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CYCLIC AMP MODULATION OF CALCIUM ACCUMULATION BY SARCOPLASMIC RETICULUM FROM FAST SKELETAL MUSCLE

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Summary

The role of cyclic 3′,5′-AMP in modulating sarcoplasmic reticulum from fast skeletal muscle was studied. The rate of Ca^{2+} uptake was stimulated in the presence of protein kinase plus 1 μM cyclic AMP. The stimulation was absent when denatured protein kinase was used. When an adenylate cyclase inhibitor was added, the uptake rates fell to 55% of control. This decrease in rate was partially overcome by 1 μM cyclic AMP. A modulating role for cyclic AMP in fast skeletal muscle is proposed.

Introduction

In recent years, the possible role of cyclic 3′,5′-AMP in the regulation of Ca^{2+} accumulation by sarcoplasmic reticulum isolated from cardiac muscle [1—9] has been extensively studied. Recently, we have experiments describing the augmentation of Ca^{2+} accumulation in the sarcoplasmic reticulum isolated from fast skeletal muscle taken from the cat [4]. This phenomenon was observed with either phosphorylase *b* kinase or with protein kinase plus 1 μM cyclic AMP. In contrast, Kirchberger and Tada [10] failed to confirm the effect of protein kinase on fast skeletal muscle sarcoplasmic reticulum isolated from the rabbit, and suggested, as a result of these experiments, that cardiac muscle is unique in its response to cyclic AMP. This property, they felt, may explain physiological responses to the hormones epinephrine and norepinephrine. The

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authors [10] attributed the protein kinase-induced stimulation of sarcoplasmic reticulum from fast skeletal muscle to an artifact because boiled protein kinase also stimulated calcium accumulation. Since these results are contrary to our own [4], we decided to re-examine the problem.

Methods

Sarcoplasmic reticulum was prepared from rabbit back (longissimus) muscle, which consists predominately of fast fibers, by a modified procedure of de Meis and Hasselbach [11]. The preparation was tested for Ca^{2+} accumulating activity by a dual wavelength spectroscopic procedure using murexide [12,13]. Sarcoplasmic reticulum (50—100 μg/ml) was incubated for 5 min at 30°C in the presence of 100 mM KCl, 40 mM Tris/maleate, pH 6.8, 10 mM $MgCl_2$, 5 mM sodium oxalate, 0.1 mM murexide, and any further additions. The total volume of the cuvette was 3.0 ml; 5 mM Na_2ATP was added and the incubation continued for an additional 5 min. Ca^{2+} uptake was initiated by the introduction of 60 μM Ca^{2+} into the cuvette. (Ca^{2+}, Mg^{2+})-ATPase was measured in the absence of oxalate and murexide with a linked enzyme system in a Gilford Recording Spectrophotometer [15]; 1 mM ethylene-bis(β-amino ethyl ether)-*N*,*N*′-tetraacetic acid (EGTA) was added to measure the (Mg^{2+})-ATPase activity.

Results

Protein kinase (50 μg) plus 10^{-6} M cyclic AMP, augments the rate of Ca^{2+} uptake (Fig. 1). On the other hand, 10^{-6} M cyclic AMP, 50 μg protein kinase alone, or 50 μg of heat denatured protein kinase plus 10^{-6} M cyclic AMP produces results which do not differ from control. The heat stable protein kinase inhibitor of Ashby and Walsh [14] completely blocks the effects of protein kinase and cyclic AMP (data not shown). This inhibition is not reversed by the addition of cyclic AMP.

A second approach to this problem has been provided through the use of an adenylate cyclase inhibitor fraction [16]. We have recently found that cardiac sarcoplasmic reticulum is partially dependent on basal cyclic AMP formation [17,26] for optimal uptake in that the accumulation process is inhibited by the adenylate cyclase inhibitor and restored to optimal activity by cyclic AMP. The adenylate cyclase inhibitor added in a 1 : 30 ratio (this inhibits the endogenous adenylate cyclase to 40% of control) reduces the rate of Ca^{2+} uptake to 55% of control (Fig. 2). Adenylate cyclase inhibitor shows a concentration-related inhibition of sarcoplasmic reticulum calcium uptake (Fig. 3). Likewise, in the presence of a constant amount of adenylate cyclase inhibitor (1 : 30), addition of cyclic AMP to the uptake system results in a concentration-dependent reversal of the inhibition. The addition of 1 μM cyclic AMP in the presence of adenylate cyclase inhibitor (1 : 30) returns the uptake rate to 79% of control. This, coupled with the lack of an effect of added cyclic AMP alone is evidence that sarcoplasmic reticulum from fast skeletal muscle is modulated by a cyclic AMP-dependent system in the same way as cardiac muscle [4,5] although, under endogenous conditions, cyclic AMP formation is not rate controlling. The (Ca^{2+}, Mg^{2+})-ATPase of fast skeletal muscle sarcoplasmic reticulum was

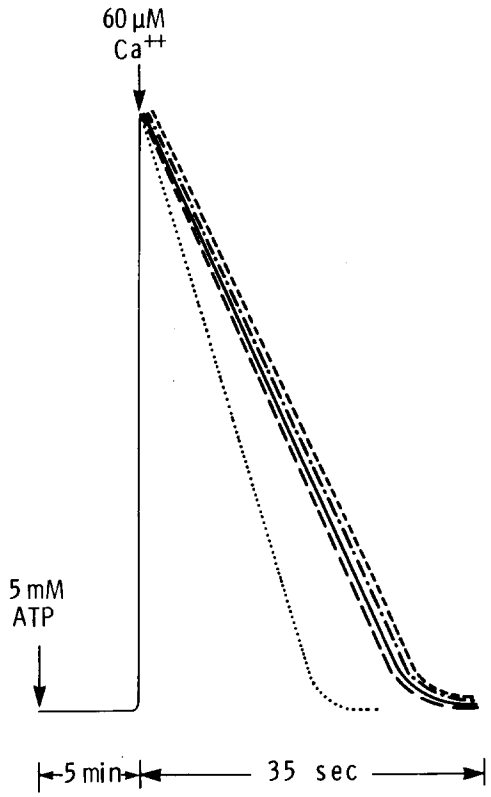

Fig. 1. The effect of protein kinase and cyclic AMP on Ca^{2+} uptake by rabbit fast skeletal muscle sarcoplasmic reticulum. Reaction conditions are as follows: 100 μg/ml sarcoplasmic reticulum, 40 mM Tris/maleate buffer, pH 6.8, 100 mM KCl, 10 mM $MgCl_2$ 100 μM murexide, 5 mM Na oxalate and 5 mM Na_2ATP. Protein kinase, when present, was 50 μg/3 ml; cyclic AMP was present in 1 μM concentration. Ca^{2+} uptake was initiated by the addition of 60 μM Ca into the cuvette: ——— = control; · — · — · = + cyclic AMP 10^{-6} M; · · · · · · = + protein kinase + cyclic AMP 10^{-6} M; — — — = + (10 min boiled) and cyclic AMP 10^{-6} M; - - - - - - = + protein kinase alone; (uptake rate in mol^{-9} Ca^{2+}/mg per min).

also studied. Although the ATPase is occasionally inhibited (⩽15%) the inhibition is not reversed by cyclic AMP. Many preparations revealed a lack of inhibition of either Mg^{2+}- or Ca^{2+}-ATPase by the inhibitor (data not shown).

Preincubation with ATP results in a significantly higher calcium uptake than that observed when calcium uptake is initiated with ATP alone rather than with calcium, after preincubation. In the absence of preincubation, the adenylate cyclase inhibitor does not inhibit calcium uptake at any concentration. Calcium accumulation in the absence of oxalate (calcium binding) is not affected by the adenylate cyclase inhibitor at any concentration used; however, since this reaction cannot be examined after preincubation with ATP [24] a firm conclusion regarding this effect cannot be made.

Discussion

This report shows that the sarcoplasmic reticulum from fast skeletal muscle is responsive to cyclic AMP stimulation and suggests that sarcoplasmic retic-

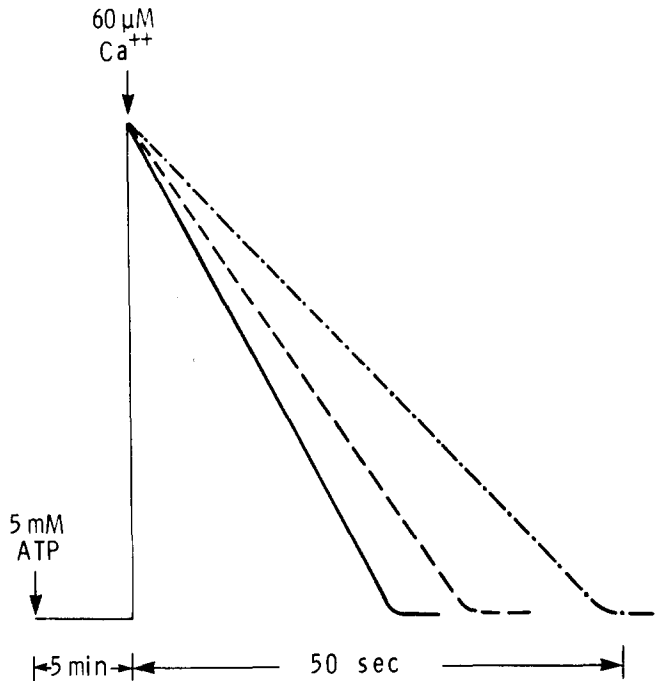

Fig. 2. The effect of adenylate cyclase inhibitor on Ca^{2+} uptake by rabbit fast skeletal muscle sarcoplasmic reticulum. Same reaction conditions as Fig. 1, except 100 mM Tris/maleate buffer, pH 6.8. Adenylate cyclase inhibitor was present in a 1 : 30 dilution: ——— = control; · — · — · = 100 µl adenylate cyclase inhibitor; - - - - - - = 100 µl adenylate cyclase inhibitor + 10^{-6} M cyclic AMP.

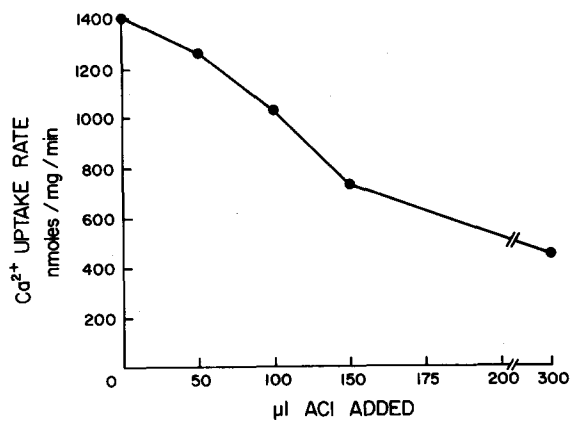

Fig. 3. The effect of various adenylate cyclase inhibitor concentrations on Ca^{2+} uptake by rabbit fast skeletal muscle sarcoplasmic reticulum. Same reaction conditions as Fig. 1, except 100 mM Tris/maleate buffer pH 6.8. ACI, adenylate cyclase inhibitor.

ulum from all muscle functions similarly. This seems to be inconsistent with the observations of Bowman and Zaimis [18] that fast skeletal muscle is relatively insensitive to catecholamine stimulation unless unphysiologic concentrations of these agents are used. There are several possible reasons for this apparent lack of correlation between the biochemical and physiological observations. The pharmacology of fast skeletal muscle has not been well delineated. There are marked differences in excitation-contraction coupling between cardiac and fast skeletal muscle. The studies of Reuter and coworkers and of several others [3,19,27] suggest that cyclic AMP-mediated agents increase the "slow current" calcium entry during phase II of the depolarization of cardiac muscle and that this might be the mechanism by which they exert their positive inotropic effect. Cardiac muscle is highly dependent on beat-to-beat calcium influx and requires external calcium in order to function [20]. In contrast to cardiac muscle, fast skeletal muscle does not require an external source of calcium, is not dependent on calcium influx for function, and the action potential does not exhibit a plateau phase [27]. In addition, the turnover of calcium within the fast skeletal muscle is very slow so that calcium exchange is not a prominent factor in the control of excitation-contraction coupling in fast skeletal muscle. Thus, two of the controlling factors of cardiac excitation-contraction coupling known to be affected by cyclic AMP-mediated agents in heart are not prominent parts of fast skeletal muscle excitation-contraction coupling. Since both of these functions are thought to be influenced by cyclic AMP-mediated agonists [3,27], a difference in response between cardiac muscle and fast skeletal muscle in terms of excitation-contraction coupling is expected. It is also possible that fast skeletal muscle lacks the sarcolemmal beta adrenergic receptor but contains an internal one located on the sarcoplasmic reticulum. Cyclic AMP-mediated regulation does occur in other biological functions of skeletal muscle such glycogenolysis [22], glycogen synthesis [23], and amino acid metabolism [24] and it is likely that calcium metabolism is intimately involved in some of these activities. Therefore, cyclic AMP regulation of Ca^{2+} uptake at a sarcoplasmic reticulum level in skeletal muscle is plausible and of potential significance.

The problems described by Kirchberger and Tada [10] regarding possible artifacts (viz., effects of boiled protein kinase and use of Millipore filters) in Ca^{2+} accumulation were not encountered in these experiments. It should be noted that the concentration of protein kinase in our experiments (viz., 50 μg/3 ml) is lower than in the experiments reported by Kirchberger and Tada (100 μg/ml). In other experiments we have observed that increasing the concentration of protein kinase above 15 μg/ml resulted in no additional stimulation. Further, the augmentation of Ca^{2+} uptake is cyclic AMP-dependent and is abolished when the protein kinase was subjected to 100°C for 10 min. Thus, it appears that the responsiveness of muscle sarcoplasmic reticulum to cyclic AMP is a mechanism functioning in fast skeletal muscle as well as in slow and cardiac muscle [4]. The inability of Kirchberger and Tada [10] to find a protein kinase effect in fast skeletal muscle sarcoplasmic reticulum preparations is probably not due to isolation procedures or to species source, since we have employed at least two different methods and cat and rabbit muscle [4]. It is possible that measurement of maximal velocity of spectroscopy as reported here is a more

sensitive index of changes than is the filtration method used by these investigators.

The lack of effect of the inhibitor on Ca^{2+}-ATPase is of interest, although inexplicable at this time. It is possible that an "uncoupling" of ATPase activity and effect on Ca^{2+} uptake has occurred. In this regard, we have been able to correlate quantitatively effects of protein kinase and cyclic AMP on Ca^{2+}-ATPase activity and calcium uptake rate. It is possible, therefore, that phosphorylation of sarcoplasmic reticulum membranes by protein kinase or phosphorylase *b* kinase produces permeability changes, but subsequently leads to an augmented Ca^{2+} transport rate.

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