ACTION OF MANGANESE IONS ON EXCITATION-CONTRACTION COUPLING OF FROG SKELETAL MUSCLE FIBRES

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Summary 1. The action of manganese ions on excitation-contraction coupling in frog skeletal muscle fibres was studied.
2. In normal Ringer's solution, twitch tension development was inhibited by the addition of 10 mM-Mn. The inhibitory effect of Mn on twitch tension development was not eliminated by addition of 1 mM caffeine.
3. In 10 mM-Mn Ringer's solution, membrane potential of the semitendinosus muscle fibre was increased by 9.1 mV and the hyperpolarizing action of Mn was observed in various concentrations of potassium up to 50 mM.
4. The action potential was still observed in 10 mM-Mn Ringer's solution and negative afterpotential was prolonged. In 10 mM-Mn Ringer's solution, an intensity of electrical current needed to elicit action potential was increased.
5. Negative afterpotential which disappeared after glycerol treatment (HOWELL and JENDEN, 1967) could not be regained by treatment of 10 mM-Mn.
6. Mn suppressed potassium contractures induced by 80 mM-K or less (constant [K] x [Cl] product). Removal of Mn from 50 mM-K Ringer's solution produced an unusual tension development. Tension development produced by Mn removal from 80 mM-K, 10 mM-Mn Ringer's solution was smaller than that produced by Mn removal from 50 mM-K, 10 mM-Mn Ringer's solution.
7. Caffeine rapid cooling contracture was not affected in the presence of Mn. After the disruption of T-tubules produced by glycerol treatment, caffeine rapid cooling contracture was also observed even in the presence of Mn.
8. No morphological changes in muscle fibres treated with Mn were recognized by electron microscopical studies.
9. From these results it was considered that Mn acted on transverse tubules to inhibit the influx of Ca, but did not directly affect the sarcoplasmic reticulum.
Through investigations of the taenia coli of guinea pig (Nomura et al., 1966), barnacle muscle (Hagiwara and Nakajima, 1966), crustacean muscle (Fatt and Ginsborg, 1958; Takeda, 1967; Chiarandini et al., 1970), and heart muscle (Rouquier et al., 1969), it has been suggested that manganese ions of certain concentrations have an inhibitory effect on Ca entry through excitable membranes.

Recently, the action of manganese ions on the skeletal muscle fibres has aroused a special interest in excitation-contraction coupling (Oota et al., 1972; Chiarandini and Stefani, 1971, 1973). In the frog sartorius muscles it has been found that manganese ions inhibit their twitch, tetanus and potassium contracture, while generation of action potentials was not affected significantly. Oota et al. (1972) suggested from their electrophysiological and biochemical studies that manganese ions might impair excitation-contraction coupling by acting on the transverse tubular system and possibly on the terminal cisternae of sarcoplasmic reticulum. Chiarandini and Stefani (1973) also reported Mn effects on electrical and mechanical properties of the frog sartorius muscles and the tibialis anticus longus. In this paper, it was stated that manganese ions could be adsorbed at the walls of the T-system, and the possible physiological role of Mn in reducing or blocking Ca permeability of surface membranes of the T-system should be considered.

The present experiments were conducted on the frog toe and the semitendinosus muscles to investigate whether manganese ions acts on T-tubules, which would cause release of “trigger Ca,” or on lateral cisternae, which has been assumed to release “activator Ca” (Sandow, 1970). Some of the results obtained in the present experiment were reported at the 49th Annual Meeting of the Physiological Society of Japan in 1972.

**MATERIALS AND METHODS**

The extensor longus digiti IV and the semitendinosus muscles of the frog, *Rana japonica* and *Rana nigromaculata*, were used in the present experiments. The experiments were performed from January to April, 1972. The animals were fed at room temperature (15–20°C) for a few days before experiments.

Mechanical responses of the muscle specimen, mounted vertically in a glass vessel which had a capacity of 50 ml, were recorded as tension development on a Nihon-Kohden direct-writing oscillograph. The lower end of the muscle specimen was fixed to the glass rod and the upper was attached to a Shinkoh U-type mechano-electric transducer with a nylon thread. By means of loop-type Ag-AgCl electrodes located at both ends of the muscle specimen, supramaximal electrical stimulation in the form of 1 msec square-wave shocks was applied to generate twitch tension. Tetani were produced by a train pulse of 500 msec at a frequency of 50/sec.
For the purpose of rapid cooling of the specimen, two glass tubes filled with the same solution, but at different temperatures (20°C and 0°C), were prepared so that Ringer's solution in the glass vessel at room temperature could be substituted rapidly for a cooled one (SAKAI, 1965).

The disruption of the T-tubules of the muscle fibres was achieved by the procedure described by HOWELL and JENDEN (1967), i.e., by immersing the fibres in 400 mM hypertonic glycerol medium for one hour and then returning them to normal Ringer's solution. When the muscle did not show twitch or tetanus tension in response to electrical stimulation, the T-tubules were considered to have been disrupted completely (HOWELL and JENDEN, 1967). The potassium contracture was elicited by immersion of muscle fibres in various high concentrations of potassium solutions by the method of HODGKIN and HOROWICZ (1960a, b).

In order to determine the electrophysiological properties of muscle membrane, conventional glass microelectrodes filled with 3 M KCl with a resistance of 10–20 MΩ were used. In this experiment, a muscle specimen was stimulated extracellularly by applying an electric current through Ag-AgCl plates which were located 5 mm apart from each other (ABE and TOMITA, 1968).

The normal Ringer's solution used in the present experiment was as follows; 117.0 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, and 2.0 mM Tris-HCl (pH 7.0). When MnCl₂ was added to the solution, the product of [K]₀ × [Cl]₀ was kept constant by adding an adequate amount of sucrose.

For electron microscopic preparations, the following procedures were carried out. The resting muscle specimens were fixed with 2% glutaraldehyde (adjusted with 0.1 M cacodylate buffer solution to pH 7.2) for 120 min and with 2% osmium oxide (adjusted with 0.1 M cacodylate buffer solution to pH 7.2) for 120 min at 15°C. Then they were dehydrated with ethylalcohol and propylene oxide. The specimens were embedded with the Epon-mixture (LUFT, 1961). An L.K.B. ultramicrotome with a glass knife was used to obtain sections of about 600 Å longitudinal and cross sections. These preparations were stained with uranyl acetate for 3 min and then with lead citrate for 3 min. The electron microscope used in the present experiment was a JEM 100 (Nihon-Denshi Co., Ltd.).

RESULTS

1) Inhibitory effect of Mn on twitch tension

When Mn was added to normal Ringer's solution, twitches in toe and semi-tendinosus muscles were immediately inhibited. By addition of 5 mM-Mn to normal Ringer's solution, twitch tension development was completely abolished within 60 sec and with 10 mM-Mn within 10 sec. After twitch tension disappeared, a slight decrease in resting tension was observed and no tetanic responses were elicited with electrical stimuli at a frequency of 50/sec. Mechanical responses were almost completely inhibited by the addition of Mn even in the muscle fibres.
treated with 1 mM caffeine. Twitch tension development recovered immediately after removal of Mn from a test solution. The above depressant effect of Mn on twitch tension development was reversible.

2) Effect of Mn on electrical properties of the skeletal muscles

(a) Resting membrane potential and action potential. The resting membrane potential of semitendinosus muscles, 87.4±4.7 mV, measured in normal Ringer’s solution, increased to 96.5±5.6 mV in the presence of 10 mM-Mn, i.e., manganese ions have an action which hyperpolarizes the resting membrane potential of the frog skeletal muscles. The above hyperpolarization of resting membrane potential in the presence of Mn was also observed in the presence of various concentrations of potassium up to 50 mM (Fig. 1). The hyperpolarizing effect of Mn was statistically significant.

The action potential was well elicited in Ringer’s solution containing 10 mM-

![Figure 1](image)

Fig. 1. Effects of Mn on electrical properties of semitendinosus muscle fibres. Upper figures show action potential produced by mass polarization method. Left figure is a control and right is action potential elicited in 10 mM-Mn Ringer’s solution. Upper trace shows 0 mV and the minimum current intensities to elicit action potential. Lower graph shows effect of Mn on resting membrane potential. Ordinate indicates resting membrane potential and abscissa extracellular K concentration. Black circles represent membrane potentials in various concentrations of potassium Ringer’s solutions containing Mn, while white circles represent control values (mean ± S.D.). After treatment of muscle specimen with Mn, membrane potentials were slightly hyperpolarized.
Mn. The electrical current intensity needed for eliciting action potential in Mn-Ringer’s solution, however, was 2.6 times greater than that in normal Ringer’s solution, indicating an increase of electrical threshold for eliciting action potential. In the presence of Mn, the amplitude of the action potential decreased slightly and the negative afterpotential was prolonged (Fig. 1). Threshold, amplitude and negative afterpotential of the action potential in the Mn-treated fibres were not affected by addition of 1 mM caffeine to the test solution (Fig. 2(A)).

(b) Effect of Mn on the T-tubules disrupted muscles. The disappearance of negative afterpotential in the T-tubules disrupted muscle fibres is accompanied by a slight depolarization of resting membrane potential (HOWELL, 1969; EISENBERG and GAGE, 1969; GAGE and EISENBERG, 1969; EISENBERG et al., 1971). This was also recognized in the present study.

In the T-tubules disrupted muscle fibres treated with 5 mM-Mn negative afterpotential, which disappeared after glycerol shock, could not be regained (Fig. 2(B)).

Fig. 2. (A) Effect of caffeine on action potential of skeletal muscle fibres treated with Mn. a) Normal action potential. b) Action potential in 5 mM-Mn Ringer’s solution. c) Action potential after addition of 1 mM caffeine to 5 mM-Mn Ringer’s solution. Upper trace is 0 mV, middle trace, action potential and lower trace, the minimum current intensity to elicit action potential. Caffeine had no effect on prolonged negative afterpotential and the minimum current intensity. (B) Effect of Mn on T-disrupted muscle fibres. a) Normal action potential. b) Action potential of T-disrupted muscle fibres. c) Action potential obtained from T-disrupted muscle fibres. After disruption of T-tubules, Mn did not prolong negative afterpotential.
3) Effect of Mn removal from high-K solutions containing Mn

The effect of Mn on the potassium contracture in 50 mM-K Ringer’s solution was examined. The height and the rate of rise of tension were observed to be reduced by 10 mM-Mn (Oota et al., 1972; Chiarandini and Stefani, 1973). Figure 3(A) shows a normal tetanus and normal 50 mM-potassium contracture. The muscle fibres were soaked in normal Ringer’s solution for 30 min and then in 50 mM-K Ringer’s solution containing Mn, after confirming the disappearance of twitch tension development in 10 mM-Mn Ringer’s solution. As shown in Fig. 3, potassium contracture was remarkably inhibited. When potassium contracture in 50 mM-K Ringer’s solution containing Mn relaxed spontaneously due to inactivation in a parallel manner with ordinary potassium contracture, Mn was removed from that solution. The Mn removal produced an unusual transient tension development (see Fig. 3). Black circles in Fig. 4 show the time course of the relaxation phase of potassium contracture obtained from frog toe muscles (N=10) induced by a solution containing 50 mM-K and 10 mM-Mn.
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Fig. 4. Tension development produced by removal of Mn. Black circles indicate relative tensions of 50 mm-potassium contractures in the falling phase of normal potassium contracture. White circles represent the relative tensions produced by removing Mn from 50 mm-K, 10 mm-Mn Ringer's solution at times indicated by arrows. Tension development produced by removing Mn was greater than that of normal potassium contractures. Relative tension values were obtained by calculation against its peak tension. Vertical bars represent standard deviations.

On the other hand, when manganese ions were removed during the falling phase of the contracture, a large transient tension development was found to take place above the tension of the previous contracture (white circles in Fig. 4). Relatively greater tension development was observed during the early stage of the falling phase in potassium contracture in response to the removal of manganese ions. It is of interest that the tension development in response to Mn removal occurred even if the previous potassium contracture had been almost relaxed.

Figure 5 illustrates 50, 60, 70, and 80 mM-K contractures in the presence or absence of 13 mm-Mn obtained from one and the same preparation. Mechanical responses were observed to take place immediately on removing Mn during the relaxation phase of potassium contracture in the presence of Mn. The peak height and the rate of rise of potassium contracture in the presence of Mn were obviously reduced. The peak tension in response to Mn removal was always smaller than that of normal potassium contracture at the same potassium concentration. The effect of Mn removal on mechanical response was more remarkable at low potassium concentrations than at higher concentrations. It is noteworthy that tension development in solutions of high potassium concentrations was not affected by 10 mm-Mn as reported by CHIARANDINI and STEFANI (1973).
Fig. 5. Effect of Mn on potassium contractures in various concentrations of potassium Ringer’s solutions. The same preparation was used in this experiment. As the concentration of potassium was increased, the rate of rise in tension and the peak tension developed in various concentrations-potassium Ringer’s solution containing 13 mm-Mn were increased, and tension development produced by removing Mn was suppressed.

4) Factors affecting tension development by Mn removal

It is known that the mechanical threshold of potassium contracture and resting membrane potential varies in the presence or absence of 10 mm-Mn, i.e., the S-shaped relation between tension of potassium contractures and log $[K]_0$ is shifted to the right by Mn. A shift of mechanical threshold from $-48$ mV to $-33$ mV by Mn has been reported by Chiarandini and Stefani (1973). Taking into consideration the above threshold change, the following experiments were conducted.

After observing potassium contracture produced by 50 mm-K Ringer’s solution containing Mn, the test solution was replaced during the relaxation phase of potassium contracture with 30 or 40 mm-K Ringer’s solution without Mn. It was considered that resting membrane potentials in 50 mm-K Ringer’s solution
Fig. 6. Effect of K concentration on tension development produced by removing Mn. All records were obtained from one preparation. (A) shows tetanus or twitches in normal Ringer’s solution and 50 mM-potassium contracture. (B) After twitches were stopped in 10 mM-Mn Ringer’s solution, the preparation was immersed in 50 mM-K Ringer’s solution containing 10 mM-Mn. During the falling phase of potassium contracture, Mn was removed and tension development was observed. (C) On removing Mn from test solution, the K concentration was changed simultaneously. After confirming the inhibitory effect of Mn on 50 mM-potassium contracture, the preparation was immersed in 40 mM-K Ringer’s solution without Mn. (D) 50 mM-K Ringer’s solution with Mn was replaced with 30 mM-K Ringer’s solution without Mn. Tension output produced by removing Mn was decreased, as the K concentration in Mn-free solution was decreased 50 mM to 30 mM.

containing Mn and in 40 mM-K Ringer’s solution without Mn were nearly the same, because the resting membrane potential was increased by Mn (see Fig. 1). Figure 6 shows the tension developed after replacement of 50 mM-K, 10 mM-Mn Ringer’s solution with 30 or 40 mM-K, Mn-free Ringer’s solution. The peak height and the rate of rise of tension were suppressed by replacing 50 mM-K, 10 mM-Mn Ringer’s solution with 30 mM or 40 mM-K, Mn-free Ringer’s solution. On the contrary, mechanical response induced by Mn removal was enhanced if
Fig. 7. Tension development produced by Mn removal in the various K concentrations. The relative tension development was calculated from the equation in the figure. After the 50 mM-potassium contracture relaxed in full, the tension development was not observed even if the potassium concentrations of the test solutions were increased up to high concentrations, higher than that in the first test solution (black circles). White circles and triangles show tension development which was observed on removing Mn from 50 mM-K, 10 mM-Mn Ringer’s solution. On removing Mn, if the preparation was rinsed out with a high concentration K Ringer’s solution without Mn, tension development was enhanced. When the preparation was rinsed out with low concentration of K Ringer’s solutions without Mn, tension developments were reduced.

50 mM-K Ringer’s solution containing Mn was replaced with Mn-free, high potassium solutions whose concentrations were higher than 50 mM (Fig. 7(C)). It is obvious from Fig. 7 that there existed a close relationship between tension development in response to Mn removal and [K]₀ in the medium, i.e., the higher the potassium concentration in Mn-free solution, the larger the tension developed. It is worthwhile to note that the above-mentioned phenomenon could be observed under a certain concentration of potassium. When 50 mM-K, 10 mM-Mn Ringer’s solution at room temperature was replaced with 50 mM-K, Ca-free Ringer’s solution without Mn at low temperature (about 2°C), tension development produced by Mn removal was also recognized and enhanced at low temperature (Fig. 8(C)). On the other hand, when the muscle specimen bathed in 50 mM-K, Ca-free Ringer’s solution with 10 mM-Mn at room temperature was immersed in 50 mM-K, Mn-free Ringer’s solution at low temperature, namely by Mn removal and application of low temperature at once, tension development was not observed at all (Fig. 8(D)).
Fig. 8. Effect of rapid cooling and Ca concentration on tension development produced by Mn removal. (Records were obtained from the same preparation). (A) Normal tetanus and 50 mM-potassium contracture. (B) 50 mM-potassium contracture in the presence of 10 mM-Mn and the effect of Mn removal. (C) On removing Mn from 50 mM-K, 10 mM-Mn Ringer's solution, the test solution was replaced with a Ca-free, 50 mM-K Ringer's solution in which the temperature had been lowered to 0°C. At low temperature, tension development produced by Mn removal was larger than that observed at room temperature even though the test solution was Ca-free. (D) After the preparation was immersed in Ca-free, 50 mM-K Ringer's solution with Mn for 70 sec, it was rinsed out with normal 50 mM-K Ringer's solution at low temperature (0°C). No effects of Mn removal and of rapid cooling were observed.

5) Effects of Mn on caffeine rapid cooling contracture

Regarding caffeine rapid cooling contracture (RCC), it has been reported by Sakai (1965), Fujii and Sakai (1969), Sakai et al. (1971) that its mechanism can be accounted for by the temperature dependence of the Ca pumping action in lateral sacs, rather than by the effect of temperature on the T-tubules.

Based on the evidence that Mn decreased the extra Ca-induced splitting of ATP in sarcoplasmic reticulum of rabbit skeletal muscles, Oota et al. (1972)
Fig. 9. Mn effect on rapid cooling contracture (RCC). After the preparation was immersed in 10 mm-Mn Ringer's solution, twitches disappeared. In Mn Ringer's solution, contraction could not be produced by rapid cooling, but the addition of 1.0 mM caffeine produced remarkable contracture in Mn Ringer's solution at low temperature (A). Caffeine-RCC's were observed also in 30 mm-K Ringer's solution containing 10 mm-Mn. Caffeine-RCC was not inhibited by Mn (B).

Fig. 10. Effect of Mn on RCC of T-disrupted muscle fibres. Glycerol treatment was performed by the same method as described by Howell and Jenden (1967). After the disruption of T-tubules, 1.5 mm caffeine was added to normal Ringer's solution (A). In the presence of 1.5 mm caffeine, T-disrupted muscle fibres produced a rapid cooling contracture. Caffeine-RCC could not be inhibited by the addition of 10 mm-Mn to the test solution.
suggested that Mn might act on internal membranes, including the T-system and terminal cisternae. On the other hand, CHIARANDINI and STEFANI (1973) suggested that Mn reduces the entry of Ca across excitable membrane involving the T-system, but does not modify mobilization of Ca from the sarcoplasmic reticulum.

In order to examine the above results, the technique for producing RCC was also applied for the present experiments on the effect of Mn. When the temperature of Ringer's solution containing Mn was rapidly lowered, no mechanical responses were observed. At low temperatures, contracture was induced by addition of 1.0 mM caffeine to Ringer's solution containing Mn. As shown in Fig. 9 (B), caffeine-RCCs could be repeatedly observed without any inhibitory effect of Mn. No noticeable effects of Mn on electrical properties were observed in the T-tubules disrupted muscles as well (Fig. 10). After disruption of T-tubules (HOWELL and JENDEN, 1967), caffeine-RCC was observed both in the presence and absence of 10 mM-Mn.

CHIARANDINI and STEFANI (1973) showed that caffeine contracture and supramaximal potassium contractures were unaffected by Mn and that Mn did not impair contractile protein and function of the sarcoplasmic reticulum.

6) Electron microscopic observations

The effect of Mn on the ultrastructure of muscle fibres was carefully examined by electron microscopic studies. Even in the fibres treated with Mn, no clear structural alterations of contractile element, sarcoplasmic reticulum and mitochondria were observed as shown in Fig. 12. The enlargement of the diameters of T-tubules and extraordinary change in distances at the triadic junction were not observed in the fibres soaked in Mn Ringer's solution. The bridge-like structure at the triadic junction which is observed clearly in the normal muscle fibres (Fig. 11) did not disappear even in the muscle fibres treated with Mn, either.

DISCUSSION

Recently, two excellent papers concerning the effects of Mn on electrical and mechanical properties of frog skeletal muscle fibres have been published (OOTA et al., 1972; CHIARANDINI and STEFANI, 1973). The effects of Mn may be explained by assuming that it acts on the mechanism of excitation-contraction coupling, though the sites of Mn action are still unknown.

The minimum potassium concentration needed for producing potassium contracture increased from 20 to 40 mM, with the addition of Mn. In the presence of Mn, the mechanical threshold potential needed to induce potassium contracture was shifted from −44 mV to −33 mV and the electric current needed to elicit the action potential was increased 2.6 times compared with that in Ringer's solution containing 10 mM-Mn. These results agree with the data
Fig. 11. Normal ultrastructure of M. extensor longus digiti IV. Longitudinal section. T-tubules, lateral cisternae and bridge-like structures are clearly observed. Glycogen particles appear as black particles. Calibration bar: 0.5 μm.

Fig. 12. Longitudinal section of the fast muscle fibres treated with Mn in E.M. No significant changes of the shapes of lateral cisternae and bridge-like structures are observed as compared with normal muscle fibres. T-tubules are not swollen significantly. Calibration bar: 0.5 μm.
reported by ORKAND (1962) and MATSUMURA (1972) that Mn increases the mechanical threshold potential in crayfish muscles.

According to FATT and GINSBORG (1958), FRANK (1960), JENDEN and REGER (1963) and HAGIWARA and NAKAJIMA (1966), Mn may act on the sites of surface membranes occupied by Ca to block Ca permeability. Regarding mechanical responses of skeletal muscles produced by depolarization, it has been considered that the influx of membrane-bound Ca or extracellular Ca may occur in the early stages of E-C coupling (BIANCHI and SHANES, 1959; FRANK, 1960; LÓRKOVIĆ, 1962; BIANCHI and BOLTON, 1967). The above-mentioned Ca ions promote the release of bound Ca of the sarcoplasmic reticulum to activate the contractile elements. CHIARANDINI and STEFANI (1973) suggested that the stabilizing action of Mn on muscle membranes might be explained by the following two hypotheses:

1. As a result of adsorption of Mn to walls of the T-system and muscle surfaces in competition with Ca, a shift of the mechanical threshold followed.
2. Mn reduces or blocks permeability of Ca through the surface membranes.

In the case of semitendinosus and toe muscles, the inhibitory effect of Mn on potassium contracture was rapidly reversed when Mn was removed at the falling phase of potassium contracture. As shown in Figs. 3, 4, and 5, tension development could be produced immediately after Mn was removed. It has also been reported by CHIARANDINI and STEFANI (1973) that the inhibitory effect of Mn on twitch is rapidly reversed when Mn is washed out. Therefore, it seems that Mn rapidly adsorbs to and is released from the surface membranes.

As illustrated in Figs. 9 and 10, however, the caffeine-RCC was not influenced by the addition of Mn to normal Ringer’s solution. The mechanism of caffeine-RCC has been considered as being due to the Ca-release from lateral sacs. Since it has already been reported that Mn does not impair caffeine contracture at room temperatures (RUBIO and SPERELAKIS, 1972; CHIARANDINI and STEFANI, 1973), the obtained results of caffeine-RCC in the presence of Mn indicate that Mn can not alter the contractile proteins, and that Ca ions may be released from the sarcoplasmic reticulum without any restriction by the Mn effect. In addition, NATORI and ISHII (1972) observed no direct effect of Mn on contractility of myofibrils in the skinned muscle fibres. No morphological alterations of triadic junction and contractile elements in our experiments will give further support to the above concept.

It has been discussed that the mechanical threshold potential is changed by the action of Mn adsorbed to the surface membranes of muscle cells, especially to the transverse tubular system. Therefore, tension development produced by Mn removal during the falling phase of the potassium contracture may be accounted for by the change in mechanical threshold.

However, it should be noted that even in the inactivation process of potassium contracture, a remarkable tension development was produced by the removal of
Mn which was made before the completion of spontaneous relaxation (Fig. 4). This phenomenon can also be explained by the mechanical threshold change induced by Mn removal. Mechanical threshold change induced by Mn may be explained in two different ways. One is that, in Ringer's solution containing Mn, depolarization of the transverse tubular system is suppressed and the "trigger Ca" can not be released adequately, while the other is that the release of the "trigger Ca" is inhibited by some unknown mechanisms even though the transverse tubular system was depolarized sufficiently to release the "trigger Ca". When the test solution was free of Ca ions at the time of Mn removal, tension development was still observed.

Furthermore, when 50 mM-K, 10 mM-Mn Ringer's solution at room temperature was replaced with 50 mM-K, Ca-free Ringer's solution at low temperature, tension development produced by Mn removal was enhanced (Fig. 8(C)). Since the enhanced release of Ca from the sarcoplasmic reticulum in skeletal muscle could be induced by lowering the temperature (WEBER and HERZ, 1968; OGAWA, 1970; NISHIJIMA et al., 1972; MATSUBARA and SAKAI, 1973), tension development produced by Mn removal might be due to the release of bound Ca in the internal membranes.

On the other hand, no tension development was produced when Ca was absent before Mn removal (Fig. 8(D)). Therefore, it should be assumed that the "trigger Ca" may have a close relation with a part of extracellular Ca in transverse tubules, or with an influx of membrane bound-Ca.

BIANCHI and BOLTON (1967), ENDO et al. (1970), and FORD and PODOLSKY (1972) have already proposed that Ca-release from transverse tubules during activation of skeletal muscle will accelerate a secondary release of Ca from the sarcoplasmic reticulum, but in the present study no information was obtained about the direct release of Ca from transverse tubules. The possibility that Mn acts on transverse tubules and inhibits the influx and the release of Ca should be taken into consideration.

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