ADENOSINE TRIPHOSPHATE-INDUCED RAPID CALCIUM RELEASE FROM FRAGMENTED SARCOPLASMIC RETICULUM

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SUMMARY

Calcium efflux from skeletal muscle fragmented sarcoplasmic reticulum was studied using a dilution technique and Millipore filtration. In the absence of Mg^{++} and external Ca^{++}, addition of 1mM adenosine triphosphate to the suspension resulted in an immediate loss of 26-55% of total vesicular calcium. The amount of calcium released was calculated to be sufficient to effect muscle contraction. After separation of the sarcoplasmic reticulum into light, intermediate and heavy vesicles, the light and heavy fractions were found to be only weakly responsive to adenosine triphosphate, whereas the intermediate fraction lost nearly half of its calcium. The significance of these results with respect to excitation-contraction coupling in muscle is discussed.

INTRODUCTION

It is generally accepted that the SR‡ controls the state of contraction in muscle by regulating the availability of Ca^{++} to the contractile proteins. Isolated membranes of the SR are capable of rapidly accumulating Ca^{++} against a concentration gradient, a process dependent on the enzymatic hydrolysis of ATP. This Ca^{++} accumulation is thought to be the *in vitro* counterpart for the sequestration of Ca^{++} responsible for muscle relaxation (1,2). Conversely, tension development *in vivo* is considered to follow the release of stored Ca^{++} from the SR upon depolarization of the T-tubule of the muscle fiber (3,4). The manner in which T-tubule depolarization results in Ca^{++} release from the SR remains an unknown link in the chain of events known as excitation-contraction coupling.

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‡Abbreviations: SR (sarcoplasmic reticulum); HSR, ISR, LSR (heavy, intermediate and light sarcoplasmic reticulum, respectively); T-tubule (transverse tubule); MOPS (morpholinopropane sulfonic acid); EDTA (ethylenediaminetetraacetate); TC (terminal cysternae).

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One approach to the problem of elucidating the mechanism of Ca^{++} release is to test the ability of various agents to induce the release of Ca^{++} from pre-loaded vesicles of isolated SR. We have found that sudden exposure of such vesicles to ATP under conditions in which hydrolysis is prevented results in a rapid release of Ca^{++} from the vesicles, suggesting that ATP may be the link between T-tubule excitation and Ca^{++} release from the SR.

MATERIALS AND METHODS

Fragmented SR was obtained by differential centrifugation of homogenized white muscle from rabbit hind leg as previously described (5).

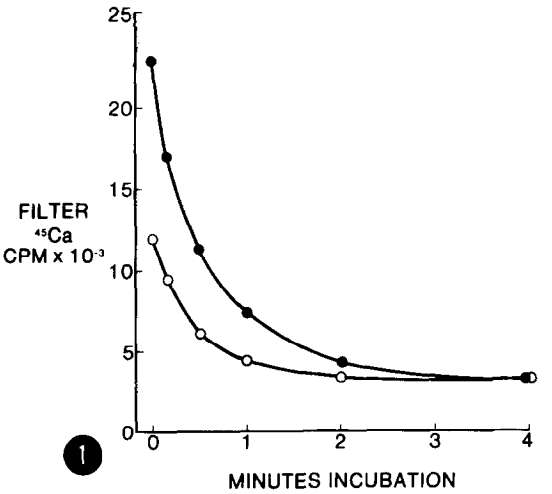
Incorporation of Ca^{++} into the SR vesicles was accomplished passively, i.e., in the absence of ATP. Unless otherwise stated, SR (10mg protein/ml) was incubated for at least 12 hr at 2° in a medium containing 0.1M KCl, 20 mM MOPS buffer, and 10mM $^{45}CaCl_2$, pH 6.80. Efflux of the incorporated Ca^{++} was then initiated by 40-fold dilution (37°) of this initial suspension with an identical solution, but without SR or Ca^{++}. In addition, 21mM EDTA was included in the diluting medium to reduce the final, extravesicular Ca^{++} concentration to 10^{-9}M, assuming an apparent dissociation constant for CaEDTA of $10^{-7.04}$M (6). Test dilution media also contained ATP at the concentrations indicated in the legends to the figures. SR $^{45}Ca^{++}$ content was monitored at appropriate intervals by filtration of aliquots of the final suspension through Millipore filters. The filters were placed in Bray's solution and counted in a liquid scintillation spectrometer. The high EDTA concentration and the omission of Mg^{++} from the incubation media ensured that negligible quantities of free Mg^{++} were present, so that the fluxes observed in the presence at ATP were purely passive (7). In addition, 10^{-9}M free Ca^{++} is below the Ca^{++} concentration threshold for the SR active transport system (8).

The minimum time interval between dilution and complete filtration of the final suspension through the Millipore filter was 2 sec at 37°. To minimize Ca^{++} efflux during this time, the initial (time-zero) sample was diluted at 0° also (9), and immediately filtered; however, at this temperature, the filtration time was increased to 5-6 sec. The above two methods for determining the SR Ca^{++} level at the start of efflux yielded essentially identical results. The lower temperature was consistently used since, although the filtration time was tripled, membrane permeability was reduced nearly 500-fold in cooling from 37° to 0° (unpublished observations). In determining the $^{45}Ca^{++}$ content of the vesicles at equilibrium, the following three methods produced identical values, within experimental error: incubation of the final suspension at 37° for at least 2 hr; incubation in the presence of 5 μM of the Ca^{++} ionophore A23187 for 1 min at 37°, or for 5 min at room temperature.

Na_2ATP was obtained as the purest grade from Sigma Chemical Co., St. Louis, Mo. The other chemicals used were analytical or reagent grade. Deionized, distilled water was used in all preparations and experiments.

RESULTS

Figure 1 shows the efflux of Ca^{++} from SR vesicles in the presence and absence of 1mM ATP. It is seen that including ATP in the diluting medium



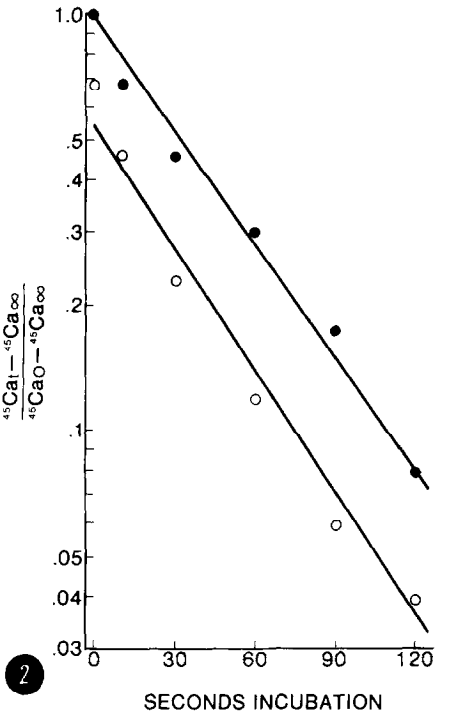


Figure 1: Time course of Ca^{++} efflux from SR vesicles in the absence (●) and presence (o) of 1mM ATP.

Figure 2: Semilogarithmic plot of Ca^{++} efflux as a function of time. Control (●) half time=32 sec. 1mM ATP (o) half time=31 sec.

results in the loss of a significant fraction of the SR Ca^{++} in the time required to filter the first sample. This rapid loss of SR Ca^{++} is not due to reversal of the Ca^{++} pump by contaminating ADP and P_i (10), since a) 1mM ADP by itself (not shown) is found to produce a smaller Ca^{++} release than does 1mM ATP; b) the requirement for Mg^{++} is not met (10); c) such facilitated Ca^{++} efflux at 10° or less has been shown to result in negligible Ca^{++} release in times up to 10 sec (11). Nor is the observed initial release due to a rapid elevation in pH, as was observed by Nakamaru and Schwartz (12), since the pH of the suspension after dilution is found to be the same whether or not ATP is present.

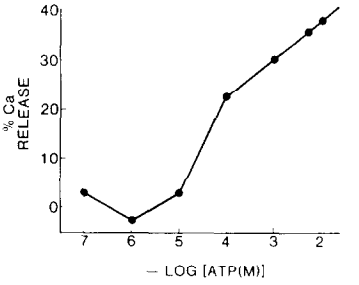


Figure 3: Percent Ca release as a function of LOG(ATP concentration).

Following the initial burst of Ca^{++} release, efflux of the remaining Ca^{++} proceeds in an apparently exponential fashion. Note that the two curves level off at the same point, suggesting that the ATP does not simply abstract Ca^{++} bound to the external surface of the membrane by complexation. This is further indicated, in that the amount of Ca^{++} rapidly released is nearly four times greater than the residual Ca^{++} remaining on the filter as equilibrium is approached. Finally, similar experiments in which the SR is omitted from otherwise identical solutions show this residual radioactivity to be due entirely to contamination of the filter, rather than to membrane-bound Ca^{++}.

That the efflux following the initial burst of Ca^{++} release is indeed exponential is verified in Figure 2, which is a semilogarithmic plot of the fraction of remaining $^{45}Ca^{++}$ in the vesicles, relative to that at time-zero, as a function of time. The straight lines indicate that, subsequent to the initial burst of Ca^{++} release, the first order character of the efflux is retained even in the presence of 1mM ATP. An early component lasting a few seconds after the initial burst may be apparent with the ATP. In fact, the rate constant (proportional to the slope) for the efflux after the initial release is identical with or without ATP. This demonstrates that the "steady state" passive permeability of the SR membrane is unaffected by ATP in the absence of Mg^{++} and extravesicular Ca^{++}, and that the Ca^{++} pump is indeed inactive under these conditions.

Table 1: % Ca release from three different fractions of SR. ATP concentration = 1mM. The sucrose densities for the separations were obtained from Meissner (14). The individual fractions were isolated using separate centrifugations with the single sucrose concentrations indicated, rather than using sucrose gradients. The particular whole SR preparation from which these fractions were obtained released 26% of its Ca^{++} rapidly. In several experiments, rapid Ca^{++} release from HSR was found to range from 0-21%, while the variation in the other fractions was much smaller.

SR MEMBRANE FRACTION (% SUCROSE, W/W)	LIGHT (< 32)	INTER-MEDIATE (32–39)	HEAVY (> 39)
% Ca RELEASE	4	49	16

One may quantitate the fractional amount of Ca^{++} which is initially released by ATP in terms of the ratio, $[^{45}Ca_0(\text{control}) - {}^{45}Ca_0(\text{ATP})]/[^{45}Ca_0(\text{control}) - {}^{45}Ca_\infty]$. When multiplied by 100, this ratio will be referred to as "% Ca release". Figure 3 shows the concentration dependence of the ATP effect. The effect becomes significant at an ATP concentration above 0.01mM, and the release increases linearly with log[ATP] for concentrations between 0.1mM and 10mM. Although Ca^{++} release is shown on a relative basis, the absolute amount of Ca^{++} released was determined from specific activity measurements. When the initial suspension contained 20mM $CaCl_2$ and the diluting medium contained 1mM ATP, 23 nanomoles Ca^{++}/mg SR protein were rapidly released. This quantity of released Ca^{++} has been calculated to be nearly sufficient (25 nanomoles/mg) to effect muscle contraction (13).

The fact that only a portion of the total SR Ca^{++} is initially released at the higher ATP concentrations may be partially explained by considering that the whole SR preparation can be resolved, by differential centrifugation, into three separate fractions of different densities (14). In characterizing the three fractions, Meissner (14) has identified the heavy fraction as originating from the TC portions of the in situ SR,

whereas the intermediate and light fractions were considered to be derived from the central portions of the longitudinal tubules. Table 1 shows the results of efflux experiments in which the different isolated SR fractions were exposed to 1mM ATP. The response of these isolated fractions is seen to be non-uniform: HSR releases a small portion of its Ca^{++}, whereas LSR is almost completely insensitive to ATP. ISR, however, which comprises about 70% of the whole SR preparation (14), releases nearly half of its Ca^{++} in response to ATP.

DISCUSSION

In light of the present results, we may consider how Ca^{++} is retained by the SR in situ in the resting state, with total rabbit muscle ATP concentration being about 8mM (15) after correcting for fiber water content (16). Burt et al. (17) have shown that most of muscle ATP is in the form MgATP, which we have found to be incapable of producing rapid Ca^{++} release under the present conditions (unpublished observations.) The physiological mechanism for Ca^{++} release may therefore involve transient, local production of increased levels of free ATP as a result of T-tubule excitation. Such free ATP levels may not be difficult to achieve. Using Chutkow's measurements of muscle Mg^{++} and water content (16), and a MgATP apparent dissociation constant of $10^{-4.39}$M (18), the cytoplasmic free ATP concentration at rest is calculated to be about 0.08mM, after correcting for Mg^{++} binding to myosin and to creatine phosphate (19). This concentration of free ATP is seen to be very near the minimum value necessary to induce rapid Ca^{++} release in isolated SR vesicles (Figure 3).

According to Meissner, the ISR has functional and structural characteristics between those of HSR and LSR, and may represent a mixture of the latter two membrane types (14). The results of Table 1 however indicate that that is not the case, at least with respect to ATP-induced rapid Ca^{++} release, since this activity appears to be rather specific for ISR.

Endo and Kitazawa recently reported a stimulatory effect of free ATP on both Ca^{++}-induced Ca^{++} release and "depolarization"-induced Ca^{++} release from the SR of skinned fibers (20). The present results show that external Ca^{++} is not a requirement for ATP-induced rapid Ca^{++} release from fragmented SR, and that the ATP effect is therefore distinct from either of the above two methods for producing Ca^{++} release.

It is interesting that the unhydrolyzable ATP analog, β,γ-methylene ATP, has been shown to induce Ca^{++} release from isolated SR, although at least one-half minute is required for the complete effect, which occurs in the presence of Mg^{++} (21).

A preliminary account of this communication has been published (22).

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