DEPOLARIZATION INDUCED CALCIUM RELEASE FROM SARCOPLASMIC RETICULUM MEMBRANE FRAGMENTS BY CHANGING IONIC ENVIRONMENT

M. KASAI and H. MIYAMOTO
Department of Biophysical Engineering, Faculty of Engineering Science, Osaka University, Toyonaka, Osaka 560, Japan

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1. Introduction

It is well known that release of Ca^{2+} from sarcoplasmic reticulum (SR) caused by electrical excitation of sarcolemma is the key signal of muscle contraction [1, 2]. However, how depolarization of the T-system causes the release of Ca^{2+} from SR is the least understood process in the excitation—contraction coupling of skeletal muscle. It is very probable that electrical excitation of T-system is transmitted to SR through electrical coupling and that release of Ca^{2+} from SR is caused by its electrical excitation [3]. However, no direct experimental evidences of electrical excitation of SR and of resulting increase of Ca^{2+} permeability are available. It is important to establish such a concept for understanding of excitation—contraction coupling in the skeletal muscle.

Many researchers tried to show the electrical excitation of sarcoplasmic reticulum fragments (SRF) and resulting release of Ca^{2+} *in vitro*. The effect of direct application of electrical stimulation on the release of Ca^{2+} by using platinum electrodes was reported by Lee et al. [4]. However, it was made clear by our re-examination that such a method always caused some irreversible denaturation of SRF [5]. There is no evidence that SRF is responsive to the electrical stimulation in the same way as *in vivo* by such a method.

In this paper, SRF membrane was depolarized by changing the ionic environment and the resulting release of Ca^{2+} was examined.

2. Materials and methods

The SRF was isolated from rabbit dorsal and hind leg muscle as a microsomal fraction by the method of Weber et al. [6] with slight modification [7]. ^{45}Ca was purchased from International Chemical Nuclear Corp. USA. ATP was obtained from Sigma Chemical Co. USA. Amount of Ca^{2+} in the SR vesicles was determined by measuring the radioactivity of SRF adhered on a Millipore filter (HAWP 025 pore size 0.45 μm) using Hitachi—Horiba liquid scintillation counter.

3. Results and discussion

3.1. *Release of Ca^{2+} from SRF by changing ionic environment*

Ca^{2+} uptake by SRF was performed for 3 min in a solution containing 0.3 M KCl or 0.3 M potassium methanesulfonate (KMS) in addition to 20 mM Tris-maleate (pH 6.5), 2 mM $MgCl_2$, 3 mM ATP, and 30 μM total Ca^{2+} ($CaCl_2$ + $^{45}CaCl_2$). After Ca^{2+} uptake, 1 ml of the suspension was filtrated through a Millipore filter by applying a negative pressure in the bottom flask. By this treatment almost all SRF were adhered on the filter. In order to change ionic environment and to remove remaining free ^{45}Ca, 3 ml of various washing media indicated in table 1 was passed through the filter respectively maintaining the osmolarity of the solution constant to avoid burst of SR vesicles. As shown in table 1, only when methanesulfonate ion (MS^-) of the solvent was replaced by Cl^-, release of Ca^{2+} from SRF vesicle was observed. The amount of released Ca^{2+} was about 50%.

Table 1
Effect of the change of ionic environment on Ca^{2+} release from SRF.

Uptake medium	Washing medium Isoosmotic (equivalent to 0.3 M KCl)						Non isoosmotic	
	KCl	KMS	NaCl	K_2SO_4	Choline Cl	Sucrose	Water	KCl (0.88 M)
KCl	100	94	101	98	98	96	35	98
KMS	53	100	59	101	94	100	28	68

SRF (2.2 mg/ml) was preincubated in the solution containing 0.3 M KCl or 0.3 M KMS in addition to 20 mM Tris-maleate (pH 6.5) and 2 mM $MgCl_2$. Then Ca^{2+} uptake was started by adding the SRF suspension (100 μl) to the medium (1 ml) containing the same solute with solution for preincubation in addition to 3 mM ATP and 30 μM total Ca^{2+} ($CaCl_2$ + $^{45}CaCl_2$). The reaction mixture was filtered through a Millipore filter 3 min after the initiation of Ca^{2+} uptake and 3 ml of various washing media of osmolarity equal to the reaction mixture was passed through the filter. (All solutions contained 20 mM Tris-maleate (pH 6.5) and 2 mM $MgCl_2$ and the salt indicated in the table.) The remaining Ca^{2+} in SRF is expressed as the percentage for the controls underlined in the table.

Membrane potential of SR is considered to be inside positive because active transport of Ca^{2+} takes place through SR membrane from outside to inside. MS^- must be less permeable than Cl^-. Therefore it is likely that depolarization of SRF membrane caused by the replacement of MS^- with Cl^- brought about the release of Ca^{2+}. In the process of Ca^{2+} release the possibility of the burst of SRF membrane caused by the decrease of the osmolarity of the solvent was completely excluded because a similar effect was observed for the replacement of 0.3 M KMS with 0.88 M KCl while no effect was observed for that of 0.3 M KCl with 0.88 M KCl. When SRF incubated in a high salt solution was washed with water containing only the buffer, the release of Ca^{2+} by the burst of SRF was always observed. The results are also shown in the table.

On the contrary, the effect of cation is ambiguous. When the SRF incubated in permeable cation such as K^+ was washed with less permeable cation such as Na^+ or choline$^+$ without changing anions, the depolarization of the membrane must be expected. However, no effect was observed as shown in the table. We cannot explain this result at present.

3.2. *Dependence of Ca^{2+} release on changing ionic composition*

In the next experiment, we studied how much change of ionic environment is required to induce Ca^{2+} release. Ca^{2+} was taken up by SRF in the solution containing 0.3 M KMS as in the case of table 1. Methods are equal to table 1 except that the washing solution is the mixture of KCl and KMS solutions. Varying the ratio of KCl and KMS with maintaining K^+ concentration constant (0.3 M), the Ca^{2+} remained in SRF were plotted against mole fraction of KMS in fig. 1. A continuous curve was obtained.

3.3. *Time course of Ca^{2+} release*

In the experiments mentioned above, Ca^{2+} was actively taken up by SRF using the chemical energy of ATP hydrolysis. In order to separate the process of Ca^{2+} release from the active transport, a concentrated SRF was incubated in the medium containing 0.3 M KMS or 0.3 M KCl. Then the suspension of SRF was diluted into about 100 vol of the medium containing 0.3 M KCl or KMS. Four cases of dilution were examined, that is, from KCl to KCl and KMS, and from KMS to KCl and KMS. After the dilution an aliquot of the suspension was filtrated through the Millipore filter at appropriate times. Time courses of Ca^{2+} release from SR vesicles in four cases were shown in fig. 2.

Rate of Ca^{2+} release from SRF was dependent on the solvent conditions of incubation and dilution. However, only when the suspension was diluted from KMS to KCl, a transient increase of the release of Ca^{2+} was observed. This result is consistent with the concept that release of Ca^{2+} is due to the depolarization of SRF membrane caused by replacement of less permeable ion, MS^-, with more permeable anion, Cl^-. The high rate of Ca^{2+} release decreased within 1

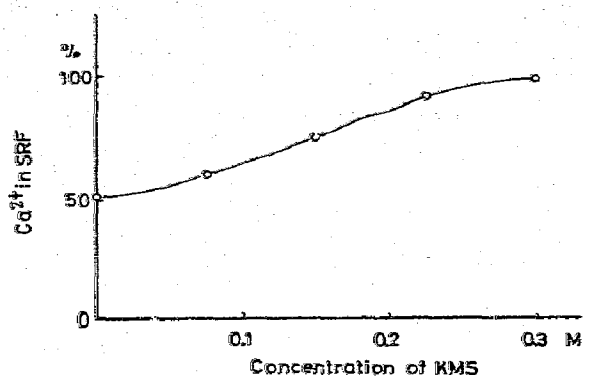

Fig. 1. Effect of the composition of KCl and KMS on Ca^{2+} release from SRF. Assay of Ca^{2+} uptake was similar to table 1 except that washing medium was a mixture of KCl and KMS solution. Total K^+ concentration of washing medium was 0.3 M. Ordinate: radioactivity of ^{45}Ca retained in SRF vesicles; abscissa: mole fraction of KMS in the washing medium.

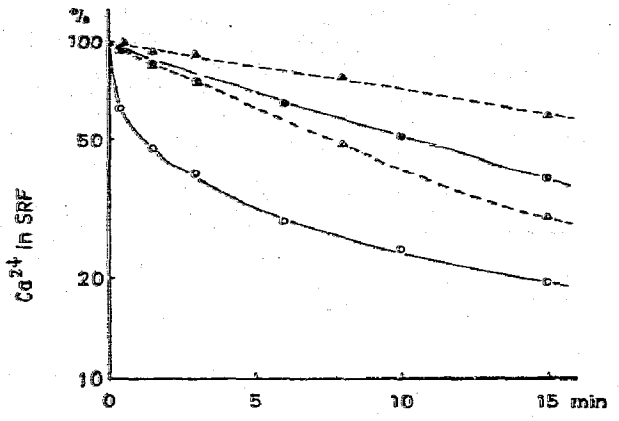

Fig. 2. Time courses of the release of Ca^{2+} from SRF in various conditions. SRF (20 mg/ml) was incubated for about 12 hr at 0°C in the solution of 0.3 M KCl or 0.3 M KMS in addition to 1 mM total Ca^{2+} ($CaCl_2$ + $^{45}CaCl_2$), 20 mM Tris-maleate (pH 6.5) and 2 mM $MgCl_2$. Release of Ca^{2+} was followed after 100-fold dilution in the solutions containing 0.3 M KCl or 0.3 M KMS, 20 mM Tris-maleate (pH 6.5) and 2 mM $MgCl_2$. One ml was withdrawn from the diluted suspension and filtered through the Millipore filter. The Millipore filter was washed with 3 ml of the same solvent of the diluted media by passing it through. Remaining Ca^{2+} was plotted against the time after the dilution. (●—●—●) Dilution from KMS to KMS; (○—○—○) from KMS to KCl; (▲---▲---▲) from KCl to KMS; (△---△---△) from KCl to KCl. The value of remaining Ca^{2+} was normalized with the value extrapolated to time zero as 100%. The amount of Ca^{2+} retained in SRF by the incubation process which can be calculated from the Ca^{2+} content extrapolated to time zero was about 6.5 μmol/g SRF in every case.

min. The amount of the released Ca^{2+} through this process was about 50%.

Such a release of Ca^{2+} in these experiments was not accompanied by any irreversible denaturation because the calcium uptake ability and ATPase activity remained unchanged. Furthermore this release of Ca^{2+} was observed repeatedly for the identical sample in the condition that Ca^{2+} was fully loaded by SRF. From these observations, the change of the ionic environment is considered to be effective merely to change the membrane potential. Finally, it is concluded that depolarization of SRF membrane caused the increase of Ca^{2+} permeability, that is, SRF were still responsive to the electrical stimulation *in vitro*.

Recently, Endo et al. [3] succeeded in demonstrating the release of Ca^{2+} from SR caused by its electrical excitation *in vivo* by using a skinned fibre of frog skeletal muscle. In that experiment, the depolarization of SR was caused by the replacement of the ionic environment. The fibre had been preincubated in KMS before the experiment was done in KCl. The result is consistent with our results.

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