microsomal ATPase activity, effects of NaN₃ on ATPase activity of the microsomal preparation were studied. The low “basic” ATPase activity, measured in the presence of 0.5 mM EGTA, was markedly inhibited by 5 mM NaN₃, whereas this concentration of NaN₃ had no significant effect on Ca²⁺-activated ATPase activity (Table I). For this reason, 5 mM NaN₃ was included in most studies to inhibit mitochondrial ATPase activity (26, 27).

#### Assays for Calcium Uptake and Calcium Binding

Oxalate-stimulated calcium uptake was determined as previously described (17, 22) in 40 mM histidine buffer (pH 6.8), 5 mM MgCl₂, 5 mM ATP, 125 mM KCl, 2.5 mM Tris-oxalate, 4Ca-EGTA buffer, and 10 to 50 μg per ml of microsomal protein at 25°C. Unless otherwise stated, the reaction was started by adding Ca²⁺-EGTA buffer, containing (in final concentration) 25 or 125 μM CaCl₂ (Ca²⁺ = 1 μM) or 0.5 mM EGTA. Assays were performed as described under “Methods.”

### Table I

<table>
<thead>
<tr>
<th>Addition</th>
<th>Total ATPase</th>
<th>Basic ATPase</th>
<th>Ca²⁺-activated ATPase</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>156</td>
<td>51</td>
<td>102</td>
</tr>
<tr>
<td>NaN₃, 5 mM</td>
<td>118</td>
<td>17</td>
<td>101</td>
</tr>
</tbody>
</table>

*a Ca²⁺-activated ATPase = total ATPase — basic ATPase.

#### Pretreatment of Microsomes with Protein Kinase or Cyclic AMP (or Both)

**Procedure I**—Microsomes (10 to 30 μg per ml) were treated with and without various concentrations of protein kinase or cyclic AMP or both for 10 min at 25°C in the standard reaction mixtures for assaying ATPase activity and calcium uptake except that the calcium-EGTA buffers were omitted. Assays for ATPase and calcium uptake were subsequently carried out by the addition of the appropriate calcium-EGTA buffers.

**Procedure II**—Microsomes (0.5 μg per ml) were incubated with and without protein kinase (1 to 2 μg per ml) or 1 μM cyclic AMP or both in 5 mM MgCl₂, 5 mM ATP, 125 mM KCl, and 40 mM histidine buffer (pH 6.8) for 10 min at 25°C. Aliquots (0.15 ml) were taken and added to the standard reaction mixtures for assaying calcium uptake and Ca²⁺-activated ATPase.

**Procedure III**—Microsomes (0.5 to 1.0 μg per ml) were pretreated with and without protein kinase (1 to 2 mg per ml) or 1 μM cyclic AMP or both under the same conditions as in Procedure II in a total volume of 10 to 20 ml. After 20 min of incubation at 25°C, the mixture was centrifuged at 105,000 × g for 20 min, and the pellet was homogenized gently with 10 ml of ice-cold 50 mM KCl and 20 mM Tris-HCl (pH 6.8) in a glass homogenizer with a Teflon pestle. The resulting suspension was centrifuged and the pellet again was homogenized gently with 1.0 ml of 50 mM KCl and 20 mM Tris-HCl (pH 6.8). Recovery of microsomal protein after this procedure was approximately 70% and did not vary with the nature of the pretreatment. Assays for calcium uptake and Ca²⁺-activated ATPase were carried out subsequently under standard conditions.

#### Results

**Properties of Ca²⁺-activated ATPase Activity and Calcium Uptake of Cardiac Microsomes**

The ATPase activity of cardiac microsomes increased with increasing Ca²⁺ concentration within the range of 0.3 to 3 μM (Fig. 1A). The “basic” ATPase activity was approximately 5% of the maximal Ca²⁺-activated ATPase activity obtained at 3 μM Ca²⁺. Maximal ATPase activity was seen at 3 to 5 μM Ca²⁺, and Ca²⁺ concentrations higher than approximately 5 μM were inhibitory (Fig. 1B). In most of the following studies submaximal Ca²⁺ concentrations of 0.75 (CaCl₂ = 125 μM; EGTA = 457 μM) and 1 μM (CaCl₂ = 125 μM; EGTA = 391 μM) were used.
Ca\textsuperscript{2+}-activated ATPase activity and calcium uptake of cardiac microsomes were measured simultaneously in 5 mM Na\textsubscript{3} to determine the ratio of moles of calcium taken up per mole of ATP hydrolyzed (Ca:Pi). The Ca:Pi ratio was estimated by comparing the initial rates of calcium uptake and Ca\textsuperscript{2+}-activated ATPase. In freshly prepared microsomes, the Ca:Pi ratios ranged between 1.8 to 2.0 in five preparations. This ratio decreased when the microsomal preparations were aged by storage on ice, becoming 1.6 within 20 hours due to a decrease in the rate of calcium uptake. In the subsequent experiments, the Ca:Pi ratios were measured within 2 hours after the preparations were completed.

**Effects of Protein Kinase and Cyclic AMP on Ca\textsuperscript{2+}-activated ATPase and Calcium Uptake**—The effects of pretreatment with protein kinase and cyclic AMP on Ca\textsuperscript{2+}-activated ATPase activity were examined under conditions similar to those where calcium uptake was stimulated (17). When cardiac microsomes were pretreated with protein kinase and cyclic AMP according to Procedure I (see “Experimental Procedure”), the initial rate of Ca\textsuperscript{2+}-activated ATPase activity in the presence of 0.75 mM Ca\textsuperscript{2+} was increased more than 2-fold (Fig. 2). Protein kinase alone caused slight stimulation of the initial rate of ATP hydrolysis, but after prolonged incubation this stimulation became more pronounced. Pretreatment with cyclic AMP alone caused no detectable activation of Ca\textsuperscript{2+}-activated ATPase activity. The “basic” ATPase activity (5 moles of P\textsubscript{i} liberated per min per mg of microsomal protein) did not vary with the nature of the pretreatment (Fig. 2).

A similar pattern of stimulation of Ca\textsuperscript{2+}-activated ATPase activity by protein kinase in the presence and absence of cyclic AMP was observed in all of eight different microsomal preparations in which three different preparations of protein kinase were examined. In these experiments, Ca\textsuperscript{2+}-activated ATPase activities (nanomoles of P\textsubscript{i} liberated per min per mg of microsomal protein) were: control, 47 ± 3; protein kinase alone, 78 ± 6; protein kinase plus cyclic AMP, 103 ± 7 (mean ± S.E.). Protein kinase preparations incubated under standard conditions used for ATPase assays in the absence of microsomes exhibited no apparent ATPase activity.

Stimulation of Ca\textsuperscript{2+}-activated ATPase activity increased when 30 µg per ml of cardiac microsomes were incubated with increasing protein kinase concentrations up to 100 µg per ml in the presence of 10\textsuperscript{-4} M cyclic AMP (Fig. 3A). Similarly, the rate of calcium uptake by a different preparation of cardiac microsomes increased with increasing protein kinase concentrations and a plateau was reached at approximately 100 µg per ml (Fig. 3B).

The extent of stimulation of Ca\textsuperscript{2+}-activated ATPase activity by protein kinase depended on cyclic AMP concentration (Fig. 4). The threshold for stimulation was below 2 × 10\textsuperscript{-4} M cyclic AMP with maximal stimulation at about 10\textsuperscript{-4} M cyclic AMP. Half-maximal stimulation occurred at approximately 10\textsuperscript{-3} M cyclic AMP.

Pretreatment of cardiac microsomes with protein kinase and cyclic AMP according to Procedure III, in which both control and treated microsomes were subsequently washed prior to study, demonstrated enhancement of the initial rates of both Ca\textsuperscript{2+}-activated ATPase activity (Fig. 5A) and calcium uptake (Fig. 5B) by protein kinase and cyclic AMP. The yield of protein after washing was not increased after pretreatment with protein kinase, indicating that the protein kinase remained in the supernatant.

**Ca\textsuperscript{2+} Sensitivity of Calcium Uptake and Ca\textsuperscript{2+}-activated ATPase Activity**—When calcium uptake by microsomes pretreated with and without cyclic AMP plus protein kinase according to Procedure II was measured at different Ca\textsuperscript{2+} concentrations, significant stimulation of calcium uptake was seen at Ca\textsuperscript{2+} concentrations up to approximately 2 µM (Fig. 6B). At higher Ca\textsuperscript{2+} concentra-
microsomal preparations.

concentrations of protein kinase, as indicated on the abscissa, in protein kinase on Ca\(^{2+}\)-activated ATPase activity (A) and calcium uptake (B) of canine cardiac microsomes. Microsomes (30 ng per ml) were pretreated according to Procedure I with different concentrations of protein kinase, as indicated on the abscissa, in the presence of 1 \(\mu\)M cyclic AMP. Assays for ATPase and calcium uptake were started subsequently by the addition of calcium-EGTA buffer containing 125 \(\mu\)M CaCl\(_2\) (A) or \(^{45}\)CaCl\(_2\) (B) (Ca\(^{2+}\) = 1 \(\mu\)M). Experiments in A and B were carried out on different microsomal preparations.

Microsomes (30 ng per ml) were pretreated according to Procedure I with 100 ng per ml of protein kinase in the presence of different concentrations of cyclic AMP as indicated on the abscissa. Assays for ATPase were subsequently started by the addition of calcium-EGTA buffer (Ca\(^{2+}\) = 1 \(\mu\)M). Each point in the figure represents an average of four determinations.

Uptake and Ca\(^{2+}\)-activated ATPase Activity—When freshly prepared microsomes were pretreated with and without protein kinase and cyclic AMP, followed by washing with buffer solution, on Ca\(^{2+}\)-activated ATPase activity (A) and calcium uptake (B). Microsomes (0.7 mg per ml) were incubated with (●) and without (○) 1.4 mg per ml of protein kinase + 1 \(\mu\)M cyclic AMP and washed with buffer solution according to Procedure III, prior to measurement of ATPase activity and calcium uptake. A, ATPase assay performed under standard conditions with 80 ng per ml of microsomes and calcium-EGTA buffer containing 125 \(\mu\)M CaCl\(_2\) (Ca\(^{2+}\) = 0.75 \(\mu\)M). Each point represents an average of duplicate determinations from which "basic" ATP hydrolysis was subtracted. B, calcium uptake was measured under standard conditions with 60 ng per ml of microsomes and calcium-EGTA buffer containing 25 \(\mu\)M \(^{45}\)CaCl\(_2\) (Ca\(^{2+}\) = 0.75 \(\mu\)M). Experiments in A and B were carried out on different microsomal preparations.


cyclic AMP concentration dependency of protein kinase-induced stimulation of Ca\(^{2+}\)-activated ATPase activity. Microsomes (30 ng per ml) were pretreated according to Procedure I with 100 ng per ml of protein kinase in the presence of different concentrations of cyclic AMP as indicated on the abscissa. Assays for ATPase were subsequently started by the addition of calcium-EGTA buffer (Ca\(^{2+}\) = 1 \(\mu\)M). Each point in the figure represents an average of four determinations.

Absence of Stimulation of Calcium Binding by Cyclic AMP-dependent Protein Kinase—Our previous observation that the steady state level of calcium binding (measured in the absence of oxalate) was not stimulated by protein kinase was based on studies in which microsomes were incubated with relatively high concentrations of protein kinase (17). Because artifacts can result from Millipore filtration at high concentrations of soluble proteins, which promote the passage of microsomal protein through the filters (28), these data were confirmed with microsomes that were pretreated with and without protein kinase and cyclic AMP and subsequently were washed according to Procedure III. Under these conditions, calcium uptake rate was approximately doubled, whereas the steady state level of calcium binding measured concurrently remained unchanged (Table II).

Effects of Protein Kinase on Stoichiometry between Calcium Uptake and Ca\(^{2+}\)-activated ATPase Activity—When freshly pre-pared microsomes were pretreated with protein kinase and cyclic AMP according to Procedure I, the stoichiometry of approximately 2 moles of Ca\(^{2+}\) taken up per mole of ATP hydrolyzed was unchanged (Table III). Similar ratios were obtained after microsomes were pretreated with either protein kinase or cyclic AMP alone. When microsomes were pretreated with MgATP and washed according to Procedure III, the control ratio of Ca\(^{2+}\) uptake was 1.6 (Table III). This slight “uncoupling” of calcium uptake from the Ca\(^{2+}\)-activated ATPase, which could be attributed to the washing procedure, was not influenced by treatment with protein kinase or cyclic AMP or both (Table III).

DISCUSSION

We have previously shown that calcium uptake by cardiac microsomes is enhanced after pretreatment with protein kinase
TABLE II

Effect of protein kinase and cyclic AMP on calcium binding

Mirosomes (1 mg per ml) were incubated with and without protein kinase (2 mg per ml) and cyclic AMP (1 μM) and were washed with buffer solution according to Procedure III, prior to measurement of calcium binding. Calcium binding and calcium uptake were measured under standard conditions described under “Experimental Procedure” in the presence of 0.75 μM Ca²⁺ (calcium-EGTA buffer containing 20 μM CaCl₂). Microsomal protein concentrations for measuring calcium binding and calcium uptake were 0.3 and 0.03 mg per ml, respectively.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Calcium binding</th>
<th>Calcium uptake</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>nmoles Ca/mg protein</td>
<td>nmoles Ca/min/mg protein</td>
</tr>
<tr>
<td>Protein kinase + cyclic AMP</td>
<td>48</td>
<td>47</td>
</tr>
</tbody>
</table>

TABLE III

Effects of protein kinase on stoichiometric relationship between calcium uptake and Ca²⁺-activated ATPase activity of canine cardiac microsomes

In Experiment A, freshly prepared microsomes (50 μg per ml) were pretreated for 10 min at 25° according to Procedure I, with and without protein kinase (100 μg per ml) and cyclic AMP (1 μM) under conditions described under “Methods” except that calcium-EGTA buffers were omitted. Assays for ATPase and calcium uptake were subsequently started by the addition of calcium-EGTA buffers (Ca²⁺ = 0.75 μM). In Experiment B, microsomes (0.7 mg per ml) from the same preparation as was used in Experiment A were treated with and without protein kinase (1.5 mg per ml) and cyclic AMP (1 mg per ml) and subsequently were washed with buffer solution according to Procedure III. Assays for ATPase and calcium uptake of these microsomes were carried out as described above. Experiment A was performed within 1 hour after preparation was completed; assays for ATPase and calcium uptake in Experiment B were carried out approximately 2 hours later than those in Experiment A due to washing procedures.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Pretreatment</th>
<th>Calcium uptake</th>
<th>Ca²⁺-activated ATPase activity</th>
<th>Stoichiometric ratio (Ca²⁺/P₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>61</td>
<td>Ca²⁺-activated ATPase activity</td>
<td>nmoles Ca/min/mg protein</td>
</tr>
<tr>
<td></td>
<td>Protein kinase</td>
<td>92</td>
<td>33</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Protein kinase + cyclic AMP</td>
<td>143</td>
<td>52</td>
<td>2.1</td>
</tr>
<tr>
<td>B</td>
<td>Control</td>
<td>81</td>
<td>50</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Protein kinase</td>
<td>120</td>
<td>71</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Protein kinase + cyclic AMP</td>
<td>167</td>
<td>103</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* Calculated by dividing the rate of calcium uptake by the rate of Ca²⁺-activated ATPase.

in the presence of cyclic AMP (17). This enhancement of calcium transport is accompanied by phosphorylation of microsomal protein (18–20) in which γ-phosphate of ATP is incorporated into a phosphoester bond that is different from that of the acyl phosphoprotein ATPase intermediate (18, 19). In view of the well established coupling between calcium uptake and Ca²⁺-activated ATPase activity in skeletal (4–7), and cardiac (29, 30) muscle sarcoplasmic reticulum, the protein kinase-induced stimulation of calcium uptake in cardiac sarcoplasmic reticulum could represent either an enhanced turnover of a normally coupled calcium transport system, or an increased efficiency of calcium transport. The present findings, which demonstrate that enhancement of cardiac microsomal calcium uptake by protein kinase is accompanied by increased Ca²⁺-activated ATPase activity, are thus in accord with thermodynamic analysis of this system in skeletal muscle (31). Maintenance of the stoichiometric coupling ratio of 2 moles of calcium taken up for each mole of ATP hydrolyzed after treatment with protein kinase and cyclic AMP (Table III) indicates that the overall rate of calcium transport, rather than its efficiency, is enhanced. This stoichiometry is the same as that reported for calcium transport by preparations of skeletal muscle sarcoplasmic reticulum (4–7). In cardiac microsomes, Entman et al. (30) observed a similar stoichiometry but Suko (29) reported a Ca²⁺/P₀ ratio of 1. We observed that aging of the microsomes decreases the Ca²⁺/P₀ ratio, so that the higher Ca²⁺/P₀ ratio of 2 is probably correct in the case of freshly prepared cardiac microsomes.

Stimulation of calcium transport by protein kinase also can be demonstrated in cardiac microsomes that were washed after phosphorylation (Fig. 5), indicating that the effect does not require either protein kinase or cyclic AMP to be present at the time that measurements of calcium uptake or Ca²⁺-activated ATPase are made. The washing procedure slightly reduces the Ca²⁺/P₀ ratio, but this ratio is not influenced by protein kinase-catalyzed phosphorylation (Table III).

Marked stimulation of calcium uptake and Ca²⁺-activated ATPase activity by protein kinase and cyclic AMP is seen at Ca²⁺ concentrations where both the sarcoplasmic reticulum calcium pump (15) and the contractile system (32) of myocardium are fully active (Fig. 6). Unlike the activity of the calcium pump, which is dependent on the Ca²⁺ concentration, the protein kinase-catalyzed phosphorylation of cardiac microsomes is relatively independent of Ca²⁺ concentration in the physiological range between 0.1 and 100 μM (18). Over this range of Ca²⁺ concentrations, both Ca²⁺-activated ATPase and calcium uptake are significantly enhanced (Fig. 6). Stimulation of Ca²⁺-activated ATPase activity (Fig. 4) and calcium uptake (17) by protein kinase is seen with cyclic AMP concentrations between 10⁻⁸ to 10⁻⁴ M, levels which have been estimated to occur in the intact myocardium (33, 34).

Slight but significant stimulation of Ca²⁺-activated ATPase activity is seen after pretreatment of cardiac microsomes with protein kinase in the absence of cyclic AMP (Fig. 2). This stimulation, initially, is less than that seen in the presence of both protein kinase and cyclic AMP, but as the reaction time is prolonged the extent of ATP hydrolysis approaches that seen with both added protein kinase and cyclic AMP (Fig. 2). These findings are qualitatively similar to the effect of protein kinase alone on both cardiac uptake (17) and phosphorylation of the microsomes (18) and may be attributed to production of cyclic AMP by an active microsomal adenylate cyclase (18, 35–37). The protein kinase concentration needed to attain maximal stimulation of calcium transport was high because the protein kinase preparations used in these studies were only partially purified through the DEAE-cellulose chromatography step. According to the data of Miyamoto et al. (21) and Rubin et al. (38), the specific activity of protein kinase partially purified through the DEAE-cellulose chromatography step can be estimated to be approximately one-fourth to one-eighth that of highly purified protein kinase.

While cyclic AMP is considered to mediate several metabolic actions of catecholamines on heart muscle (e.g. Ref. 39), its role in mediating the positive inotropic response to catecholamines,
which was first postulated by Sutherland and Rall (40), has not
been entirely clarified (41-45). Considerable evidence, however,
exists to suggest such a role for cyclic AMP (46, 47). The pres-
ent evidence together with our previous communications (17, 18),
which document cyclic AMP-dependent protein kinase-induced
stimulation of calcium transport by the cardiace sarcoplas-
mic reticulum and phosphorylation of these membranes,
may provide a biochemical basis for the mediation by cyclic
AMP of the two principal mechanical effects of catecholamines:
abnormal contraction of systole because of the increased rate at
which calcium would otherwise be lost during diastole. This
increased calcium storage could add to the amount of calcium
available for delivery to the contractile system in subsequent beats, thus
promoting augmentation of myocardial contractility. Such a con-
ideration is supported by the recent findings obtained by Rolett
(49) who found that the initial mechanical response of the heart
to norepinephrine is a shortening of systole without an increase in
tension, the rise in tension occurring later. Augmentation of
myocardial contractility due to catecholamines thus may be
related, at least in part, to the stimulation of calcium transport
by the sarcoplasmic reticulum. There is additional evidence,
however, that the increased myocardial contractility may also be
related to catecholamine-induced enhancement of calcium
influx across the plasma membrane (50-52).

If stimulation of calcium transport by cyclic AMP-dependent
protein kinase reflects a physiological response of the intact myo-
cardium, then agents other than catecholamines which activate
adenylate cyclase should also produce mechanical effects similar
to catecholamines. Glucagon, previously shown to increase
adenylate cyclase activity (53), also increases contractility (34,
54, 55). However, its ability to abbreviate systole has been
questioned (54, 55). These findings have been discussed by
Epstein et al. (47) who found that highly purified glucagon
causes a slight but significant abbreviation of systole in the cat
papillary muscle that is similar to the shortening of contraction seen
with concentrations of norepinephrine that produce comparable
increase in developed tension. These investigators also
found that, in the presence of a fixed concentration of theophyl-
line, glucagon could reduce time to peak tension by up to one-
third, this abbreviation of systole being similar to that induced
by concentrations of norepinephrine that caused comparable
tension increases.

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