Biochimica et Biophysica Acta, 984 (1989) 289–292
Elsevier

BBAMEM 74560

Rapid Ag^+-induced release of Ca^{2+} from sarcoplasmic reticulum vesicles of skeletal muscle: a rapid filtration study

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(Received 20 March 1989)
(Revised manuscript received 8 June 1989)

Key words: Calcium ion release; Silver ion effect; Sarcoplasmic reticulum; (Rabbit skeletal muscle)

Using a rapid filtration method, we show that Ag^+ is able to trigger Ca^{2+} release from sarcoplasmic reticulum vesicles at a rate as fast as that induced by Ca^{2+} itself. The Ag^+ concentration dependence of the rate constant of Ca^{2+} release presents a bell shape, similar to that of Ca^{2+}-induced Ca^{2+} release, with a maximum at 30 μM free Ag^+. The rapid phase of Ca^{2+}-release induced by Ag^+ is activated by millimolar ATP and inhibited by 5 μM ruthenium red. Moreover, micromolar Ca^{2+} produces a shift of the Ag^+ concentration dependence of the Ca^{2+} release rate. All these results suggest that Ag^+ acts on the same sites as Ca^{2+} to regulate the release of Ca^{2+}.

Introduction

The sarcoplasmic reticulum (SR) forms an intracellular membrane compartment that regulates the contraction-relaxation cycle of skeletal muscle by releasing and reabsorbing Ca^{2+} [1,2]. How Ca^{2+} is released from SR, still remains unclear. In this context, understanding the mechanism, by which different reagents trigger Ca^{2+} release from SR, may give important information regarding the mechanism by which physiological Ca^{2+} release occurs. Abramson, Salama and co-workers [3,4] have shown that micromolar concentrations of Ag^+ can trigger Ca^{2+} release from skeletal muscle SR vesicles. They suggested that Ag^+ acts at the physiological site of release by binding to sulfhydryl groups associated with the Ca^{2+} release channel.

In the present report, we examine Ag^+-induced Ca^{2+} release using a rapid filtration method that we previously described [5,6] and we compare to Ca^{2+}-induced Ca^{2+} release. Our results suggest that the Ag^+ and Ca^{2+} binding sites which are responsible for the activation of Ca^{2+} release strongly resemble each other, and are probably identical sites. Moreover we describe an inhibitory effect of high concentrations of Ag^+ similar to that observed with high Ca^{2+}.

Materials and Methods

Preparation of SR vesicles

SR vesicles were prepared from rabbit skeletal muscle as in Ref. 5. The vesicles (about 20 mg of protein/ml) were suspended in 300 mM sucrose, 20 mM Mops, 4 mM BTP at pH 6.8. Samples (0.5 or 1 ml) were rapidly frozen in liquid nitrogen where they were stored until used. Protein concentration was determined spectrophotometrically at 280 nm in the presence of 1% sodium dodecyl sulfate as described in Ref. 7.

Solutions

The experiments have been performed with special ionic conditions, as compared to those generally employed for measurements of Ca^{2+} release [5,8]. No chloride was used to avoid precipitation of AgCl. Based on the results of our previous work [5] the ionic conditions used were Mops/BTP during the loading and K/Mops during the release (see next paragraph for details). Free Ca^{2+} and Ag^+ concentrations were calculated in the presence of EGTA and ATP using a computer program as previously described in Ref. 5. The Ag^+/EGTA dissociation constant of 1.12 mM was derived from Smith and Martell [9]. The apparent dissoci-

Abbreviations: SR, sarcoplasmic reticulum; Mops, 3-(*N*-morpholino) propanesulfonic acid; BTP, bis-Tris propane or 1,3-bis[tris(hydroxymethyl)methylamino]propane; EGTA, [ethylenebis(oxyethylenenitrilo)] tetraacetic acid.

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ation constant for Ag^+/ATP was determined using a Ag^+-selective electrode in a buffer containing 160 mM Mops, 50 mM KOH, pH 6.8. The value obtained was 1.3 mM at pH 6.8. The contaminating Ca^{2+} was supposed to be no more than 5 μM.

Rapid filtration measurements of Ca^{2+} release

SR vesicles (3 mg of protein/ml) were passively loaded by incubation for 1 h at room temperature in 2.5 mM $^{45}Ca^{2+}$, 160 mM Mops buffered at pH 6.8 with 30 mM BTP and 90 mM sucrose (60 mM coming from the storing medium). Ca^{2+} release measurements were performed as described by Moutin and Dupont [5]. Release solutions contained 160 mM Mops, 50 mM KOH at pH 6.8 and various ATP, free Ag^+ and Ca^{2+} concentrations (see legend of the figures). In the absence of any inducer no leakage of Ca^{2+} was observed (Fig. 1). This indicates that dilution of the vesicles, loaded in the presence of 90 mM sucrose, with the release solution that contains no sucrose does not break them.

Since the release experiments are performed on filters, we have tested the binding of Ag^+ to the filter. Two prefiltered Ag^+ solutions of 7 and 22 μM (concentrations verified by atomic absorbtion) were tested. 300 μl of each solution were passed through 0.65 μm Millipore filters. Ag^+ retained by the filters was determined by atomic absorbtion after their incineration. For both solutions the amount of Ag^+ remaining on the filter was estimated to be less than that contained in the hydrated volume of the filter. Thus binding of Ag^+ on 0.65 μm Millipore filter seems to be negligible. We conclude that during the rapid filtration experiments, SR vesicles absorbed on the filter are submitted to the exact Ag^+ concentration of the release solution.

Results

Evidence for a rapid Ca^{2+} release induced by Ag^+

In the presence of 2 mM EGTA (1 nM Ca^{2+}) and 1 mM ATP, 30 μM free Ag^+ trigger rapid Ca^{2+} release from SR vesicles passively loaded with $^{45}Ca^{2+}$ (Fig. 1). The release presents an initial rate of about 300 nmol · (mg protein)$^{-1}$ · s^{-1}. In the absence of Ag^+, only a small amount of Ca^{2+} is released during the first 200 ms (Fig. 1). In the same conditions (no chloride, 1 mM ATP), we have also measured the kinetics of Ca^{2+} release induced by 1 μM Ca^{2+} (which corresponds to the maximal effect, see Ref. 5) (Fig. 1). The apparent rate constant (calculated as in Ref. 5) for Ca^{2+} release induced by Ca^{2+} is 2.5 s^{-1} whereas it is 6 s^{-1} for Ag^+-induced Ca^{2+} release. The rate constant induced by Ca^{2+} here is 4-times slower than that observed in the same conditions but with KCl in the loading and release media [5].

Salama and Abramson [4] previously observed Ag^+-induced Ca^{2+} release from SR vesicles using a spectrophotometric method with arsenazo III. The Ca^{2+} release rates were slower than observed in this study, probably as a consequence of the lower concentrations of ATP and higher Mg^{2+}.

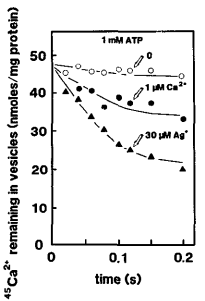

Fig. 1. Measurement of a rapid Ca^{2+} release induced by Ag^+. Passive loading and Ca^{2+} release measurements were performed as described in Materials and Methods. Release solutions contained 160 mM Mops, 50 mM KOH, pH 6.8, 1 mM ATP and (○) 2 mM EGTA (1 nM free Ca^{2+}), (▲) 2 mM EGTA and 100 μM $AgNO_3$ (30 μM free Ag^+) or (●) 0.2 mM EGTA and 0.15 mM calcium gluconate (1 μM free Ca^{2+}).

ATP dependence

The rate of Ca^{2+} release induced by Ag^+ is strongly dependent upon the concentration of ATP. In the experiments shown in Fig. 2, we examine the ATP concentration dependence of the Ca^{2+} release rate induced by 15–40 μM free Ag^+. Millimolar ATP concentrations increase the Ca^{2+} release rate constant. Half-maximal

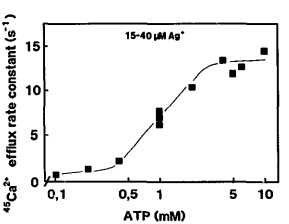

Fig. 2. Activation of the Ag^+-induced Ca^{2+} release by ATP. Passive loading and Ca^{2+} release measurements were performed as described in Materials and Methods. Release solutions contained 160 mM Mops, 50 mM KOH, pH 6.8, 2 mM EGTA (1 nM free Ca^{2+}), 100 μM $AgNO_3$ (15 to 40 μM free Ag^+ depending on the ATP concentration) and the indicated ATP concentrations.

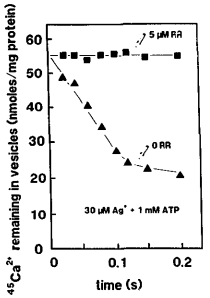

Fig. 3. Inhibition of the Ag^+-induced Ca^{2+} release by Ruthenium red. Passive loading and Ca^{2+} release measurements were performed as described in Materials and Methods. Release solutions contained 160 mM Mops, 50 mM KOH, pH 6.8, 2 mM EGTA (1 nM free Ca^{2+}), 1 mM ATP, 100 μM $AgNO_3$ (30 μM free Ag^+) and 0 (▲) or 5 μM Ruthenium red (■).

activation is obtained at about 1 mM ATP ($K_{1/2} = 1$ mM), while the maximal effect is observed with 5 mM ATP or more. This experiment was performed at a total $AgNO_3$ concentration of 100 μM. Correcting for Ag^+ binding to ATP yields that the free Ag^+ concentration varied between 40 μM at low ATP and 15 μM at high ATP. A small underestimation of the $K_{1/2}$ value at high ATP should therefore be expected.

As in the case of Ca^{2+}-induced Ca^{2+} release [5,8], the initial phase of Ca^{2+} release induced by Ag^+ is strongly dependent upon the presence of millimolar ATP. The $K_{1/2}$ observed is identical to that we previously obtained for Ca^{2+}-induced Ca^{2+} release [5].

Inhibition by ruthenium red

Ruthenium red, which is known to inhibit Ca^{2+}-induced Ca^{2+} release [10], inhibits in the same way the rapid release of Ca^{2+} induced by Ag^+. Fig. 3 shows the time course of Ca^{2+} release triggered by 30 μM free Ag^+ and 5 mM ATP in the absence and in the presence of 5 μM Ruthenium red. The initial phase of Ca^{2+} release is totally inhibited by 5 μM Ruthenium red.

Dependence of Ca^{2+} release on external Ag^+. Effect of micromolar Ca^{2+}

ATP activated Ca^{2+} release in the presence of 1 nM Ca^{2+} or 1 μM Ca^{2+} is influenced by the free extravesicular Ag^+ concentration as shown in Fig. 4. In the 'absence' of Ca^{2+} (1 nM free Ca^{2+}), Ca^{2+} release begins to be activated at about 5 μM Ag^+. The rate constant of Ca^{2+} release reaches its maximal value at 30 μM free Ag^+. Higher Ag^+ concentrations result in an inhibition of Ca^{2+} release. This inhibition at high concentrations of Ag^+ has not been reported previously. It closely ressembles the inhibition produced by high concentrations of Ca^{2+} and gives to Ag^+-induced Ca^{2+} release a similar bell-shaped concentration dependence as previously reported for Ca^{2+}-induced Ca^{2+} release [5,8]. The SR membrane is less sensitive to Ag^+ than to Ca^{2+}. 30 μM free Ag^+ is required to maximally activate Ca^{2+}, while only 1 μM free Ca^{2+} [5] is required to maximally stimulate Ca^{2+}-induced Ca^{2+} release.

The addition of 1 μM Ca^{2+} in the release medium produces a displacement of the curve to higher Ag^+ concentrations: the $K_{1/2}$ of activation by Ag^+ is shifted from 10 to 30 μM, the $K_{1/2}$ of inhibition from 80 to 130 μM. The maximal level of Ag^+ stimulation remains unaffected in the presence of 1 μM Ca^{2+}. The shift observed in the first part of the curve suggests that Ag^+ and Ca^{2+} compete for the triggering of Ca^{2+} release, Ca^{2+} having a higher affinity but displaying a slower rate constant. The right shift produced by 1 μM Ca^{2+} in the Ag^+-induced inhibition is more difficult to understand, since the affinity of Ca^{2+} for the inhibition site(s) was shown to be largely higher than 1 μM [5]. It may be explained by the addition of an activating effect produced by Ca^{2+} on the inhibiting effect of Ag^+.

Discussion

In the present work we demonstrate that Ag^+ is able to induce a rapid release of Ca^{2+} from sarcoplasmic reticulum vesicles passively loaded with Ca^{2+}. The measurements were made over a larger range of Ag^+ concentration and under conditions different from those previously reported [4,11,12]. The SR vesicles are im-

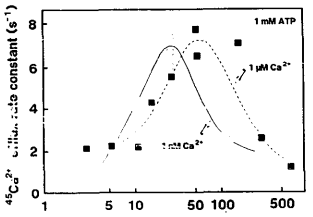

Fig. 4. Dependence of Ca^{2+} release on free Ag^+. Effect of 1 μM free Ca^{2+}. Passive loading and Ca^{2+} release measurements were performed as described in Materials and Methods. Release solutions contained 160 mM Mops, 50 mM KOH, pH 6.8, 1 mM ATP with various $AgNO_3$ concentrations, and (□) 2 mM EGTA (1 nM free Ca^{2+}) or (■) 0.2 mM EGTA and 0.15 mM Ca^{2+} (1 μM free Ca^{2+}).

mobilized on a filter and submitted to Ag^+ solutions for no more than 200 ms. Whereas in the experiment described by Salama, Abramson et al. [4,12] and Tatsumi et al. [11] the SR vesicles were suspended in solutions and submitted to Ag^+ for at least several seconds.

We observed similarities between Ag^+-induced Ca^{2+} release and Ca^{2+}-induced Ca^{2+} release: a similar bell shape of the concentration dependence of the rate, an activation by ATP (with the same $K_{1/2}$), an inhibition by Ruthenium red and by high concentrations of Ca^{2+} and Mg^{2+} (not shown). Moreover, the Ag^+-concentration dependence of the Ca^{2+} release is displaced by micromolar free Ca^{2+}. These results suggest that Ag^+ and Ca^{2+} may act on the same site(s), however, our data cannot at this moment support this idea unambiguously.

In their recent study, Tatsumi et al. [11] found no shift in the Ca^{2+}-induced Ca^{2+}-release rate caused by 1 μM Ag^+, in the absence of ATP. They conclude that Ag^+ binds to an activation site of the Ca^{2+}-induced Ca^{2+}-release channel different from that of Ca^{2+}. According to our experiments (Fig. 4) no effect should be expected with such a low Ag^+ concentration. Their result is thus no inconsistent with ours showing that Ca^{2+} ions (at their maximal concentration) shift the Ag^+-concentration dependence of the Ca^{2+} release.

At the present time, there is no simple scheme able to describe the mechanism of the competitive effect between Ag^+ and Ca^{2+}. Indeed, even the bell-shaped concentration dependence (with a very steep response of the activation and inhibition phases) of the Ca^{2+}- and Ag^+-induced Ca^{2+} release can not be described by single activating and inhibitory sites. Activation and inhibition of the Ca^{2+} release by Ca^{2+} [5] and Ag^+ (Fig. 4) probably involve several interacting sites.

Finally, Gould and collaborators have proposed that the Ca^{2+}-ATPase may be the pathway for the Ca^{2+} efflux triggered by Ag^+ [13]. This is in contradiction with what was proposed by several authors [3,4,11,14] and more generally with the well-accepted idea that calcium release in sarcoplasmic reticulum membranes is mediated by a specific calcium channel. Whether Ag^+ is reacting with the Ca^{2+}-ATPase or the Ca^{2+}-releasing channel to trigger Ca^{2+} release from the SR vesicles can not be concluded definitively from the present work. However, the high values of the initial rates of Ca^{2+} release that we measured and the fact that the experiments were carried out in the absence of Mg^{2+} are incompatible with a reverse function of the Ca^{2+}-ATPase.

Acknowledgments

This work was supported by grants from the Association Française contre les Myopathies, from the Institut National de la Santé et de la Recherche Médicale (CRE 856009), from the Muscular Distrophy Association and from the American Heart Association (Grant 87 915). J. Abramson is an Established Investigator of the American Heart Association (84 130). We thank Dr. J. Cronin for his technical assistance in measuring the Ag^+/ATP dissociation constant.

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