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INFLUENCE OF MONOVALENT CATIONS ON THE Ca^{2+}-ATPase OF SARCOPLASMIC RETICULUM ISOLATED FROM RABBIT SKELETAL AND DOG CARDIAC MUSCLES

AN INTERPRETATION OF TRANSIENT-STATE KINETIC DATA

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Transient-state kinetics of phosphorylation and dephosphorylation of the Ca^{2+}-ATPase of sarcoplasmic reticulum vesicles from rabbit skeletal and dog cardiac muscles were studied in the presence of varying concentrations of monovalent and divalent cations. Monovalent cations affect the two types of sarcoplasmic reticulum differently. When the rabbit skeletal sarcoplasmic reticulum was Ca^{2+} deficient, preincubation with K^+ (as compared with preincubation with choline chloride) did not affect initial phosphorylation at various concentrations of Ca^{2+}, added with ATP to phosphorylate the enzyme. This is in contrast to preincubation with K^+ of the Ca^{2+}-deficient dog cardiac sarcoplasmic reticulum, which resulted in an increase in the phosphoenzyme level. When Ca^{2+} was bound to the rabbit skeletal sarcoplasmic reticulum, K^+ inhibited E ~ P formation; but under the same conditions, E ~ P formation of dog cardiac sarcoplasmic reticulum was activated by K^+ at 12 μM Ca^{2+} and inhibited at 0.33 and 1.3 μM Ca^{2+}. Li^+, Na^+ and K^+ also have different effects on E ~ P decomposition of skeletal and cardiac sarcoplasmic reticulum. The latter responded less to these cations than the former. Studies with ADP revealed differences between the two types of sarcoplasmic reticulum. For rabbit skeletal sarcoplasmic reticulum, 40% of the phosphoenzyme formed was 'ADP sensitive', and the decay of the remaining E ~ P was enhanced by K^+ and ADP. Dog cardiac sarcoplasmic reticulum yielded about 40–48% ADP-sensitive E ~ P, but the decomposition rate of the remaining E ~ P was close to the rate measured in the absence of ADP. Thus, these studies showed certain qualitative differences in the transformation and decomposition of phosphoenzymes between skeletal and cardiac muscle which may have bearing on physiological differences between the two muscle types.

Introduction

The Ca^{2+}-ATPase activity of sarcoplasmic reticulum provides energy for the active transport of cytoplasmic calcium into the sarcoplasmic reticulum lumen, which results in muscle relaxation [1,2]. Several approaches have been useful in studying the relationship between sarcoplasmic reticulum properties and twitch characteristics of a muscle. For example, Heilmann and Pette [3] have recently shown that stimulation (for 75 days) of the fast-twitch tibialis anterios muscle of the rabbit with a frequency pattern similar to that of a motor neuron which innervates a slow muscle brings about parallel time courses of changes in contractile properties of the muscle and changes in molecular and functional properties of the sarcoplasmic reticulum. The stimulated fast-twitch muscle was converted into a slow-twitch type and the sarcoplasmic reticulum resembled that isolated from the slow-twitch soleus.

Our approach focuses on measurements of transient-state kinetics of the formation and decomposition of phosphorylated enzymes (E ~ P) under various ligand conditions, using sarcoplasmic reti-

culum isolated from fast- and slow-twitch muscles [4–6]. We have previously found that the longer the half-life of isometric relaxation of a muscle, the slower are two reaction steps in the transient phase of ATP hydrolysis by sarcoplasmic reticulum isolated from the muscle. These two steps include phosphorylation of Ca^{2+}-deficient sarcoplasmic reticulum ($E + Ca^{2+} + ATP \rightarrow E \sim P$) and decomposition of the phosphorylated protein ($E \sim P \rightarrow E + P_i$) [4]. Therefore, it is conceivable that certain ligands that may be involved in regulating sarcoplasmic reticulum activities may exert their influence primarily on these two differentiating reaction steps.

This paper illustrates differences in effects of monovalent cations, K^+ in particular, on the kinetic properties of rabbit skeletal and dog cardiac sarcoplasmic reticulum.

Methods

Materials. [γ-^{32}P]ATP (1 mCi in 0.03 μmol) was purchased from New England Nuclear. ADP (disodium salt) was obtained from Sigma and ATP (disodium salt) from Boehringer Mannheim. All other chemical reagents were of analytical grade and stock solutions were prepared in distilled deionized water.

Preparation of sarcoplasmic reticulum vesicles. The methods were the same as our previously reported procedures for preparing sarcoplasmic reticulum vesicles from dog cardiac and rabbit back skeletal muscles [5] with slight modification [7]. For the experiments carried out in the presence of K^+, the final sarcoplasmic reticulum preparation was suspended in 10 mM Tris-maleate (pH 6.8) containing 100 mM K^+, and for those carried out in the absence of K^+, the sarcoplasmic reticulum preparation was washed in a K^+-free buffer at 143 000 $\times g$ for 45 min and the isolated sarcoplasmic reticulum vesicles were suspended in 30 mM Tris-maleate (pH 6.8). Protein was determined by the biuret method, using bovine serum albumin (Sigma) as standard. All preparations were stored on ice and used within 20 h. The stability of the sarcoplasmic reticulum Ca^{2+}-ATPase prepared by this procedure was high. The skeletal sarcoplasmic reticulum stored at 0°C for 1 week and cardiac sarcoplasmic reticculum for 3 days did not significantly lose the Ca^{2+}-ATPase activity. Electron micrographs showed vesicular structures of these sarcoplasmic reticulum preparations. Sarcolemmal contamination was low as judged by biochemical markers. For example, the cardiac sarcoplasmic reticulum preparation bound a maximum of 10 pmol ouabain/mg protein while a relatively purified dog cardiac sarcolemmal preparation bound about 200 pmol/mg protein (Adams, R., personal communication).

Transient-state experiments. Rapid-mixing experiments were carried out using chemical quench-flow apparatus described previously [4–6,8]. The procedure for isolation of the acid-stable intermediate for ^{32}P counting was the same as previously described [5]. The concentration of free calcium was calculated from an apparent EGTA-calcium binding constant of $6.93 \cdot 10^5$ M^{-1} [9]. The vehicle solution used for the sarcoplasmic reticulum and substrate contained 3 mM $MgCl_2$, 5 mM NaN_3, and 20 mM Tris-maleate (pH 6.8). The temperature was controlled at 20°C.

Effects of monovalent cations on phosphorylation of sarcoplasmic reticulum

Ca^{2+}-deficient sarcoplasmic reticulum. Rabbit skeletal and dog cardiac sarcoplasmic reticulum was studied under the same conditions. Calcium-deficient sarcoplasmic reticulum vesicles were preincubated with or without monovalent cations before phosphorylation with ATP and Ca^{2+}. In the former case, the enzyme syringe contained 1 mg/ml of sarcoplasmic reticulum in the standard vehicle solution including 100 mM K^+ and 100 μM EGTA; the substrate syringe contained 100 mM choline chloride, 20 μM ATP (including 20 μl of [γ-^{32}P]ATP/25 ml), 100 μM EGTA, and 40, 100 or 200 μM $CaCl_2$ (to give a final free [Ca^{2+}] of 0.33, 1.3, or 12 μM); and the perchloric acid syringe contained 9% perchloric acid and 1% sodium polyphosphate. When the sarcoplasmic reticulum was not preincubated with K^+, the 'sarcoplasmic reticulum syringe' contained choline chloride instead of KCl and the choline chloride in the 'substrate syringe' was replaced with KCl. The final concentration of KCl and choline chloride in both cases was 50 mM.

Ca^{2+}-bound sarcoplasmic reticulum. The previously described experimental procedures [4] for the calcium-preloaded sarcoplasmic reticulum were used. Both enzyme and substrate syringes contained 100 mM K^+, Na^+, or choline chloride and also 100 μM

EGTA and 20, 50 or 100 μM $CaCl_2$ (to give a final free [Ca^{2+}] of 0.33, 1.3 or 12 μM).

Effects of monovalent cations on E ~ P decomposition under various conditions

E ~ P formation in 100 mM Li^+ and decomposition in 67 mM Li^+ plus 33 mM Li^+, Na^+, or K^+. The enzyme syringe contained 1 mg/ml sarcoplasmic reticulum, 100 mM LiCl, 100 μM EGTA and 100 μM $CaCl_2$ (free [Ca^{2+}] 12 μM); the substrate syringe contained 20 μM ATP (including [γ-^{32}P]ATP), 100 mM LiCl, 100 μM EGTA and 100 μM $CaCl_2$; the EGTA syringe contained 6 mM EGTA and 100 mM LiCl, NaCl or KCl. All the above ligands were dissolved in the standard vehicle solution. The reaction of sarcoplasmic reticulum with ATP proceeded for 116 ms before quenching free Ca^{2+} with EGTA to initiate E ~ P decomposition. The time course of E ~ P decomposition was measured from 0 to 277 ms. A 9% perchloric acid solution including 1% sodium polyphosphate was used to quench the reaction. The entire sequence of reaction was carried out at 20°C.

Effect of ADP. The experimental procedure were the same as described above for the E ~ P decomposition with the EGTA syringe containing 4 mM ADP. Formation of E ~ P was quenched with EGTA and ADP at 20.5 ms and the time courses of the subsequent E ~ P disappearance were measured from 0 to 277 ms at 20°C.

Results

Effects of K^+ on E ~ P formation in Ca^{2+}-deficient sarcoplasmic reticulum

The Ca^{2+}-deficient rabbit skeletal sarcoplasmic reticulum, whether or not preincubated with 100 mM KCl, gave the same E ~ P level in the initial phase (0–40 ms) of phosphorylation by ATP and Ca^{2+} (Fig. 1). In each case the final concentrations of all ligands in the reaction medium were kept identical. The Ca^{2+}-deficient dog cardiac sarcoplasmic reticulum, on the other hand, was activated by K^+. At a final concentration of 12 μM Ca^{2+} (added with ATP), the K^+-incubated sarcoplasmic reticulum gave an E ~ P level (at 10 ms) about 32% higher than the choline chloride-preincubated sarcoplasmic reticulum. In 1.3 μM Ca^{2+}, the difference was only 23%. In 0.33 μM Ca^{2+}, no significant difference was observed.

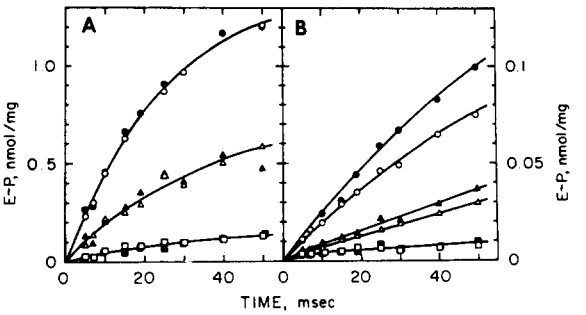

Fig. 1. Time courses of the initial formation of the phosphorylated enzyme, E ~ P, from calcium-free sarcoplasmic reticulum preincubated with choline chloride or KCl. A, rabbit skeletal sarcoplasmic reticulum; B, dog cardiac sarcoplasmic. The phosphorylation reaction was carried out at 20°C. Sarcoplasmic reticulum was preincubated with 100 mM choline chloride (open symbols) or K^+ (solid symbols with Ca^{2+} and K^+ or Ca^{2+} and choline chloride added together with ATP. The final concentrations were: 0.5 mg/ml sarcoplasmic reticulum, 10 μM ATP, 3 mM $MgCl_2$, 5 mM NaN_3, 50 mM K^+, 50 mM choline chloride, 20 mM Tris-maleate (pH 6.8), and 0.33 μM (□——□ and ■——■), 1.3 μM (△——△ and ▲——▲) or 12 μM (○——○ and ●——●) Ca^{2+}.

Effects of K^+ and Na^+ on E ~ P formation in Ca^{2+}-bound sarcoplasmic reticulum

Rabbit skeletal sarcoplasmic reticulum vesicles containing bound Ca^{2+} gave E ~ P levels in 100 mM K^+ or Na^+ that were lower than the E ~ P levels in 100 mM choline chloride. This decrease in E ~ P level by K^+ and Na^+ was dependent on the Ca^{2+} concentration in the medium. In 12 μM Ca^{2+}, the E ~ P levels obtained (at 10 ms) in the presence of K^+ and Na^+ were 75 and 65%, respectively, of that obtained in choline chloride. However, in 1.3 μM Ca^{2+}, the percentage of the E ~ P level obtained in K^+ decreased to 55% of that obtained in choline chloride and, in Na^+, the percentage decreased to 38%. In 0.33 μM Ca^{2+}, 18 and 13% were obtained, respectively (Fig. 2). For the Ca^{2+}-bound dog cardiac sarcoplasmic reticulum vesicles, K^+ or Na^+ enhanced the E ~ P formation at high [Ca^{2+}] and inhibited it at low [Ca^{2+}]. In 12 μM Ca^{2+}, the E ~ P levels obtained in 100 mM K^+ and Na^+ were 180 and 150%, respectively, of that obtained in 100 mM choline chloride. In 1.3 μM Ca^{2+}, the percentages decreased to 90% in K^+ and 70% in Na^+ and, in 0.33 μM Ca^{2+}, to 60% in both K^+ and Na^+ (Fig. 3).

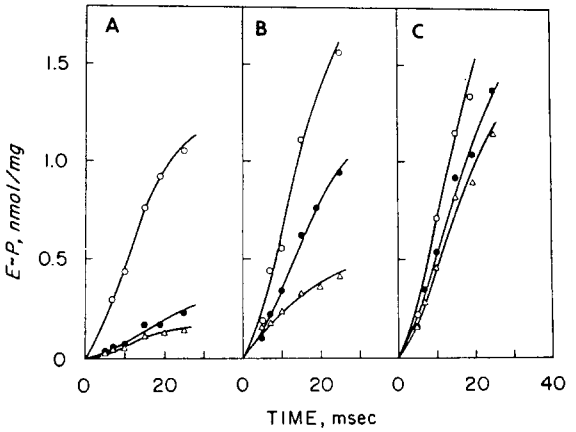

Fig. 2. Effects of Na^+ and K^+ on the initial phosphorylation of rabbit skeletal sarcoplasmic reticulum at various Ca^{2+} concentrations: A, 0.33 μM; B, 1.3 μM; C, 12 μM. Other reaction conditions were: 0.5 mg/ml sarcoplasmic, 10 μM ATP, 3 mM $MgCl_2$, 5 mM NaN_3, 20 mM Tris-maleate (pH 6.8), and 100 mM choline chloride (○———○), Na^+ (△———△) or K^+ (●———●).

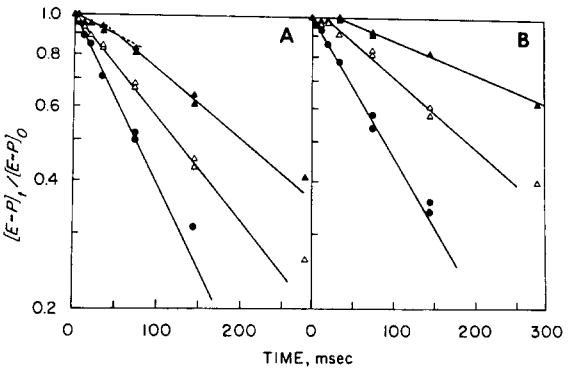

Fig. 4. Semilogarithmic plots of E ~ P decomposition with respect to time. A, rabbit skeletal sarcoplasmic reticulum; B, dog cardiac sarcoplasmic reticulum. E ~ P was formed at 20°C in 20 mM Tris-maleate buffer (pH 6.8) containing 10 μM ATP, 12 μM Ca^{2+}, 3 mM $MgCl_2$, 5 mM NaN_3 and 100 mM Li^+. At 116 ms a buffer solution containing 3 mM $MgCl_2$, 5 mM NaN_3, 6 mM EGTA and 100 mM Li^+, Na^+ or K^+ was added to initiate E ~ P decomposition. Final concentrations of monovalent cations in the decomposition medium were; ▲———▲, 100 mM Li^+; △———△, 67 mM Li^+ and 38 mM Na^+; ●———●, 67 mM Li^+ and 33 mM K^+.

Effects of monovalent cations on E ~ P decomposition

The phosphorylated enzyme, E ~ P, formed in 100 mM Li^+, decomposed at different rates in the presence of Li^+, Na^+ or K^+. For the skeletal E ~ P, there was a slow, initial phase of E ~ P decomposition in the presence of 100 mM Li^+, whereas for the cardiac E ~ P, induction periods were observed for 20–30 ms in 100 mM Li^+ or in 67 mM Li^+ and 38 mM Na^+ (Fig. 4). The rate constant for decomposition of the major portion for the skeletal E ~ P was greater than that for cardiac E ~ P under identical conditions: 3.8 vs. 1.8 s^{-1} in 100 mM Li^+; 5.7 vs. 4.0 s^{-1} in 67 mM Li^+ and 38 mM Na^+; and 9.4 vs. 7.8 s^{-1} in 67 mM Li^+ and 33 mM K^+.

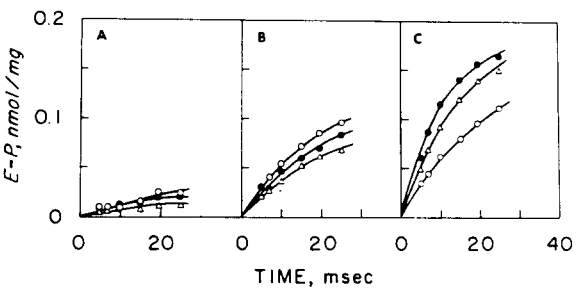

Fig. 3. Effects of Na^+ and K^+ on the initial phosphorylation of dog cardiac sarcoplasmic reticulum at various Ca^{2+} concentrations: A, 0.33 μM; B, 1.3 μM; C, 12 μM. Choline chloride (○———○), Na^+ (△———△), and K^+ (●———●). Reaction conditions were as in Fig. 2.

Effects of ADP on E ~ P decomposition

In the medium containing 100 mM choline chloride, ADP added after E ~ P formation reacted rapidly with about 40% of the total skeletal E ~ P formed, and the remaining E ~ P decomposed biphasically with rate constants of 23 and 7.3 s^{-1} for the first and the second phase, respectively. Under the same conditions, 40% of the cardiac E ~ P also reacted rapidly with ADP, but the remaining E ~ P decomposed monophasically within 277 ms with a relatively small rate constant of 1.5 s^{-1} (Fig. 5). In the medium containing 100 mM K^+, the fraction of total E ~ P in the skeletal sarcoplasmic reticulum that reacted rapidly with ADP was the same and, when K^+ was substituted for choline chloride, the decomposition of the remaining E ~ P was enhanced by 1.3 mM ADP with the rate constant of the initial phase increasing from 23 s^{-1} in choline chloride to about 58 s^{-1} in K^+. In the cardiac sarcoplasmic reticulum under the same conditions, the fraction of the total

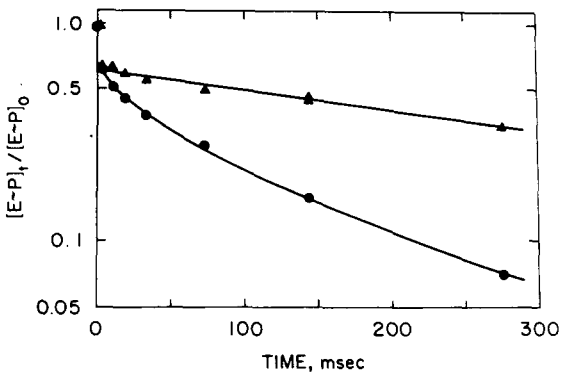

Fig. 5. Semilogarithmic plots of E ~ P decay with respect to time: effects of ADP in the presence of choline chloride. E ~ P was formed at 20°C in 20 mM Tris-maleate buffer (pH 6.8) containing 10 μM ATP, 12 μM Ca^{2+}, 3 mM $MgCl_2$, 5 mM NaN_3 and 100 mM choline chloride. At 20.5 ms, a buffer solution containing 3 mM $MgCl_2$, 5 mM NaN_3, 100 mM choline chloride, 6 mM EGTA, and 4 mM ADP was added to initiate E ~ P decay. Final concentrations of ADP was 1.33 mM. Dog cardiac sarcoplasmic reticulum, ▲——▲; rabbit skeletal sarcoplasmic reticulum, ●——●.

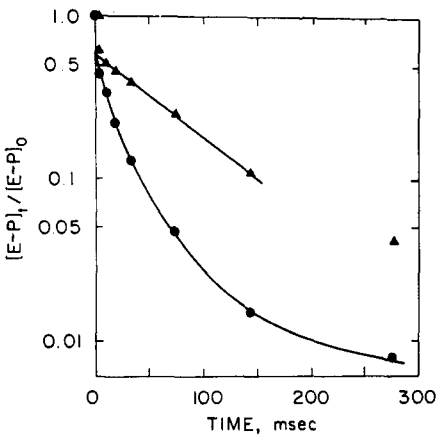

Fig. 6. Semilogarithmic plots of E ~ P decay iwth respect to time; effect of ADP in the presence of K^+. E ~ P was formed at 20°C in 20 mM Tris-maleate buffer (pH 6.8) containing 10 μM ATP, 12 μM Ca^{2+}, 3 mM $MgCl_2$, 5 mM NaN_3 and 100 mm K^+. At 116 ms, a buffer solution containing 3 mM $MgCl_2$, 5 mM NaN_3, 100 mM K^+, 6 mM EGTA and 4 mM ADP was added to initiate E ~ P decay. Final concentration of ADP was 1.33 mM. Dog cardiac sarcoplasmic reticulum, ▲——▲; rabbit skeletal sarcoplasmic reticulum, ●——●.

E ~ P that reacted rapidly with ADP was about 48%, and the rate of the decomposition of the remaining E ~ P increased from 1.5 s^{-1} in choline chloride to 11 s^{-1} in K^+. In choline chloride or K^+, ADP enhanced the E ~ P decomposition in the skeletal sarcoplasmic reticulum, as compared with decomposition in the absence of ADP. The cardiac E ~ P appeared to be not affected by ADP; the rate of the decomposition in choline chloride or K^+ was close to that obtained in the absence of ADP (data not shown).

Discussion

Intracellular concentrations of K^+ in skeletal and cardiac muscle cells are high (about 150 mM [10]) and probably change very little during the excitation-concentration-relaxation process. Thus, from the viewpoint of beat-to-beat regulation of cardiac sarcoplasmic reticulum or stimulation activity of skeletal sarcoplasmic reticulum, K^+ may appear unimportant in its regulatory functions. However, one may assess the possibility of 'K^+ regulation' by studying different responses of sarcoplasmic reticulum of different muscle types from normal and diseased muscles to the same K^+ concentrations. On the other hand, the effects of K^+ we have observed may reflect fundamental aspects of sarcoplasmic reticulum structure that in itself may be representative of a specific muscle type.

These differences between K^+ effects on skeletal and cardiac sarcoplasmic reticulum can be tabulated in several ways. The skeletal sarcoplasmic reticulum Ca^{2+}-ATPase does not appear to require K^+ for its maintenance of the activity for E ~ P formation when the enzyme is in a Ca^{2+}-deficient state. However, the ATPase activity of the Ca^{2+}-deficient cardiac sarcoplasmic reticulum is enhanced by K^+. The results suggest that K^+ in skeletal muscle cells may not regulate the rate of the conversion of the sarcoplasmic reticulum Ca^{2+}-ATPase from the Ca^{2+}-deficient state (presumably 'relaxation state' in the contraction-relaxation cycle) to the Ca^{2+}-bound state ('contraction state'). K^+ in cardiac muscle cells, on the other hand, may increase the rate of the conversion. Furthermore, the inhibitory effects of K^+ on the Ca^{2+}-bound skeletal and cardiac sarcoplasmic reticulum, especially at low $[Ca^{2+}]$, indicate that K^+ may participate in lowering the Ca^{2+} pump activity at low $[Ca^{2+}]$ to prevent excessive decrease in Ca^{2+} near the

end of relaxation [11] and to maintain the threshold of Ca^{2+} for contraction. K^+ is inhibitory at low [Ca^{2+}], presumably because K^+ may allosterically [12] affect the affinity of sarcoplasmic reticulum Ca^{2+}-ATPase for Ca^{2+} and cause a significant decrease in the amount of Ca^{2+} bound to the enzyme. At 12 μM Ca^{2+}, K^+ enhances the activity of the Ca^{2+}-bound cardiac sarcoplasmic reticulum Ca^{2+}-ATPase. The enhancement may be due to an activation by K^+ on sarcoplasmic reticulum similar to that which it does to the Ca^{2+}-deficient state of the enzyme when, at 12 μM Ca^{2+}, the Ca^{2+} sites are occupied and not interfered with by K^+. That K^+ enhances the cardiac but not the skeletal sarcoplasmic reticulum Ca^{2+}-ATPase at 12 μM Ca^{2+} suggests a greater amplification of sarcoplasmic reticulum activity in cardiac muscle due to its smaller density of Ca^{2+} pump sites.

K^+ may also regulate the activities of phosphoenzyme intermediates (E ~ P), suggesting a possible regulation of Ca^{2+} translocation in the process [13]. The skeletal sarcoplasmic reticulum E ~ P decomposes faster than the cardiac sarcoplasmic reticulum E ~ P in various, arbitrarily chosen media (E ~ P formed in Li^+ and decomposed in Li^+, Na^+ or K^+). Such differences reveal a possible regulatory point for K^+. An examination of the E ~ P properties toward ADP also shows a difference between the skeletal and cardiac sarcoplasmic reticulum. After the initial, rapid consumption of E ~ P by ADP [14–16] (a common property for both types of sarcoplasmic reticulum), ADP enhances the disappearance of the remaining E ~ P in the skeletal sarcoplasmic reticulum but not in case of cardiac sarcoplasmic reticulum. Part of the influence, which ADP exerts on the skeletal sarcoplasmic reticulum E ~ P, may be in the reversed pump direction that is accompanied by release of Ca^{2+} from the sarcoplasmic reticulum vesicles [14–16]. In this regard, the observation that ADP has a greater influence on skeletal than on cardiac sarcoplasmic reticulum may suggest a physiological relevance. Since the skeletal sarcoplasmic reticulum is the major source of intracellular Ca^{2+} [17–19], it may be important for the sarcoplasmic reticulum Ca^{2+}-ATPase to possess also a property responsive to ADP in the reversed pump action for the release of Ca^{2+} for muscle contraction. In cardiac muscle cells, since extracellular Ca^{2+} is required for contraction [17–19], the sarcoplasmic reticulum Ca^{2+}-ATPase may be less responsive to ADP in its regulation of the reversed pump action for Ca^{2+} release.

The above kinetic findings clearly show that the rabbit skeletal and dog cardiac sarcoplasmic Ca^{2+}-ATPases have different properties towards K^+ and ADP. Factors giving rise to these differences are unknown. Nevertheless, the results allow us to speculate that changes in the factors characterizing each type of sarcoplasmic reticulum may bring about a change in the response of sarcoplasmic reticulum activities toward the environment. As a consequence, altered regulatory effects of the intracellular environment on the sarcoplasmic reticulum function can be expected. Thus, it is important to sort out those factors that differentiate sarcoplasmic reticulum systems manifested in the kinetic properties. A knowledge of such factors is essential in understanding how the activities of the sarcoplasmic reticulum may be altered by pharmacological agents or muscle diseases.

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