Heparin induces Ca^{2+} release from the terminal cysterns of skeletal muscle sarcoplasmic reticulum

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Received 20 June 1985

Using a Ca^{2+}-selective electrode and the chlorotetracycline fluorescence technique, the effects of heparin on Ca^{2+} transport in the sarcoplasmic reticulum (SR) of skeletal muscles in the absence of oxalate were investigated. It was shown that heparin (0.5–10 μg/ml) causes a rapid release of 40–50 nmol Ca^{2+}/mg protein from the terminal cystern SR vesicles bound to 130–150 nmol/mg protein of Ca^{2+} in the presence of ATP. However, heparin has practically no effect on the longitudinal cystern fraction of SR. The effects of heparin can be prevented by ruthenium red. No influence of heparin is observed in the case of the Ca^{2+}-induced release of Ca^{2+} from the terminal cysterns. When the Ca^{2+} release is induced by heparin, no Ca^{2+}-induced release of Ca^{2+} takes place.

Sarcoplasmic reticulum *Ca^{2+} transport* *Chlorotetracycline fluorescence technique* *Heparin*

1. INTRODUCTION

The molecular mechanism of Ca^{2+} release from the sarcoplasmic reticulum (SR) of skeletal muscles and myocardium upon excitation is still unknown. Some authors believe that the release of Ca^{2+} is induced by the depolarization of SR membranes [1]. However, such a mechanism is hardly possible because of the absence of an electrical potential difference on the SR membranes due to their high permeability for monovalent cations and chloride [2]. Recent studies have shown that the internal ionic composition of SR cysterns is the same as that of the myoplasm [3]. Therefore, assuming that the electric stimulation of SR membranes is the true cause of Ca^{2+} release, it seems more reasonable to suppose that the latter process is the result of drastic changes in the surface charge rather than the depolarization of the SR membranes. To test this assumption, we examined the effects of the natural polyanion, heparin, on Ca^{2+} transport in different fractions of rabbit skeletal muscle SR under the stipulation that heparin binding to the membrane increases the negative charge on its surface.

2. MATERIALS AND METHODS

The experiments were carried out on 2 fractions of SR, i.e., on the terminal (TC) and longitudinal cystern (LC) fractions isolated from rabbit hind limb skeletal muscle homogenate. The tissue was homogenized as described [4]. To increase the membrane yield, 10 mM caffeine was added to the homogenization medium. The total membrane fraction obtained by centrifugation at 10000–36000 × *g* was extracted in the cold, using a medium that contained 0.6 M KCl, 0.1 mM EDTA, 0.2 mM $CaCl_2$, 0.6 mg/ml human serum albumin and 5 mM histidine, pH 7.4. The membrane suspension was centrifuged at 11000 × *g* for 20 min to let the TC fraction sediment and then at 40000 × *g* for 60 min to obtain the LC fraction. The resulting pellets were suspended in a medium containing 25% glycerol (w/v), 0.1 mM EDTA, 0.2 mM $CaCl_2$ and 10 mM histidine, pH 7.2 (4°C). For further purification, the fractions were layered on top of the same medium (4 ml) filling the centrifuge tubes and spun at 36000 × *g* for 60 min. The TC fraction formed a pellet, whereas the LC fraction sedimented as a dense suspension on the bottom of the tubes.

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00145793/85/$3.30

The transport of Ca^{2+} was studied, using an 'Orion' 93-20 Ca^{2+}-selective electrode or by chlorotetracycline (10 μM) fluorescence on a Hitachi SP-850 spectrofluorimeter in a medium containing 100 mM KCl, 4 mM $MgCl_2$, 2 mM ATP, 5 mM creatine phosphate, 2–3 IU creatine kinase, 30–60 μg/ml SR protein and 10 mM Hepes, pH 6.8 (28°C). Protein concentration was determined in a biuret reaction.

3. RESULTS AND DISCUSSION

Fig.1 shows the kinetics of Ca^{2+} transport in the TC and LC fractions as measured by a Ca^{2+}-selective electrode. As can be seen from the figure, an addition of ATP to the incubation medium containing SR membranes results in a rapid uptake of Ca^{2+}. In the absence of SR membranes, ATP causes only a slight decrease of Ca^{2+} concentration in the assay medium due to Ca^{2+} binding. To evaluate the extent of Ca^{2+} uptake, the values of the electrode response to ATP additions in the absence of SR membranes were subtracted from that of the total response during Ca^{2+} transport. The amount of Ca^{2+} bound to the SR vesicles was found to be equal to 130–150 nmol/mg protein.

Heparin (10 μg/ml) was added to the incubation medium after the cessation of Ca^{2+} transport causes a rapid release of 40–50 nmol Ca^{2+}/mg protein from the TC fraction of SR, but practically does not affect the LC fraction. The minimum concentration of heparin capable of inducing such an effect is 0.5 μg/ml, the maximum is 10 μg/ml. The release of Ca^{2+} induced by heparin is due to the polyanionic properties of the latter, e.g. uncharged dextran (T-500) does not cause any Ca^{2+} release (fig.1) whereas dextran sulfate (2 μg/ml) releases Ca^{2+} as heparin (not shown). Owing to the low time resolution of the Ca^{2+}-selective electrode, one cannot determine with a high degree of accuracy the rate of Ca^{2+} release. As can be seen from fig.1, this rate is high enough and is comparable to that of Ca^{2+} uptake. To test the reversibility of heparin-induced Ca^{2+} release we used multicharge cation ruthenium red for the binding and neutralizing of heparin. The influence of such a compound on the heparin-induced release of Ca^{2+} was investigated in fluorescence experiments, using chlorotetracycline as an indicator for Ca^{2+}

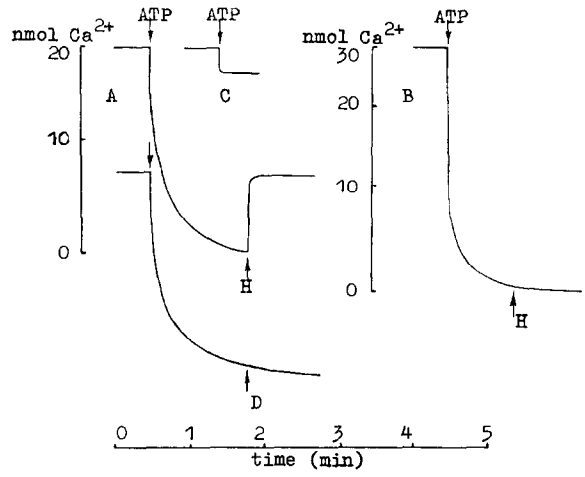

Fig.1. Transport of Ca^{2+} in the terminal cystern (A) and longitudinal cystern (B) fractions of SR membranes measured by a Ca^{2+}-selective electrode. ATP was added to the incubation medium containing SR membranes (A, 30 μg/ml; B, 45 μg/ml). Addition: 10 μg/ml heparin (H), 10 μg/ml dextran (D). (C) Addition of ATP in the absence of SR membranes.

concentration inside the SR vesicles [5]. This method has a good advantage over the Ca^{2+}-selective electrode technique, since it permits one to follow the changes in Ca^{2+} concentration inside the SR vesicles and to study the Ca^{2+} release induced by different compounds capable of changing the electrical potential of the Ca^{2+}-sensitive electrode or causing a damaging influence on its function. The kinetics of binding and release of Ca^{2+} as measured by chlorotetracycline fluorescence is shown in fig.2. An addition of SR membranes to the incubation medium containing ATP and chlorotetracycline causes a temporary increase in the fluorescence. The increase in fluorescence intensity is much lower than the rate of Ca^{2+} binding measured by the Ca^{2+}-selective electrode, due to the low permeability of the SR vesicles to chlorotetracycline [6]. An injection of the Ca^{2+} ionophore, X537A, into the incubation medium results in an immediate release of Ca^{2+} from the SR vesicles. Heparin exerts a similar effect, except that, firstly, this compound, when used at high concentrations, cannot cause the release of all the Ca^{2+} from the SR vesicles, and secondly, its effect is directed only at the TC fraction of SR. After addition of ruthenium red following the heparin-

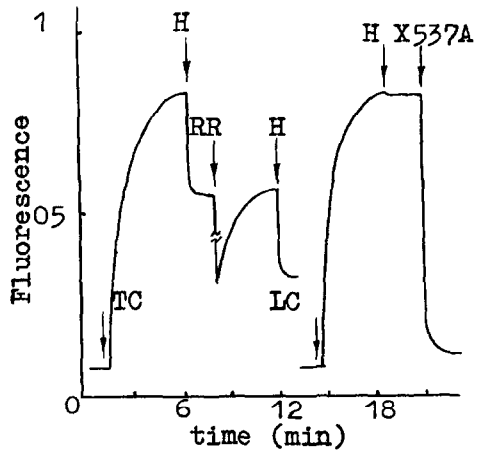

Fig.2. Release of Ca^{2+} from the terminal cystern (TC) and longitudinal cystern (LC) fractions of SR induced by heparin (H) (first addition: 10 μg/ml, second addition: 40 μg/ml for binding of ruthenium red) and X537A (10 μM), as determined from chlorotetracycline fluorescence. SR membranes (60 μg/ml) were added to the incubation medium containing Mg · ATP (see section 2). Ruthenium red (RR), 3 μM.

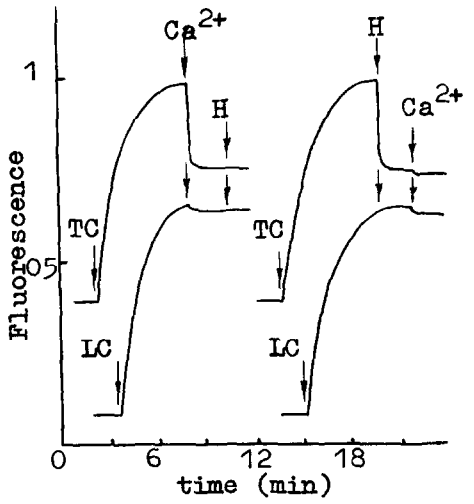

Fig.3. Release of Ca^{2+} from the terminal cystern (TC) and longitudinal cystern (LC) fractions of SR membranes induced by 25 μM Ca^{2+} and 10 μg/ml heparin (H), as determined from chlorotetracycline fluorescence. SR membranes (60 μg/ml) were added to the incubation medium containing Mg · ATP (see section 2).

induced Ca^{2+} release, the uptake of Ca^{2+} is resumed. Subsequent additions of excess heparin cause a release of Ca^{2+}. In the absence of heparin, ruthenium red does not influence Ca^{2+} transport under the conditions used.

It is known that Ca^{2+} release from the TC fraction of SR can also be induced by increasing Ca^{2+} concentration up to 10^{-5}–10^{-4} M [7]. In this connection, it seemed reasonable to investigate the interrelationship between the heparin- and Ca^{2+}-induced release of Ca^{2+}. In our studies, we used the chlorotetracycline fluorescence method to follow the changes in Ca^{2+} release induced by Ca^{2+}. As can be seen from fig.3, an addition of 25 μM Ca^{2+} to the incubation medium after the appearance of a plateau in the fluorescence plots leads to a rapid release of Ca^{2+} from the TC fraction of SR, while the LC fraction remains unaffected. Heparin added to the incubation medium after the Ca^{2+}-induced release of Ca^{2+} does not cause any further release of Ca^{2+}. When the Ca^{2+} release is induced by heparin, the latter effect is absent. In contrast, ruthenium red does not cause any blocking effect on the Ca^{2+} release induced by Ca^{2+}. Presumably, ruthenium red blocks the heparin-induced release of Ca^{2+} by forming an electrically neutral complex with them.

Hence, the experimental results demonstrate that heparin is capable of inducing a Ca^{2+} release from the TC of SR. The specific action of this compound on the TC fraction may suggest that heparin activates the physiological mechanism of Ca^{2+} release. Heparin- and Ca^{2+}-induced releases of Ca^{2+} from the SR vesicles are closely interrelated processes, i.e., in both cases either a common pool of Ca^{2+} or a common channel is involved in the process.

The mechanism of heparin-induced release of Ca^{2+} demands further verification and experiment. The key role in this process belongs, in all probability, to the negative charge of the heparin molecule. Evidence for this assumption can be derived from the fact that in the case of uncharged dextran the effect is absent and that the heparin-induced release of Ca^{2+} can be prevented by the multicharge cation (ruthenium red). Heparin binding to the SR membrane should thus increase its surface negative charge and provide for the electrical asymmetry of the SR membrane. It is also possible that the true cause of the opening of Ca^{2+}

channels is the changes in the electrical field of the SR membrane proper. The specific release of Ca^{2+} induced by heparin is in good agreement with the reports from Schneider and Chandler's laboratory [8]: these authors demonstrated that Ca^{2+} release from muscle SR can be preceded by a charge transfer in the T-system membrane in the region of the membrane junction with the TC of SR. This transfer may reflect the interaction of a T-system membrane-linked polyanion with the SR membrane.

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