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S-100a_0 protein stimulates Ca^{2+}-induced Ca^{2+} release from isolated sarcoplasmic reticulum vesicles

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S-100a_0 protein, the $\alpha\alpha$-isoform of the S-100 family, stimulates Ca^{2+}-induced Ca^{2+} release from terminal cisternae isolated from rat skeletal muscle cells. The stimulatory effect of S-100a_0 is maximal at ~5 μM S-100a_0 and half maximal at ~0.1 μM S-100a_0, at 1.8 μM free Ca^{2+} in the presence of 5 mM Mg^{2+} plus 0.1 M KCl. The effect of the protein on Ca^{2+}-induced Ca^{2+} release is completely inhibted by the calcium release blocker, ruthenium red.

Protein S-100a_0; Ca^{2+} release; Regulation; (Muscle cell)

1. INTRODUCTION

Ca^{2+}-induced Ca^{2+} release has been hypothesized to be one possible mechanism by which small increases in the concentration of sarcoplasmic Ca^{2+} in the proximity of the sarcoplasmic reticulum (SR) in muscle cells will trigger the release from SR of the additional Ca^{2+} required for muscle contraction [1–6]. The terminal cisternae of SR represent the subcellular compartment involved in Ca^{2+}-induced Ca^{2+} release [4,7,8]. This activity is brought about by a distinct molecular entity, the Ca^{2+} release channel protein, which has been purified to homogeneity [9]. Ca^{2+}-induced Ca^{2+} release is stimulated by adenine nucleotides [10] and inhibited by Mg^{2+}, ruthenium red, and the ubiquitous intracellular Ca^{2+} receptor, calmodulin [11–13].

S-100a_0 protein is the $\alpha\alpha$-isoform of S-100 proteins, a group of 3 closely related Ca^{2+}-binding proteins of the EF-hand type [14,15]. High levels of the S-100α, but not the S-100β, subunit are expressed in striated muscle cells [16–18]. Immunocytochemical and immunochemical analyses revealed that S-100a_0 protein is found associated with the sarcolemma and with SR membranes, and in the sarcoplasm facing these membranes [18,19], suggesting the possibility that this protein might be involved in the regulation of one or more membrane activities. We report here that S-100a_0 protein stimulates Ca^{2+}-induced Ca^{2+} release from SR in the presence of Mg^{2+}.

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2. MATERIALS AND METHODS

S-100a_0 protein was purified from porcine heart [18].

Terminal cisternae (R4) were obtained as in [20] from rat hindlimb muscles and washed twice with 0.2 mM EGTA. R4 was shown to be highly enriched in terminal cisternae by a number of criteria [20–23]. R4 vesicles (0.25 mg of protein/ml) were actively loaded with $CaCl_2$ in 20 mM imidazole, 0.2 mM EGTA, 20 mM potassium oxalate, 5 mM ATP, 5 mM $MgCl_2$, 0.18 mM $CaCl_2$, 0.1 M KCl, pH 7.4, in the presence of 5 μM $^{45}CaCl_2$ (specific activity 1 mCi/nmol) at 25°C. Ca^{2+}-induced Ca^{2+} release was studied by Millipore filtration technique. After the plateau of Ca^{2+} uptake had been attained (6 min), the medium was replaced with the release solution. This consisted of the above medium minus ATP, containing 1.8 μM free Ca^{2+} unless stated otherwise plus or minus other additions as indicated. The released Ca^{2+} was calculated on filtered solutions by taking the differences between the value measured at the end of Ca^{2+} uptake and those recorded at the time points indicated

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after the addition of the release solution. Protein was measured as in [24] against a standard solution of bovine serum albumin.

3. RESULTS AND DISCUSSION

In the presence of 1.8 μM free Ca^{2+} and 5 mM $MgCl_2$, S-100a_0 protein produced a time- and dose-dependent stimulation of Ca^{2+} release from R4 vesicles, with half-maximal effect around 0.1 μM S-100a_0 (figs 1 and 2). The extent of Ca^{2+} release was ~70% stimulated by 1 μM S-100a_0, whereas the initial rate of Ca^{2+} release was ~65% stimulated, under the above conditions. In the presence of 180 μM free Ca^{2+} (table 1), the percent Ca^{2+}-induced Ca^{2+} release in the absence of S-100a_0 protein was ~70% smaller than at 1.8 μM free Ca^{2+}, as expected [10]. Under the same conditions, in the presence of 1 μM S-100a_0 protein a strong stimulation of Ca^{2+} release was observed at all time points considered (table 1).

Since the SR vesicles used in these experiments were actively loaded with Ca^{2+} in the presence of ATP, experiments were conducted to examine the possibility that the S-100a_0 effect described above were due to an effect of the protein on the Ca^{2+}-ATPase activity associated with R4. In the presence of 1.8 μM free Ca^{2+}, the specific activity of the enzyme measured in the absence of S-100a_0 protein was 0.94 μmol P_i/min/mg of protein, and that of the enzyme measured in the presence of 1 μM S-100a_0 protein was 0.91 μmol P_i/min/mg of protein. Thus S-100a_0 protein did not affect the ATPase activity in R4 vesicles.

The stimulatory effect of S-100a_0 protein on Ca^{2+}-induced Ca^{2+} release was prevented by the Ca^{2+} release channel blocker, ruthenium red (table 2). Also, ruthenium red, when added to R4 vesicles induced to release Ca^{2+} in the presence of 1.8 μM free Ca^{2+} plus 1 μM S-100a_0 protein, blocked further S-100a_0-dependent Ca^{2+}-induced Ca^{2+} release (fig.3). The reverse experiment was also performed in which S-100a_0 protein was added to R4 vesicles actively loaded with Ca^{2+} and then induced to release Ca^{2+} in the presence of ruthenium red. No significant effect of S-100a_0 protein was registered under these conditions (fig.3).

S-100 proteins (S-100a_0, S-100a, and S-100b) are Ca^{2+}-binding proteins of the EF-hand type, and are $\alpha\alpha$, $\alpha\beta$, and $\beta\beta$ in subunit composition, respectively [14,15]. S-100 proteins are widely, but differently distributed in animal cells. They have been shown to regulate a number of activities, including the assembly–disassembly of microtubules, several kinase and phosphoprotein phosphatase activities, the phosphorylation of a number of proteins by interacting with the substrates rather than with the kinases, a brain aldolase activity, and the adenylate cyclase activity (reviewed in [14,15]).

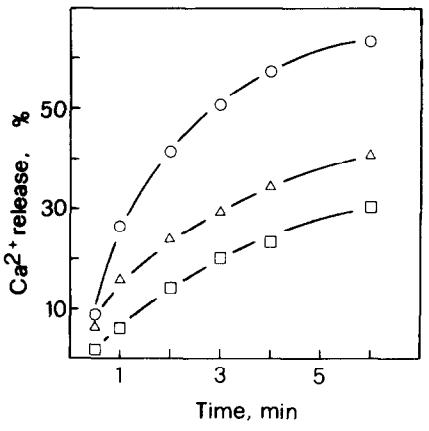

Fig.1. Time course of Ca^{2+}-induced Ca^{2+} release from R_4 vesicles in the absence of additions (△) and in the presence of either 1 μM S-100a_0 protein (○) or 60 μM ruthenium red (□). Results are expressed as the percent Ca^{2+} release vs time. At the end of Ca^{2+} uptake, vesicles contained 49 ± 6 nmol Ca^{2+}/0.1 mg protein. Maximal SD was ± 5% (n = 3).

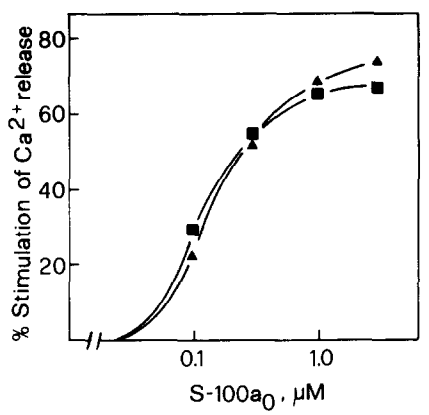

Fig.2. Effect of increasing concentrations of S-100a_0 protein on Ca^{2+}-induced Ca^{2+} release from R_4 vesicles. The release solution contained increasing concentrations of S-100a_0 protein. Results are expressed as the percent stimulation of Ca^{2+} release 2 min (▲) and 4 min (■) after the starting of Ca^{2+} release. At the end of Ca^{2+} uptake, R_4 vesicles contained 45 ± 5 nmol Ca^{2+}/0.1 mg protein. Maximal SD was ± 6% (n = 3).

Table 1

Ca^{2+}-induced Ca^{2+} release from R_4 vesicles in the presence of 180 μM free C^{2+} with or without S-100a_0 protein

Additions	Time (min)		
	0.5	2	4
None	0.7 ± 0.2	2.9 ± 0.3	5.0 ± 0.4
S-100a_0 (1 μM)	1.7 ± 0.5	6.2 ± 0.6	10.6 ± 1.2

Conditions were as described in the legend to fig.1, except that the free Ca^{2+} concentration in the release medium was 180 μM. Figures represent the percent Ca^{2+} release at the time points indicated (n = 3 ± SD). At the end of Ca^{2+} uptake (6 min) R_4 vesicles contained 62 ± 4 nmol Ca^{2+} 0.1 mg^{-1} protein

Table 2

Ca^{2+}-induced Ca^{2+} release from R_4 vesicles in the presence of various combinations of S-100a_0 protein and ruthenium red

Substances	Percent Ca^{2+} release in 4 min
None	30.7 ± 2.3
S-100a_0 (1 μM)	58.9 ± 3.5
Ruthenium red (60 μM)	20.3 ± 1.6
Ruthenium red (60 μM) + S-100a_0 (1 μM)	19.8 ± 1.8

Conditions were as described in the legend to fig.1. Substances were added to the release solution to the final concentrations indicated. At the end of Ca^{2+} uptake (6 min) R_4 vesicles contained 47 ± 6 nmol Ca^{2+} 0.1 mg^{-1} protein (n = 3 ± SD)

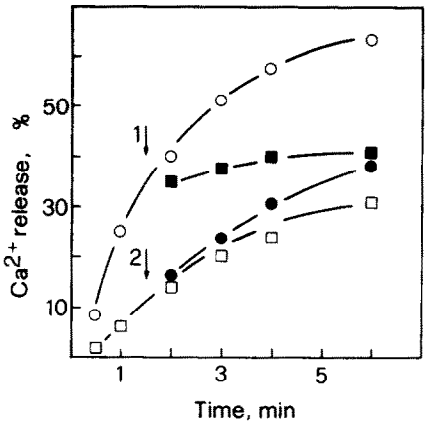

Fig.3. Effects of additions of ruthenium red or S-100a_0 protein on Ca^{2+}-induced Ca^{2+} release from R_4 vesicles in the presence of S-100a_0 protein or ruthenium red, respectively. (○) 1.5 min after starting Ca^{2+} release in the presence of 1 μM S-100a_0 protein (arrow 1), ruthenium red was added to 60 μM (■). (□) 1.5 min after starting Ca^{2+} release in the presence of 60 μM ruthenium red (arrow 2), S-100a_0 protein was added to 1 μM (●). Ruthenium red blocked the stimulatory effect of S-100a_0 protein on Ca^{2+}-induced Ca^{2+} release, whereas S-100a_0 protein did not significantly change the inhibitory effect of ruthenium red on Ca^{2+}-induced Ca^{2+} release. Results are expressed as the percent Ca^{2+} release vs time. At the end of Ca^{2+} uptake, R_4 vesicles contained 42 ± 6 nmol Ca^{2+}/0.1 mg protein. Maximal SD was ± 7% (n = 3).

Thus S-100 proteins are candidate to constitute a multifunctional protein fraction. As anticipated, striated muscle cells express almost exclusively the $\alpha\alpha$-isoform (S-100a_0). In these cells, the S-100a_0 concentration is estimated to be 1–2 μM [18], but it is expected to be considerably higher in the proximity of muscle membranes, since no S-100a_0 protein is found associated with the contractile elements [18]. S-100a_0 protein has been recently reported to stimulate the basal (Mg^{2+}-activated) adenylate cyclase activity in muscle membranes [23]. The data presented in this report suggest that this protein might also play a role in the regulation of Ca^{2+}-induced Ca^{2+} release from SR. S-100a_0 protein stimulates Ca^{2+}-induced Ca^{2+} release from R4 vesicles in the presence of 1.8 μM free Ca^{2+} plus 0.1 M KCl, i.e. under conditions where this protein in solution would not bind Ca^{2+} [14,15]. Thus our findings suggest that, if S-100a_0 protein exerts its effects on this system because of its Ca^{2+}-binding properties, as is reasonable to assume, then the conclusion can be drawn that the Ca^{2+}-binding affinity of S-100a_0 protein increases by several orders of magnitude once it has come into contact with its targets. This suggestion is supported by recent observations on interactions between S-100 proteins and tubulin [25], microtubule-associated τ-proteins and mellitin [26], artificial membranes [27], and hydrophobic matrices [18], all of which strongly indicate that the Ca^{2+}-binding affinities of individual S-100 isoforms depend on their conformation and increase upon their binding to targets. At relatively high free Ca^{2+} concentrations, Ca^{2+} release from SR occurs at a reduced rate and to a much smaller extent than at low free Ca^{2+} concentrations [10]. The observation that S-100a_0 protein stimulates Ca^{2+}-induced Ca^{2+} release by 100% at 180 μM free Ca^{2+} clearly indicates that the protein does not act on this system by chelating free Ca^{2+}.

Interestingly, S-100a_0 protein and calmodulin appear to exert opposite effects on Ca^{2+}-induced Ca^{2+} release, in spite of their belonging to the same

superfamily of Ca^{2+}-binding proteins of the EF-hand type. There is evidence that calmodulin binds to the Ca^{2+} release channel protein [12,21,28]. We are working at identifying the molecular target(s) of S-100a_0 protein in terminal cisternae.

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