EFFECT OF Mn^{2+} ON THE SARCOPLASMIC RETICULUM IN SKINNED SKELETAL MUSCLE FIBERS

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Mn^{2+} at a concentration of a few millimolar has a remarkable inhibitory effect on Ca^{2+} influx across various excitable membranes (1–3), whereas it has been supposed to have no significant effect in skeletal muscle. According to Oota et al. (4), however, a high concentration of Mn^{2+} (10 mM) depressed both potassium- and caffeine-contraction, but had no effect on the latter contraction in the depolarized muscle. Using a single fiber, Chiarandini and Stefani (5) reported that 10 mM Mn^{2+} strongly inhibited the potassium-contraction at low but not high concentration of K^+ and that the caffeine-contraction was insensitive to Mn^{2+} . Since these results obtained with intact fibers were complex, in the present study, the effect of Mn^{2+} on Ca^{2+} release from the sarcoplasmic reticulum (SR) by Ca^{2+} -releasing stimuli was examined using the skinned fibers in the hopes of obtaining more clear evidence on the mode of action of Mn^{2+} on skeletal muscle.

A single fiber was isolated from the semitendinosus of the frog, *Rana nigromaculata*; and all or part of the sarcolemma was removed in a standard relaxing solution containing 130 mM K propionate, 20 mM Tris-maleate, 4 mM $MgCl_2$, 4 mM ATP and 2 mM EGTA, pH 6.8 at 20°C. The mechanically skinned fibers thus obtained were mounted in a force-measuring apparatus, and isometric tension was recorded according to the method described in our previous paper (6). In buffered Ca^{2+} solutions, various Ca^{2+} concentrations were calculated by assuming

an apparent binding constant of $10^6 M^{-1}$ for the Ca-EGTA complex at pH 6.8, 20°C, while keeping the total EGTA concentration fixed at 2 mM (6). Since a high concentration of caffeine, such as 25 mM, releases most of the Ca^{2+} present in the SR (7), the amount of Ca^{2+} in the SR was estimated by releasing Ca^{2+} by applying 25 mM caffeine in the presence of 2 mM EGTA and measuring the area under the caffeine-induced contraction.

The Ca^{2+} in the SR was depleted by 25 mM caffeine in the presence of 2 mM EGTA. Then the SR was loaded with Ca^{2+} by immersing the skinned fibers in the buffered Ca^{2+} solution containing $5 \times 10^{-7} M$ Ca^{2+} for 2 min and rinsed in the relaxing solution with 0.5 mM EGTA. When the skinned fibers were exposed to the relaxing solution containing 2 mM $MnCl_2$ and 0.5 mM EGTA after the treatment of the Ca^{2+} loading, the fibers showed a sustained contraction (Fig. 1b). It is impossible to distinguish whether the contraction is induced by a direct effect of Mn^{2+} on the contractile system or by an indirect effect of an increase in concentration of Ca^{2+} in the solution due to the formation of the Mn-EGTA complex. In any case, the amount of Ca^{2+} remaining in the SR after the exposure to Mn^{2+} for such a short time was almost the same as that of the control experiment in which the same procedures were followed except for exposure to Mn^{2+} (Fig. 1a). Therefore, it was shown that the remaining Ca^{2+} in the SR remained almost unchanged after the exposure to Mn^{2+} . The effect of Mn^{2+} on the Ca^{2+} release

Fig. 1. Effect of Mn^{2+} on the Ca^{2+} release from the SR by Ca^{2+} -releasing stimuli in the same preparation. (a) and (d): Ca^{2+} release from the SR induced with 25 mM caffeine in the presence of 2 mM EGTA after loading with 5×10^{-7} M Ca^{2+} . (b): Effect of 2 mM MnCl_2 on the SR in the presence of 0.5 mM EGTA. (c) and (f): Effect of 2 mM MnCl_2 on caffeine- and "depolarization"-induced Ca^{2+} release in the presence of 0.5 mM EGTA. The "depolarization" was produced by quickly replacing 130 mM propionate with an equimillimolar concentration of chloride in the solution (C1). (e): Ca^{2+} release from the SR induced by "depolarization". (g): Effect of 10^{-5} M Ca^{2+} on the "depolarization"-induced Ca^{2+} release. Ca, G and Caf represent Ca^{2+} loading with 5×10^{-7} M Ca^{2+} , washing with relaxing solution containing 0.5 mM EGTA, and application of 25 mM caffeine, respectively. Remaining Ca^{2+} values in the SR shown in parentheses were represented as values relative to that of the control experiment (a and d).

induced by 25 mM caffeine, as seen in Fig. 1c, clearly shows that Mn^{2+} inhibited only 10% of the amount of Ca^{2+} released from the SR. Even if the concentration of MnCl_2 was increased up to 10 mM in the experiment shown in Fig. 1c, the degree of inhibition of the caffeine-induced Ca^{2+} release by Mn^{2+} was no more than 40%. A low concentration of caffeine, such as 5 mM, releases about 50% of the Ca^{2+} present in the SR. MnCl_2 (2 mM) had little or no effect on this caffeine-induced Ca^{2+} release.

A sudden change in the ionic composition of a solution immersing the SR enables us to directly and effectively alter the membrane potential of the SR (8–10). If 130 mM propionate in the relaxing solution was quickly replaced by a supposedly more

permeant anion (130 mM chloride), the lumen of the SR should become more negative with respect to the outside. Thus the potential change of the SR membrane is termed "depolarization" (11). As shown in Fig. 1e, the skinned fibers showed a transient contraction by the ionic replacement in the presence of 0.5 mM EGTA. The amount of Ca^{2+} remaining in the SR after the treatment was about 50% of that before the application of the Ca^{2+} -releasing stimulus (Fig. 1d). It was proved that the 2 mM MnCl_2 depressed this "depolarization"-induced Ca^{2+} release (Fig. 1f). On the other hand, the "depolarization"-induced Ca^{2+} release was unaffected by the addition of 10^{-5} M Ca^{2+} , i.e., it was certain that the inhibitory effect of Mn^{2+} on "depolarization"-induced Ca^{2+}

release could not be accounted for by an increase in Ca^{2+} in the solution due to the formation of Mn-EGTA complex, but by a direct action of Mn^{2+} on the SR.

These results indicate that the "depolarization"-induced Ca^{2+} release mechanism of the SR is inhibited by Mn^{2+} at a few millimolar concentration, but the caffeine-induced Ca^{2+} release mechanism is insensitive to the presence of Mn^{2+} . In contrast to this, it is generally accepted that the Mg^{2+} specifically inhibits the latter Ca^{2+} release mechanism (9, 11). Although the cause for the substantial difference between Mg^{2+} and Mn^{2+} in their effects on the SR is not clear at present, Mn^{2+} may be a useful tool for elucidating the Ca^{2+} release mechanism of the SR.

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REFERENCES

- 1) Nonomura, Y., Hotta, Y. and Ohashi, H.: Tetrodotoxin and Manganese ions: Effects on electrical activity and tension in taenia coli of guinea pig. *Science* **152**, 97–98 (1966)
- 2) Hagiwara, S. and Nakajima, S.: Differences in Na and Ca spike as examined by application of tetrodotoxin, procaine and manganese ions. *J. gen. Physiol.* **49**, 793–806 (1966)
- 3) Rougier, O., Vassort, G., Garnier, D., Gargouil, Y.M. and Coraboeuf, E.: Existence and role of a slow inward current during the frog atrial action potential. *Pflüger Arch.* **308**, 91–110 (1969)
- 4) Oota, I., Takauji, M. and Nagai, T.: Effect of manganese ions on excitation-contraction coupling in frog sartorius muscle. *Japan. J. Physiol.* **22**, 379–392 (1972)
- 5) Chiarandini, D.J. and Stefani, E.: Effects of manganese on the electrical and mechanical properties of frog skeletal muscle fibers. *J. Physiol.* **232**, 129–147 (1973)
- 6) Saida, K. and Nonomura, Y.: Characteristics of Ca^{2+} - and Mg^{2+} -induced tension development in chemically skinned smooth muscle fibers. *J. gen. Physiol.* **72**, 1–14 (1978)
- 7) Endo, M. and Blinks, J.R.: Inconstant association of aequorin luminescence with tension during calcium release in skinned muscle fibers. *Nature New Biol.* **246**, 218–221 (1973)
- 8) Costantin, L.L. and Podolsky, R.J.: Depolarization of internal membrane system in the activation of frog skeletal muscle. *J. gen. Physiol.* **50**, 1101–1124 (1967)
- 9) Ford, L.E. and Podolsky, R.J.: Regenerative calcium release within muscle cells. *Science* **167**, 58–59 (1970)
- 10) Nakajima, Y. and Endo, M.: Release of calcium induced by "depolarization" of the sarcoplasmic reticulum membrane. *Nature New Biol.* **246**, 216–218 (1973)
- 11) Endo, M.: Calcium release from the sarcoplasmic reticulum. *Physiol. Rev.* **57**, 71–108 (1977)