Characterization of Calmodulin Effects on Calcium Transport in Cardiac Microsomes Enriched in Sarcoplasmic Reticulum†

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ABSTRACT: Calmodulin prepared from red cell hemolysates was found to significantly increase Ca^{2+} uptake into cardiac microsomal preparations enriched in sarcoplasic reticulum in a dose-dependent manner. The stimulation of calcium uptake by calmodulin was additive to that stimulation produced by maximal stimulatory concentrations of adenosine cyclic 3′,5′-phosphate (cAMP) dependent protein kinase and cAMP, indicating separate mechanisms of action and potentially different modulatory roles for these two systems in the control of calcium transport. K^+ significantly decreased calmodulin stimulation of calcium uptake, while in the absence of calmodulin, K^+ increased Ca^{2+} uptake. In the absence of K^+, calmodulin increased Ca^{2+} uptake to levels observed at maximal K^+ concentrations without calmodulin present. Na^+ produced effects similar to those of K^+ in this preparation both in the presence and absence of calmodulin. The effect of calmodulin on the intermediate steps of the (Mg^{2+},Ca^{2+})ATPase in cardiac sarcoplasmic reticulum was also investigated. Calmodulin was found to reduce the steady-state level of the Ca^{2+}-dependent phosphoprotein ($E_{Ca}P$) and increase the (Mg^{2+},Ca^{2+})ATPase activity of this preparation. Dephosphorylation of $E_{Ca}P$ in the presence of Tris–ATP (0.5 mM) was significantly stimulated by calmodulin. These studies indicate that calmodulin stimulates Ca^{2+} transport in cardiac sarcoplasmic reticulum by increasing the turnover rate of the transport process.

A Ca^{2+}-dependent protein activator of cyclic nucleotide phosphodiesterase (3′:5′-cyclic-AMP 5′-nucleotidohydrolase, EC 3.1.4.17) has been isolated from all eukaryotic cells examined, including bovine brain (Cheung, 1971; Kakiuchi et al., 1972), bovine heart (Stevens et al., 1976; Watterson et al., 1976), and rat testis (Dedman et al., 1978).

A soluble Ca^{2+}-dependent activator protein has been isolated from erythrocyte hemolysates; this protein has been shown to stimulate (Ca^{2+},Mg^{2+})ATPase (ATP phosphohydrolase, EC 3.6.1.3) in preparations of red cell membranes (Gopinath & Vincenzi, 1977; Luthra et al., 1976; Katz et al., 1979; Jarrett & Penniston, 1977) and Ca^{2+} transport in "inside-out" red cell vesicle preparations (Larsen & Vincenzi, 1979). Characterization of this protein shows a chemical and functional similarity to the phosphodiesterase activator protein (Gopinath & Vincenzi, 1977; Jarrett & Penniston, 1977). It is generally agreed that these proteins are identical, and the name calmodulin has been established to describe them.

Studies in our laboratory have shown a significant stimulation of ATP[1]-dependent calcium transport in dog cardiac microsomes enriched in sarcoplasmic reticulum by calmodulin isolated from bovine brain (Katz & Remtulla, 1978). The purpose of these present studies was to further characterize the effects of calmodulin on Ca^{2+} transport in these preparations and to investigate the effect of calmodulin on the formation and decomposition of the Ca^{2+}-dependent phosphoprotein ($E_{Ca}P$), the membrane-associated intermediate event of the Ca^{2+} transport pathway.

Materials and Methods

Materials. Tris–ATP, cAMP, protein kinase (Type 1), EGTA, and EDTA were purchased from the Sigma Chemical Co. [γ-^{32}P]ATP (10–40 Ci/mmol) and $^{45}CaCl_2$ (10 Ci/mmol) were purchased from Amersham Co. [^{3}H]ATP (29 Ci/mmol) and Aquasol scintillation fluid were purchased from New England Nuclear.

Calmodulin Preparations. Calmodulin was prepared from the red cells of outdated human blood essentially by the method of Vincenzi and co-workers (Jung, 1978), except that following elution from the DEAE-Sephadex A50 column the fractions found to stimulate red cell (Mg^{2+},Ca^{2+})ATPase activity were concentrated and passed through a Sephadex G-25 column. The preparations were stored at −80 °C and used within 3 weeks. In order to compare the results obtained with this preparation of calmodulin, we also conducted some experiments with a calmodulin preparation from bovine brain obtained from the Sigma Chemical Co. This preparation became available during the course of these studies and is similar to that preparation used in our previous observations of calmodulin stimulation of calcium transport in cardiac sarcoplasmic reticulum (Katz & Remtulla, 1978). In all cases, the calmodulin preparation from red cell hemolysates used produced results similar to that of the purified bovine brain calmodulin preparations.

Miscellaneous Methods. The preparation of EDTA-washed red cell membranes and the method of measurement of (Mg^{2+},Ca^{2+})ATPase activity have been described previously (Katz et al., 1979; Katz & Blostein, 1975).

Preparation of Cardiac Microsomal Preparations. Dog cardiac microsomes enriched in sarcoplasmic reticulum were prepared by the method of Harigaya & Schwartz (1969) with slight modifications. Frozen pieces (1–2 g) of dog ventricle were homogenized with a Polytron P20 homogenizer (3 passes of 5 s at 1500 rpm). The homogenate was then fractionated by differential centrifugation, followed by 0.6 M KCl wash to reduce actomyosin contamination. The final preparation was suspended in a medium consisting of 40% sucrose and 40 mM Tris-HCl, pH 7.2, and used immediately.

Measurement of Calcium Transport. ATP-dependent calcium uptake was measured by the method of Tada et al. (1974) with a few modifications. Oxalate-facilitated calcium uptake was determined in an incubation medium containing 40–50 μg of protein of the microsomal preparation, 40 mM histidine hydrochloride, pH 6.8, 5 mM $MgCl_2$, 110 mM KCl,

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[1] Abbreviations used: ATP, adenosine 5′-triphosphate; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; cAMP, adenosine cyclic 3′,5′-phosphate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; DEAE, diethylaminoethyl; $NaDodSO_4$, sodium dodecyl sulfate.

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5 mM Tris–ATP, 2.5 mM Tris–oxalate, and $CaCl_2$ containing $^{45}CaCl_2$ (10 Ci/mmol). The desired free calcium concentration was maintained by the addition of ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetate (EGTA), and the free calcium concentrations present were determined by the equations of Katz et al. (1970). Following a preincubation of 7 min at 30 °C in the presence and absence of calmodulin (2 μg/0.5-mL final incubation volume, unless otherwise stated) the reaction was started by the addition of $^{45}CaCl_2$. After 5 min, the reaction was terminated by filtering an aliquot of the reaction mixture through a millipore filter (HA 45, Millipore Co.). The filter was then washed twice with 15 mL of 40 mM Tris-HCl, pH 7.2, dried, and counted for radioactivity in Aquasol (New England Nuclear Co.) by using standard liquid scintillation counting techniques.

Phosphorylation of Microsomal Preparations Enriched in Sarcoplasmic Reticulum. Phosphoprotein formation was measured by the method of Katz & Blostein (1975). Microsomal membranes were first incubated at 37 °C for 10 min in the presence of 0.1 mM Tris–EGTA, pH 7.4, and then transferred to a shaking water bath at 10 or 14 °C and incubated for a further 10 min prior to the start of the reaction. The reaction mixture contained 40 mM histidine hydrochloride, pH 6.8, 10 μM $MgCl_2$, 0.10 mM Tris–EGTA, pH 7.4, and 2 μM ATP consisting of [γ-^{32}P]ATP (sp act. of 1000–3000 cpm/pmol), [8-^{3}H]ATP (500–1000 cpm/pmol) to monitor for nonspecific binding, and Tris–ATP. When calcium was added, it was present in a concentration of 1.8 μM free Ca^{2+} as determined by the equations of Katz et al. (1970). The reaction was started by the addition of the preincubated membranes (90–150 μg of tissue/final incubation volume of 0.2 mL) to the sample tubes followed by vigorous shaking. The reaction proceeded for a total of 15 s and then was terminated by the addition of 5% trichloroacetic acid containing 5.0 mM Na_2ATP and 2 mM KH_2PO_4. The samples were mixed, 200 μL of 0.5% bovine serum albumin was added, and the samples were centrifuged at 3000 rpm for 5 min. The resulting pellets were washed 3 times. The samples were then resuspended in 0.2 M phosphate buffer and 0.2% $NaDodSO_4$, pH 7.2, and counted for radioactivity by using a β liquid scintillation counter.

In experiments where dephosphorylation was investigated, the reaction proceeded as described; following the 15-s incubation period either 2 mM Tris-HCl (control), 2 mM EGTA–Mg, or 0.5 mM Tris–ATP was added, the tubes were shaken vigorously, and the reaction was continued for an additional 5 s. The reaction was then terminated as described above. Ca^{2+}-dependent phosphorylation is designated as that amount of phosphorylation observed in the presence of 1.8 μM free Ca^{2+} following subtraction of the amount noted in the presence of 0.1 mM Tris–EGTA.

In these experiments, (Mg^{2+},Ca^{2+})ATPase activity was determined by removing an aliquot of the supernatant following the first centrifugation and subjecting it to charcoal absorption (Fisher Norit A; 1.5 g/10 mL in the presence of 5% trichloroacetic acid). Following incubation for 1 h at 0 °C with mixing every 15 min, the samples were centrifuged and an aliquot of the clear supernatant was counted for radioactivity. (Mg^{2+},Ca^{2+})ATPase activity is designated as that activity following subtraction of the activity noted in the presence of 0.1 mM Tris–EGTA.

When cAMP-dependent protein kinase (Sigma Type 1) was added to the medium, it was present in a concentration of 25 μg/0.5 mL, along with 1.0 μM cAMP. Protein concentrations were measured by the method of Lowry et al. (1951). Student's *t* test for unpaired, common variance data (Wonnacott & Wonnacott, 1977) was used as a measure of significance. Standard error of the mean (SEM) was used as a measure of variation.

Table I: Effect of Red Cell Calmodulin and cAMP-Dependent Protein Kinase on Ca^{2+} Uptake in Cardiac Microsomal Preparations Enriched in Sarcoplasmic Reticulum[a]

	Ca^{2+} uptake (nmol mg^{-1} min^{-1})
control	12.2 ± 0.4[b]
plus calmodulin (2 μg/0.5 mL)	16.5 ± 0.5[c]
plus cAMP-dependent protein kinase	18.2 ± 0.4[c]
plus cAMP-dependent protein kinase and calmodulin (2 μg/0.5 mL)	21.6 ± 0.5[d]

[a] Ca^{2+} uptake was assayed as described under Materials and Methods in the presence of 110 mM KCl and 1 μM free calcium. [b] Mean ± SEM of at least three observations in each group. [c] Significant ($P < 0.001$) when compared to the control. [d] Significant ($P < 0.001$) when compared to that activity in the presence of calmodulin alone.

Results

Comparison of Red Cell Calmodulin Preparations Used and Bovine Brain Calmodulin. Previous studies had been conducted with calmodulin purified from bovine brain (Katz & Remtulla, 1978). In these present studies calmodulin prepared from red cell hemolysates was used. In preliminary investigations the effect of bovine brain calmodulin and red cell calmodulin on the Mg^{2+}-ATPase and (Mg^{2+},Ca^{2+})ATPase activity of EDTA-washed red cell membrane preparations (Katz et al., 1979; Katz & Blostein, 1975) was determined. Maximal concentrations of these preparations stimulated (Mg^{2+},Ca^{2+})ATPase activity to a similar degree (not shown). Neither preparation of calmodulin affected the Mg^{2+}-ATPase activity of the red cell preparation or possessed indigenous ATPase activity. Bovine brain calmodulin from commercial source (Sigma) produced similar results to the red cell calmodulin preparation used in experiments on Ca^{2+} uptake into inside-out red cell vesicle preparations and cardiac microsomal preparations enriched in sarcoplasmic reticulum reticulum (not shown).

Effect of Red Cell Calmodulin on Calcium Transport in Cardiac Microsomal Preparations Enriched in Sarcoplasmic Reticulum. The effects of varying red cell calmodulin concentrations on Ca^{2+} uptake in cardiac microsomal preparations enriched in sarcoplasmic reticulum were investigated (not shown). These experiments were done in the presence of 110 mM KCl as described under Materials and Methods. Peak activation of calcium uptake occurred at 2 μg of the calmodulin preparation (final incubation volume of 0.5 mL).

As has been noted in our previous studies (Katz & Remtulla, 1978) and in those of other laboratories (Tada et al., 1974), cAMP-dependent protein kinase in the presence of cAMP stimulated Ca^{2+} uptake in cardiac microsomal preparations. The addition of maximal stimulatory concentrations of cAMP-dependent protein kinase and cAMP in the presence of calmodulin significantly stimulated Ca^{2+}-uptake activity over that activity noted in the presence of calmodulin alone (Table I).

Effect of Monovalent Cations on Calmodulin Stimulation of Ca^{2+} Transport in Microsomal Preparations Enriched in Sarcoplasmic Reticulum. The effect of red cell calmodulin on Ca^{2+} uptake in microsomal preparations enriched in sarcoplasmic reticulum in the presence and absence of 110 mM KCl or LiCl is shown in Figure 1. In the presence of 110 mM KCl, Ca^{2+} uptake activity is enhanced compared to that ac-

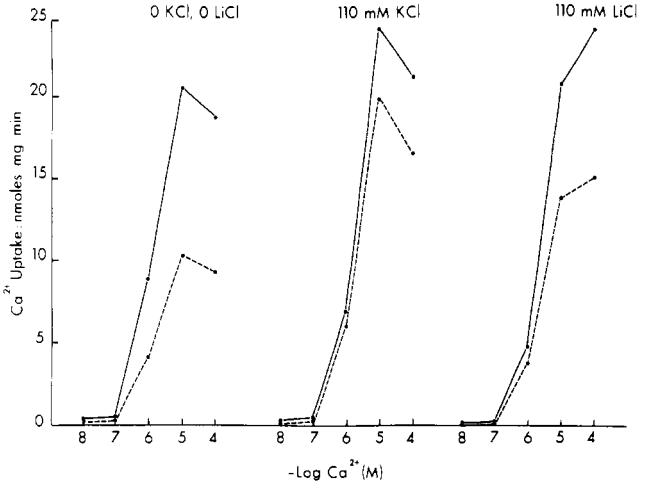

FIGURE 1: Effect of monovalent cations on Ca^{2+} uptake in the presence and absence of calmodulin. Ca^{2+} uptake was determined as described under Materials and Methods in the presence and absence of 110 mM KCl, 110 mM LiCl, or 200 mM sucrose (0 KCl, 0 LiCl) with (●–●) and without (●---●) calmodulin (3.0 μg/0.5-mL final incubation volume). The result shown is a typical experiment.

tivity noted in the presence of 110 mM LiCl or in the presence of no monovalent cations (200 mM sucrose used to maintain osmolarity) at every free calcium concentration studied. In the presence of 110 mM KCl, calmodulin increased Ca^{2+} uptake activity by 20–25% as noted previously (Katz & Remtulla, 1978). However, in the presence of 110 mM LiCl or in the absence of monovalent cations, calmodulin stimulated Ca^{2+} uptake to a much greater degree. In fact, in the absence of KCl calmodulin restored Ca^{2+} uptake to the maximum activity noted in the presence of 110 mM KCl alone. Under the experimental conditions utilized, Ca^{2+} uptake was found to be linear with respect to time in both the presence and absence of 110 mM KCl at all free Ca^{2+} concentrations studied (0.1–30 μM free Ca^{2+}).

The effects of increasing K^+ concentrations on calmodulin stimulation of calcium transport in cardiac microsomal preparations enriched in sarcoplasmic reticulum was investigated (Figure 2). Increasing the K^+ concentration increased the degree of calcium uptake to a maximum at 30 mM KCl, after which Ca^{2+} uptake decreased slightly. Calmodulin, though, stimulated Ca^{2+} uptake to a greater degree in the absence of added KCl. As the KCl concentration was increased, the degree of calmodulin stimulation of calcium uptake decreased, from over 100% stimulation at zero K to 15–25% at 110 mM KCl. A qualitatively similar result was obtained with increasing NaCl concentrations (Figure 3). In our hands, maximal calcium uptake occurred at 100 mM NaCl. Calmodulin produced the highest stimulatory effect at zero NaCl, and no stimulation was noted at NaCl concentrations above 80 mM.

Effect of Calmodulin on the Ca^{2+} Transport Intermediate. The effect of calmodulin on the steady-state level of phosphoprotein present in cardiac microsomal preparations enriched in cardiac sarcoplasmic reticulum is shown in Figure 4. Under the assay conditions used (2 μM ATP, 15-s incubation at 10 °C), cAMP and cAMP-dependent protein kinase did not stimulate the level of phosphorylation above that noted in the presence of 0.1 mM EGTA (not shown). In the presence of calmodulin concentrations that stimulate (Mg^{2+},Ca^{2+})ATPase activity in this preparation, a slight decrease in the steady-state level of phosphoprotein was noted. The v/EP ratio, the turnover rate of this reaction, increased from 5.6 to 10.5 at concentrations of calmodulin previously found to maximally

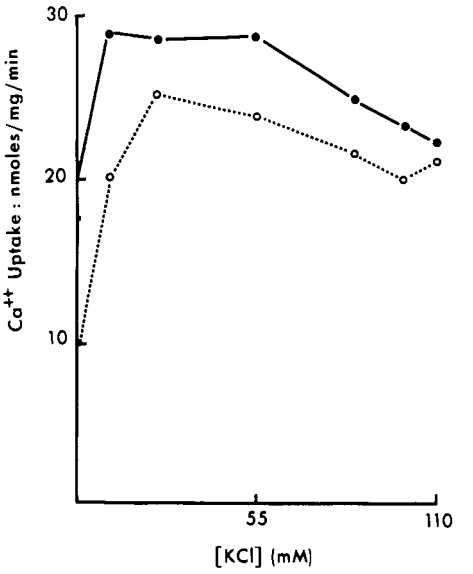

FIGURE 2: Effect of K^+ on Ca^{2+} uptake in the presence of red cell calmodulin. Ca^{2+} uptake was determined as described under Materials and Methods at 1 μM free Ca^{2+} in the presence (●) and absence (○) of red cell calmodulin (3.0 μg/0.5-mL final incubation volume). The result shown is a typical experiment.

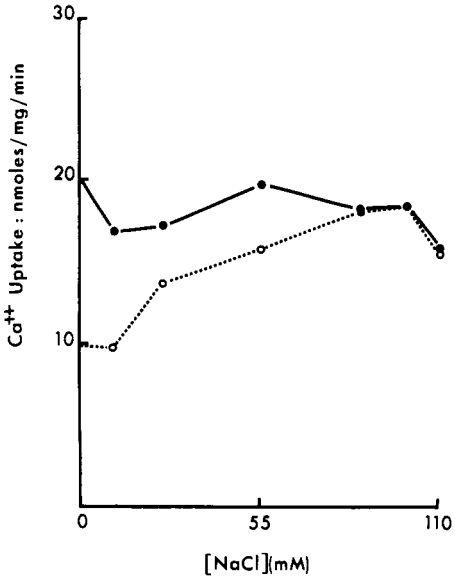

FIGURE 3: Effect of Na^+ on Ca^{2+} uptake in the presence and absence of red cell calmodulin. Ca^{2+} uptake was determined as described under Materials and Methods at 1 μM free Ca^{2+} in the presence (●) and absence (○) of red calmodulin (3.0 μg/0.5-mL final incubation volume). The result shown is a typical experiment.

stimulate Ca^{2+} uptake in this preparation.

In view of these observations on the turnover rate of the Ca^{2+} pump, the effect of calmodulin on the dephosphorylation of the Ca^{2+}-dependent phosphoprotein intermediate was investigated. Where indicated, calmodulin was present throughout the reaction procedure. Dephosphorylation of the Ca^{2+}-dependent phosphoprotein in the presence of EGTA–Mg (2 mM) was slightly but not significantly enhanced in the presence of calmodulin (Figure 5). In the presence of 0.5 mM Tris–ATP, calmodulin significantly increased the Ca^{2+}-dependent phosphoprotein decomposition compared to levels noted in the absence of added calmodulin ($P < 0.02$).

Discussion

A red calmodulin which stimulates (Mg^{2+},Ca^{2+})ATPase activity and Ca^{2+} transport in red blood cell preparations

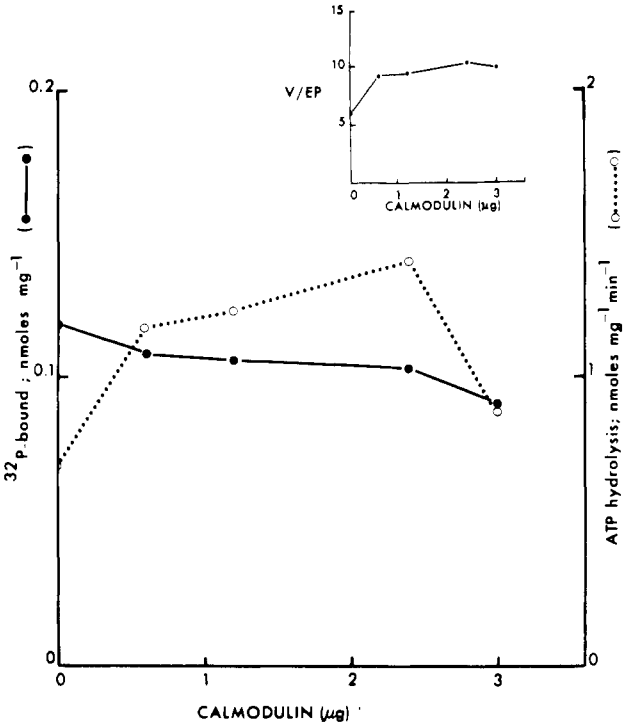

FIGURE 4: Effect of calmodulin concentration on Ca^{2+}-dependent phosphorylation and (Mg^{2+},Ca^{2+})ATPase activity in microsomal preparations enriched in sarcoplasmic reticulum. Dog cardiac microsomal preparations were phosphorylated at 10 °C as described under Materials and Methods in the presence of Ca^{2+} (1.8 μM free Ca^{2+}) varying concentrations of calmodulin (0.5–2.5 μg/0.2-mL final incubation volume). Following termination of the reaction and centrifugation, an aliquot of the supernatant was assayed to determine the (Mg^{2+},Ca^{2+})ATPase activity. The insert indicates the v/EP ratio at each calmodulin concentration. The result shown is a typical experiment of three similar experiments done on different preparations. Phosphorylation refers to the Ca^{2+}-stimulated component following subtraction of values measured in the absence of added $CaCl_2$ (~70 pmol/mg in the presence of 0.1 mM EGTA).

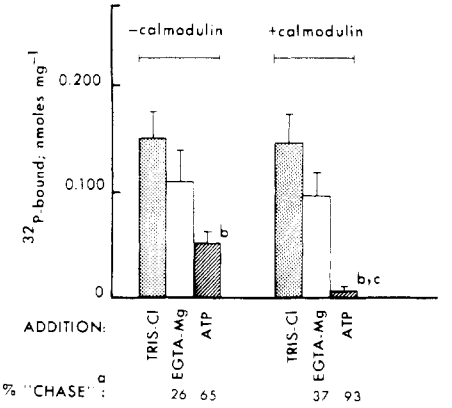

FIGURE 5: Effect of calmodulin on Ca^{2+}-dependent phosphoprotein decomposition in microsomal preparations enriched in sarcoplasmic reticulum. Dog cardiac microsomal preparations were phosphorylated as described under Materials and Methods in the presence of Ca^{2+} (1.8 μM free Ca^{2+}) and in the presence and absence of calmodulin (1.2 μg). The reaction proceeded at 10 °C for 15 s when either 2 mM Tris-HCl, 2 mM EGTA–Mg, or 0.5 mM Tris–ATP was added to the reaction medium. The reaction was continued for an additional 5 s and then terminated as described under Materials and Methods. The results shown are a mean ± SEM of four similar experiments done on different microsomal preparations. Phosphorylation refers to the Ca^{2+}-stimulated component following subtraction of values measured in the absence of added $CaCl_2$ (~70 pmol/mg in the presence of 0.1 mM EGTA). (a) Percent dephosphorylation compared to that noted in the presence of 2 mM Tris-HCl; (b) significant ($P < 0.01$) when compared to the control (2 mM Tris-HCl); (c) significant ($P < 0.02$) when compared to the level noted with 0.5 mM Tris–ATP in the absence of calmodulin.

(Gopinath & Vincenzi, 1977; Larsen & Vincenzi, 1979) was also shown to stimulate Ca^{2+} transport in microsomal preparations enriched in sarcoplasmic reticulum. The mechanism by which calmodulin stimulates calcium transport remained to be defined. Studies on purified calmodulin from bovine heart (Teo et al., 1973; Teo & Wang, 1973) or brain (Lin et al., 1974) have shown that these proteins are Ca^{2+}-dependent and that the active form is a Ca^{2+}–activator complex. In these present studies we have shown that calmodulin increases the calcium uptake velocity in these preparations at all free calcium concentrations tested. We have also shown that calmodulin increases the turnover rate (v/EP) of the calcium pump. This effect is produced by the stimulation of the decomposition of the phosphoprotein intermediate, the rate limiting step of the reaction sequence. These studies therefore indicate that calmodulin stimulates calcium transport by increasing the rate of the calcium pump mechanism. This is due to a direct effect of calmodulin on the dephosphorylation of the Ca^{2+}-dependent phosphoprotein intermediate.

It has been shown that cAMP-dependent protein kinase stimulates the phosphorylation of a 20 000-dalton component of cardiac sarcoplasmic reticulum membranes and thereby affects the Ca^{2+}-transport process (Tada et al., 1974; Kirchberger et al., 1974; Kirchberger & Chu, 1976). In these present studies, it was found that under the assay conditions used (2 μM ATP, low temperature, 15-s incubation), cAMP-dependent protein kinase and cAMP did not stimulate phosphoprotein formation above control levels (0.1 mM EGTA). It is thus suggested that cAMP-dependent protein kinase mediated phosphorylation of phospholamban does not play a role in the calmodulin effect on Ca^{2+}-dependent phosphoprotein formation and decomposition noted. Le Peuch et al. (1979) have recently presented evidence that phospholamban is also a substrate for a Ca^{2+}–calmodulin-dependent kinase, at a phosphorylation site distinct from the site which is a substrate of cAMP-dependent protein kinase, and postulate that this is the mechanism by which calmodulin enhances calcium uptake in this preparation. It was not determined in the present studies if a Ca^{2+}–calmodulin-dependent protein kinase was present in the sarcoplasmic reticulum preparations used.

Le Peuch et al. (1979) observed an increase in calcium uptake into sarcoplasmic reticulum in the presence of calmodulin but no concomitant increase in the (Mg^{2+},Ca^{2+})ATPase activity of this preparation. This is contrary to our observations that indicate that calmodulin concentrations that stimulate Ca^{2+} uptake also increase (Mg^{2+},Ca^{2+})ATPase activity in sarcoplasmic reticulum preparations. Similar findings were obtained previously in red blood cell membrane preparations where both Ca^{2+} uptake and (Mg^{2+},Ca^{2+})ATPase activity were significantly increased by calmodulin (Gopinath & Vincenzi, 1977; Larsen & Vincenzi, 1979; Jarrett & Penniston, 1977). These observations and those indicating an effect of calmodulin on the rate of decomposition of the Ca^{2+}-transport phosphoprotein intermediate suggest an interaction of calmodulin with the Ca^{2+} pump. Calmodulin mediation of calcium transport by mechanisms other than via the ATP-dependent calcium transport system has to date not been reported by other investigators.

It was observed that the calmodulin effect on the decomposition of the Ca^{2+}-dependent phosphoprotein was more marked when Tris–ATP (0.5 mM) was the dephosphorylation agent rather than EGTA–Mg (1 mM). This could be due to the fact that calmodulin itself requires calcium for activation

(Teo et al., 1973; Teo & Wang, 1973; Lin et al., 1974) and not enough calcium is present in the incuation medium following the addition of the high EGTA concentrations to satisfy this requirement.

The red blood cell calmodulin utilized in these studies was similar to preparations used to study calcium transport and (Mg^{2+},Ca^{2+})ATPase activity in red cell membrane preparations (Gopinath & Vincenzi, 1977; Luthra et al., 1976; Katz et al., 1979; Jarrett & Penniston, 1977). That calmodulin from red cells can stimulate Ca^{2+} transport in a muscle system indicates the similarity in the calcium transport systems of various tissues and their mechanism(s) of regulation.

It was found that calmodulin stimulated calcium transport in these preparations to a greater degree in the absence rather than in the presence of K^+ ions. In the absence of K^+, calmodulin increased calcium transport in these preparations to approximately the level noted at high K^+ concentrations. K^+ has been found to stimulate Ca^{2+} uptake and (Mg^{2+},Ca^{2+})-ATPase activity in sarcoplasmic reticulum preparations (Shigekawa & Pearl, 1976; Jones et al., 1978). The possible mechanism of this effect is an increased turnover of the phosphorylated intermediate of the Ca^{2+} pump (Jones et al., 1978). It is possible that calmodulin stimulates Ca^{2+} uptake in a manner similar to that of K^+ and thus does not function maximally in the presence of high K^+ concentrations. It is also possible that K^+ may be affecting calmodulin stimulation of calcium transport by altering its binding to the sarcoplasmic reticulum. Na^+ produced effects similar to those of K^+ in this preparation although quantitative and qualitative differences in the effects of these two cations may be present.

The calmodulin–K^+ interactions noted in these studies may indicate the physiological relevance of calmodulin in the control of calcium transport. The sarcoplasmic reticulum in the cardiac cell is bathed in high concentrations of K^+. Thus under normal physiological conditions, it would appear that calmodulin may not be active. It is conceivable, though, that under altered conditions, possibly in disease states, the calmodulin–K^+ balance may be changed and calmodulin regulation of Ca^{2+} transport may then become important. Na^+ appears to compete with calmodulin for Ca^{2+}-transport stimulation as well, but in normal physiological conditions in the cardiac cell this is unlikely to be an important interaction. These present studies do not indicate if in fact calmodulin is present near, or is bound to, the sarcoplasmic reticulum and also do not elaborate on the effect that high K^+ concentrations may have on the binding of calmodulin. The elucidation of these questions would further clarify the physiological relevance of calmodulin in the regulation of calcium transport in cardiac cells.

Acknowledgments

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