EFFECT OF MANGANESE IONS ON EXCITATION-CONTRACTION COUPLING IN FROG SARTORIUS MUSCLE

Isao OOTA, Masa TAKAUJI, and Torao NAGAI

Department of Physiology, Sapporo Medical College, Sapporo, Japan

Summary Manganese ions are known to inhibit the calcium spike in crayfish and barnacle. In frog sartorius muscle, we found that Mn^{++} inhibits the peak tension of twitch, tetanus, and potassium contracture and also the rate of rise and relaxation of tension. The inhibiting effect of Mn^{++} increased with increasing concentration (1–10 mM). The magnitude of resting potential and the amplitude of action potential were not significantly affected, but the negative afterpotential was enhanced by 10 mM Mn^{++} . At this concentration the peak tension of twitch, tetanus, and potassium contracture was almost completely inhibited. In 10 mM Mn^{++} , the steep initial rise of tension in caffeine contracture was abolished, but the subsequent slow rise in tension was not affected. The peak tension was reduced by about 30%. Mn^{++} actually activated the Mg^{++} -activated ATPase of myofibrils from rabbit skeletal muscle and decreased the extra Ca-induced splitting of ATP by the sarcoplasmic reticulum. These results indicate that manganese ions inhibit excitation-contraction coupling by acting on the transverse tubular system and possibly terminal cisternae of the sarcoplasmic reticulum.

Recently, specific action of manganese ions on certain muscles has been noticed. FATT and GINSBORG (1958), and HAGIWARA and NAKAJIMA (1966) reported that manganese ions inhibit the calcium spike in the muscles of crayfish and barnacle. On the other hand, FRANK (1960) reported that manganese ions can be substituted for external calcium ions in frog skeletal muscle, and JENDEN and REGER (1963) showed that manganese ions in low concentration prevent the deterioration of membrane potential of frog skeletal muscle in calcium-free solution. In these reports, however, the effect of manganese ions on the excitation-contraction coupling in this muscle were not studied in detail.

Contraction and relaxation in skeletal muscle are generally considered to be caused by calcium release and uptake by the sarcoplasmic reticulum. However,

Received for publication February 3, 1972

太田 勲, 高氏 昌, 永井寅男

the detailed mechanisms of these phenomena have not yet been satisfactorily clarified.

In the present paper, the effect of manganese ions on the excitation-contraction coupling in frog sartorius muscle is investigated.

A summary on this work was presented at the U. S.-Japan seminar in Tokyo (November 1-3, 1971).

MATERIALS AND METHODS

Whole sartorius muscles of frog, *Rana japonica*, were used. Frogs were supplied from a commercial source and were maintained in a breeding room (7°C) at Sapporo Medical College. All experiments were performed between April and October, 1971.

Paired sartorius muscles were dissected by splitting the pelvic bone without damaging the tendinous attachment. Muscles were mounted horizontally in a rectangular bath of 7 ml capacity. The pelvic bone was fixed to a hook and the distal tendon was attached by means of a silk thread to a resistance wire strain gauge (Nihon Kohden, SB-1T). The mechanical responses were recorded with an ink-writing oscillograph (Nihon Kohden, WI-180). In the experiments on twitch and tetanus, muscles were stimulated supramaximally with rectangular pulses of 0.5 msec duration through massive platinum electrodes located 7 mm apart. Contractures were produced by replacing a conventional solution with a contracture solution. When the effects of various agents were to be examined, muscles were kept for 30 min in the solution containing these agents. Resting tension was 1 g in all experiments. In order to minimize individual differences among muscles, we omitted those muscles which developed extremely large or small twitch tension.

Resting and action potentials were recorded with intracellular glass microelectrodes filled with 3 M KCl and having a resistance of about 10 MΩ.

Normal Ringer's solution of the following composition (in mM) was used: NaCl, 105; KCl, 2.5; CaCl₂, 1.8; Tris, 5; adjusted with HCl between pH 7.0 and 7.2. Except for the absence of CaCl₂, the composition of Ca-free solution was identical with normal Ringer's solution. Potassium Ringer's solution was prepared by replacing all NaCl in normal Ringer's solution with isosmotic KCl. All experiments were performed at room temperature (18°-24°C).

Myofibrils and microsomes from rabbit muscle were prepared according to the procedure followed by NAGAI *et al.* (1960). Myofibrils were glycerinated and thymol treated to eliminate ATP splitting by the sarcoplasmic reticulum. For determination of ATPase activity of these proteins, inorganic phosphate was measured according to the method of FISKE and SUBBAROW (1925). The concentration of protein was determined by the biuret reaction as calibrated by nitrogen determination.

The crystalline disodium salt of ATP was purchased from Sigma Chemical Co. Other chemicals were commercial products of the best reagent grade available.

RESULTS

1. Effect of Mn^{++} on twitch and tetanus

Figure 1 shows the effect of Mn^{++} on twitch tension of the muscles which were immersed for 30 min in Ringer's solution containing various concentrations of Mn^{++} . The tension fell to about 40% of control tension at 3 mM Mn^{++} , and to about 10% at 5 mM Mn^{++} . The rate of rise and relaxation of tension decreased even at 1 mM Mn^{++} , and became marked with the increase in the concentration of Mn^{++} .

Fig. 1. Effect of Mn^{++} on twitch tension. A: twitch in normal Ringer's solution. B-F: twitches recorded 30 min after immersing sartorius muscles in solutions with various concentrations of Mn^{++} . The Mn^{++} concentration was 1 mM in B, 3 mM in C, 5 mM in D, 7 mM in E, and 10 mM in F.

The time course of decrease of twitch tension in the presence of Mn^{++} and its recovery after removal of Mn^{++} are demonstrated in Fig. 2. At 1 mM Mn^{++} , peak tension was almost unchanged. At 3 mM Mn^{++} , tension fell to about 40% of control tension after 10 min, and the new value was maintained during exposure of muscle to the solution. At 5 mM Mn^{++} , tension was reduced to about 10% after 5 min and remained unchanged. It required only a few minutes to reach complete inhibition at the higher Mn^{++} concentrations of 7 and 10 mM. The recovery of twitch tension after removal of Mn^{++} was observed within 20 min at lower Mn^{++} concentrations. At higher Mn^{++} concentrations, it took more than 30 min to recover control twitch tension. The inhibiting effect of Mn^{++} was completely reversible.

Experiments were performed to determine whether the inhibiting effect of

Fig. 2. Time course of decrease and recovery of twitch tension of sartorius muscle. The muscle was immersed in solution with Mn^{++} for the first 30 min and then in solution without Mn^{++} . $\odot$, 1 mM; $\bullet$, 3 mM; $\otimes$, 5 mM; $\bullet$, 7 mM; $\circ$, 10 mM.

Fig. 3. Effect of Mn^{++} on twitch tension in the presence of high concentrations of external Ca^{++} . The Ca^{++} concentration was 5 mM in A and 10 mM in B. a: twitch in normal Ringer's solution. b-d: twitches recorded 30 sec, 1 min, and 3 min after immersing muscle in solution with 10 mM Mn^{++} .

Mn^{++} on twitch was removed by a high concentration of external Ca^{++} . We found that concentration of 5 and 10 mM external Ca^{++} were ineffective (Fig. 3).

Tetanic responses were elicited with stimuli given at 100 Hz. The tetanus disappeared within a few minutes after immersion of muscle in a solution containing 10 mM Mn^{++} , but it recovered completely when the muscle was reimmersed in normal Ringer's solution. The effect of Mn^{++} on the rate of rise and relaxation of tetanus tension was similar to that in the case of twitch.

2. Effect of Mn^{++} on potassium contracture

The effect of various concentrations of Mn^{++} on potassium contracture is shown in Fig. 4. Peak tension was reduced to about 75% of control tension by

Fig. 4. Effect of Mn^{++} on potassium contracture. A: contracture in normal potassium Ringer's solution. B-H: contractures recorded 30 min after immersing muscle in solution with various concentrations of Mn^{++} . The Mn^{++} concentration was 1 mM in B, 3 mM in C, 5 mM in D, 7 mM in E, 10 mM in F, 15 mM in G, and 20 mM in H.

even such a low concentration as 1 mM Mn^{++} . At 3 mM Mn^{++} the tension was reduced to 50% and at 10 mM Mn^{++} to below 10%. Complete inhibition was observed at 20 mM. Moreover, it was clearly observed that the rate of rise and relaxation of tension of the contracture decreased markedly.

Figure 5 shows that the peak tensions of both twitch and potassium contracture were inhibited by Mn^{++} . However, it is noticeable that these two curves did not coincide. The curve of potassium contracture was shifted to left at concentrations lower than about 3 mM Mn^{++} and to right at the higher concentrations. This behavior at lower concentration of Mn^{++} means that the potassium contracture was more easily inhibited by Mn^{++} than the twitch.

FRANK (1960) and LÜTTGAU (1963) reported that potassium contracture is eliminated by soaking the muscle in a Ca-free solution and that divalent cations can be substituted for Ca^{++} . Results similar to theirs were obtained in our experiment; the peak tension of potassium contracture was largely decreased in Ca-

Fig. 5. Dependence of twitch and potassium contracture tensions on Mn^{++} concentration. ○, twitch; ●, potassium contracture.

free solution, and the decreased contracture was partially recovered by exposing the muscle to a solution containing 1.8 mM Mn^{++} (Fig. 6, II, B, C). However, elevation of the Mn^{++} concentration from 1.8 to 10 mM markedly inhibited the peak tension and the rate of rise and relaxation of tension in a way similar to the inhibition in the presence of 1.8 mM Ca^{++} (Fig. 6, II, D). In contrast, 10 mM Ca^{++} did not affect the peak tension at all (Fig. 6, II, E).

Several experiments were done to compare the effects of Mn^{++} with those of tetrodotoxin (TTX) and procaine on twitch and potassium contracture (Fig. 7). TTX abolishes the Na spike in frog skeletal muscle fibers (FURUKAWA *et al.*, 1959; NARAHASHI *et al.*, 1960; NAKAJIMA *et al.*, 1962). Procaine also suppresses the Na spike (TAYLOR *et al.*, 1959). In our experiments, twitch responses disappeared within 30 sec in a solution with 2×10^{-6} g/ml TTX and within 1 min in a solution with 10 mM procaine. The peak tension and the shape of potassium contracture induced in a solution containing TTX were the same as those in normal potassium Ringer's solution. This result is in good agreement with the fact that potassium contracture is not influenced in choline Ringer's solution (FRANK, 1960). In the case of procaine, peak tension was decreased but the rate of rise and relaxation of tension remained almost unchanged. This result is similar to that of FEINSTEIN (1963) and indicates that the effect of procaine on potassium contracture differs from that of Mn^{++} .

3. Effect of Mn^{++} on caffeine contracture

In caffeine contracture (Fig. 8 A), the rate of initial tension development was markedly decreased by 10 mM Mn^{++} (Fig. 8 B). The peak tension was reduced to about 70% of control tension. Furthermore, it is interesting that the inhibiting

Fig. 6. Effects of Mn^{++} on potassium contractures at various concentrations of external Ca^{++} . I, II, and III indicate that potassium contractures were recorded 30 min, 60 min, and 90 min after immersion of muscle in solution recorded below each record. A: control. B: Ca-free. C: Ca-free + 1.8 mM Mn^{++} . D: Ca-free + 10 mM Mn^{++} . E: 10 mM Ca^{++} .

Fig. 7. Effects of TTX and procaine on twitch and potassium contracture. A, A': TTX (2×10^{-6} g/ml). B, B': procaine (10 mM). a, a': twitch and potassium contracture in normal Ringer's solution. b, c: twitches recorded 30 sec and 1 min after immersion of muscle in solution with TTX or procaine. b': contractures recorded 30 min after immersion of muscle in solution containing TTX or procaine.

effect of Mn^{++} on caffeine contracture was almost completely absent when the muscle was fully depolarized by a high concentration of K^+ (Fig. 8 C). In addition, it is demonstrated that the contracture induced by 5 mM caffeine and those induced after depolarization with K^+ were completely inhibited by 10 mM procaine (Fig. 8 D).

Fig. 8. Effect of Mn^{++} on caffeine contracture. A: contracture induced by 5 mM caffeine in normal Ringer's solution. B, C, D: contractures recorded 30 min after immersion of muscle in solution with 10 mM Mn^{++} (B), 10 mM Mn^{++} and 105 mM K^+ (C), or 10 mM procaine and 105 mM K^+ (D).

4. Effect of Mn^{++} on resting and action potentials

Resting and action potentials recorded from a fiber immersed into normal Ringer's and 10 mM Mn^{++} -containing solution are shown in Fig. 9. The magnitude of the resting potential and the amplitude of the action potential in Mn^{++} -treated fibers were similar to those in the control (Table 1). However, the negative afterpotential was considerably greater in presence of Mn^{++} : 14.29 ± 0.72 in control and 34.11 ± 1.34 in Mn^{++} -treated fibers. This enhancement of negative afterpotential was observed a few minutes after the fiber was immersed in 10 mM Mn^{++} -containing solution and was maintained for the entire time of immersion.

When muscle was exposed to potassium Ringer's solution, the membrane potential fell to about -20 to -10 mV. The reduction was not altered by 10 mM Mn^{++} .

Fig. 9. Effect of Mn^{++} on action potential in a representative experiment. A: action potential in normal Ringer's solution. B: action potential recorded 30 min after immersion of muscle in solution containing 10 mM Mn^{++} .

Table 1. Effect of Mn^{++} on magnitude of resting potential and amplitudes of action potential and negative afterpotential. Results are given as the mean $\pm$ S. E. of mean. The number of fiber penetrations is in parentheses.

	Normal fiber	Mn^{++} -treated fiber
Resting potential (mV)	80.37 ± 1.13 (35)	78.71 ± 1.09 (35)
Action potential (mV)	103.71 ± 2.07 (35)	100.86 ± 1.92 (35)
Negative afterpotential (mV)	14.29 ± 0.72 (35)	34.11 ± 1.34 (35)

5. Effect of Mn^{++} on isolated myofibrils and microsomes

The effect of Mn^{++} on contractile proteins was also examined by using myofibrils isolated from rabbit skeletal muscle. Mg^{++} -activated ATPase activity of the myofibrils was increased by Mn^{++} in a concentration-dependent manner (Fig. 10). This is compatible with our previous result that Mn^{++} does not inhibit the ATP-induced tension development of glycerol-extracted muscle fiber (NAGAI

Fig. 10. Dependence of ATPase activity of rabbit skeletal myofibrils on Mn^{++} concentration. Conditions: 50 mM Tris-acetate buffer (pH 7.0), 64 mM K^+ , 1 mM $MgCl_2$, 1 mM ATP, and 1 mg of myofibrils/ml. Reaction time, 2 min. Temp., 22°C.

Fig. 11. Effect of Mn^{++} on ATPase activity of rabbit microsomes in the presence of oxalate. Conditions: 66.7 mM Tris-acetate buffer (pH 7.0), 68 mM K^+ , 2 mM $MgCl_2$, 2 mM ATP, 2 mM oxalate, 0.2 mg protein/ml. Total volume of reaction mixture, 2 ml. Temp., 20°C. ○, ●: without $MnCl_2$. △, ▲: with 10 mM $MnCl_2$. Arrow indicates addition of calcium (0.2 mM).

et al., 1965). According to HASSELBACH and MAKINOSE (1961), in the presence of oxalate the extra splitting of ATP by isolated sarcoplasmic reticulum (microsomes) after addition of Ca^{++} is correlated with Ca uptake. We studied the effect of Mn^{++} on microsomal ATPase activity (Fig. 11). Ten mM Mn^{++} increased the basic ATPase activity and completely inhibited the extra splitting of ATP by addition of Ca^{++} . The inhibition was dependent on the Mn^{++} concentration.

DISCUSSION

It is currently held that Mn^{++} acts mainly on the surface membrane of muscle fibers, as seen in the findings of FATT and GINSBORG (1958), FRANK (1960), JENDEN and REGER (1963), and HAGIWARA and NAKAJIMA (1966). However, our results suggest that Mn^{++} acts on internal membranes including the transverse tubular system (T-system) and terminal cisternae of sarcoplasmic reticulum.

We found that Mn^{++} inhibits twitch, tetanus, and potassium contracture and caffeine-induced contracture (Figs. 1, 4, and 8). All these effects of Mn^{++} were completely reversible when Mn^{++} was removed and replaced by normal Ringer solution (Figs. 2 and 6). Since resting and action potentials (Fig. 9) and the extent of depolarization by K^+ were not affected by Mn^{++} , it appears that the inhibition of these mechanical responses by Mn^{++} is not due simply to a change of these membrane potentials. Since Mn^{++} did not inhibit the ATPase activity of isolated myofibrils (Fig. 10), it does not appear to influence the contractile proteins. These findings suggest that Mn^{++} acts on the excitation-contraction coupling process in frog sartorius muscle and in this way inhibits the mechanical responses. However, this excitation-contraction uncoupling by Mn^{++} differs from that induced by hypertonic solution (HODGKIN and HOROWICZ, 1957), because caffeine contracture normally occurs in frog sartorius muscle treated with hypertonic solution (MATSU-MURA, 1959; CAPUTO, 1966).

It has been considered that the excitation-contraction coupling is closely related to internal membranes, including the T-system and terminal cisternae. As shown in Fig. 9, Mn^{++} considerably enhanced the negative afterpotential. Evidence that the negative afterpotential can be attributed to the presence of a T-system in frog skeletal muscle has been presented by EISENBERG and GAGE (1969) and GAGE and EISENBERG (1969). Therefore, it is suggested that Mn^{++} influences the T-system. In addition, a longer time is required for recovery of twitch after removal of Mn^{++} than for the appearance of the inhibiting action of Mn^{++} . On the basis of the fact that K^+ depolarization required a longer time than repolarization, HODGKIN and HOROWICZ (1960) suggested that K^+ acts on the internal membrane of single muscle fibers. According to GAGE and EISENBERG (1969), the negative afterpotential disappears in T-disrupted muscle. In contrast, Mn^{++} enhanced the negative afterpotential (Fig. 9 and Table 1). It is, therefore, considered that ultrastructural changes in the T-system may occur after treatment with Mn^{++} . Evidence that caffeine contracture is induced as a result of direct action of the drug on terminal cisternae in T-disrupted muscle has been presented by HOWELL (1969). As mentioned above, caffeine contracture is inhibited by Mn^{++} in contrast to the case of excitation-contraction uncoupling by hypertonic solution. These facts strongly suggest that Mn^{++} also acts on terminal cisternae of the sarcoplasmic reticulum.

The mechanism of the action of Mn^{++} on the T-system and terminal cisternae

has not yet been clarified in detail. It is well known that frog skeletal muscle fiber is one of the most typical excitable cells having a Na spike (NASTUK and HODGKIN, 1950). TTX, a special inhibitor of Na spike, abolished the twitch (Fig. 7). On the other hand, the action of Mn^{++} clearly differs from that of TTX. Mn^{++} inhibited twitch without changing the rate of rise and amplitude of action potential. Since 10 mM procaine inhibited not only twitch but also potassium and caffeine contractures (Fig. 7), the action of procaine seems to resemble that of Mn^{++} . However, procaine, but not Mn^{++} , had almost no effect on the rate of rise and relaxation of tension of potassium contracture. Procaine also affected caffeine contracture differently. Caffeine contracture in potassium-depolarized muscle was almost uninfluenced by Mn^{++} but completely inhibited by procaine (Fig. 8). From these results, it is considered that the mechanism of action of Mn^{++} is distinctly different from that of either procaine or TTX.

In agreement with the report of FRANK (1960), 1.8 mM Mn^{++} , which is equal to the concentration of Ca^{++} in normal Ringer's solution, can be partially substituted for Ca^{++} in the case of potassium contracture (Fig. 6). However, under Ca-free conditions, 10 mM Mn^{++} markedly inhibited potassium contracture (Fig. 6). Therefore, we cannot support Frank's conclusion that Mn^{++} simply substitutes for external Ca^{++} .

According to FRANK (1960), and BIANCHI and BOLTON (1967), the influx of membrane-bound Ca or extracellular Ca^{++} triggers release of Ca^{++} from terminal cisternae in the case of twitch and potassium contracture. HOWELL (1969) reported that twitch and potassium contracture are inhibited in T-disrupted muscle. These findings suggest that the trigger Ca^{++} enters into the fiber through the T-system. On the other hand, caffeine contracture was not inhibited in T-disrupted muscle (HOWELL, 1969). This fact supports the concept of BIANCHI (1962) that caffeine penetrates the fiber and activates the Ca^{++} release, and it also suggests that the T-system is not required for penetration of caffeine into the fiber. Our data suggests that Mn^{++} acts primarily on the T-system and perhaps terminal cisternae to suppress the influx of trigger Ca or the release of Ca. Another possible site of Mn^{++} action is the junction between the T-system and cisternae. The mechanism of transmission of excitation across this junction is not yet known. Mn^{++} activated the basic ATPase activity of microsomes and inhibited the extra splitting of ATP (Fig. 11). Although these results are regarded as evidence that Mn^{++} acts on terminal cisternae, this problem should be clarified by further studying the effect of Mn^{++} on Ca-release from microsomes by caffeine.

As shown in Fig. 8, inhibition of caffeine contracture by Mn^{++} was not observed in K^+ depolarized muscle. Although this interesting fact has not been clearly explained, two possibilities can be considered. One is that caffeine contracture is potentiated by K^+ (MATSUSHIMA *et al.*, 1962; CAPUTO, 1966). The other is that K^+ antagonizes the action of Mn^{++} on terminal cisternae. However, the fact that potassium contracture was inhibited by Mn^{++} (Fig. 4) contradicts

the latter possibility. Consequently, we consider that the site of action of Mn^{++} in potassium contracture is probably mainly on the T-system. Taking into consideration the fact that membrane potentials were almost unaffected by Mn^{++} (Fig. 9), it is suggested that electrical properties of the surface membrane differ from those of the membranes of the T-system.

On the basis of our results and conclusions mentioned above, it seems that the internal membrane system of frog sartorius muscle is functionally similar to the surface membrane of crayfish and barnacle muscles. However, initiation of the Ca spike is competitively inhibited by Mn^{++} in barnacle muscle (HAGIWARA and NAKAJIMA, 1966), whereas inhibition of twitch and tetanus in frog sartorius muscle by Mn^{++} are not blocked by elevating the concentration of external Ca^{++} (Fig. 3). Therefore, it is considered that there is a difference in the mechanism of action of Mn^{++} between these muscles.

REFERENCES

- BIANCHI, C. P. (1962) Kinetics of radiocaffeine uptake and release in frog sartorius. *J. Pharmacol. Exp. Ther.*, **138**: 41–47.
- BIANCHI, C. P. and BOLTON, T. C. (1967) Action of local anesthetics on coupling systems in muscle. *J. Pharmacol. Exp. Ther.*, **157**: 388–405.
- CAPUTO, C. (1966) Caffeine- and potassium-induced contractures of frog striated muscle fibers in hypertonic solutions. *J. Gen. Physiol.*, **50**: 129–139.
- EISENBERG, R. S. and GAGE, P. W. (1969) Ionic conductances of the surface and transverse tubular membranes of frog sartorius fibers. *J. Gen. Physiol.*, **53**: 279–297.
- FATT, P. and GINSBORG, B. L. (1958) The ionic requirements for the production of action potentials in crustacean muscle fibres. *J. Physiol.*, **142**: 516–543.
- FEINSTEIN, M. B. (1963) Inhibition of caffeine rigor and radiocalcium movements by local anesthetics in frog sartorius muscle. *J. Gen. Physiol.*, **47**: 151–172.
- FISKE, C. H. and SUBBAROW, Y. (1925) The colorimetric determination of phosphorus. *J. Biol. Chem.*, **66**: 375–628.
- FRANK, G. B. (1960) Effects of changes in extracellular calcium concentration in the potassium-induced contracture of frog's skeletal muscle. *J. Physiol.*, **151**: 518–538.
- FURUKAWA, T., SASAOKA, T., and HOSOYA, Y. (1959) Effects of tetrodotoxin on the neuromuscular junction. *Jap. J. Physiol.*, **9**: 143–152.
- GAGE, P. W. and EISENBERG, R. S. (1969) Action potentials, afterpotentials, and excitation-contraction coupling in frog sartorius fibers without transverse tubules. *J. Gen. Physiol.*, **53**: 298–310.
- HAGIWARA, S. and NAKAJIMA, S. (1966) Differences in Na and Ca spike. As examined by application of tetrodotoxin, procaine, and manganese ions. *J. Gen. Physiol.*, **49**: 793–806.
- HASSELBACH, W. and MAKINOSE, M. (1961) Die Calciumpumpe der "Erschlaffungsgrana" des Muskels und ihre Abhängigkeit von der ATP-Spaltung. *Biochem. Z.*, **333**: 518–528.
- HODGKIN, A. L. and HOROWICZ, P. (1957) The differential action of hypertonic solutions on the twitch and action potential of a muscle fiber. *J. Physiol.*, **136**: 17P–18P.
- HODGKIN, A. L. and HOROWICZ, P. (1960) Potassium contractures in single muscle fibres. *J. Physiol.*, **153**: 386–403.
- HOWELL, J. N. (1969) A lesion of the transverse tubules of skeletal muscle. *J. Physiol.*, **201**: 515–533.

- JENDEN, D. J. and REGER, J. F. (1963) The role of resting potential changes in the contractile failure of frog sartorius muscles during calcium deprivation. *J. Physiol.*, **169**: 889-901.
- LÜTTGAU, H. C. (1963) The action of calcium ions on potassium contractures of single muscle fibres. *J. Physiol.*, **168**: 679-697.
- MATSUMURA, M. (1959) The mechanism of caffeine contracture. *Juntendo Med. J.*, **5**: 265-268 (in Japanese).
- MATSUSHIMA, T., FUJINO, M., and NAGAI, T. (1962) Effects of anomalous anions on the caffeine contracture. *Jap. J. Physiol.*, **12**: 106-112.
- NAGAI, T., MAKINOSE, M., and HASSELBACH, W. (1960) Der physiologische Erschlaffungsfaktor und die Muskelgrana. *Biochem. Biophys. Acta*, **43**: 233-238.
- NAGAI, T., TAKAHASHI, H., and TAKAUJI, M. (1965) On the accumulation of divalent cations by skeletal muscular microsomes. In *Molecular Biology of Muscular Contraction* (BBA Library, Vol. 9), ed. by EBASHI, S., OOSAWA, F., SEKINE, T., and TONOMURA, Y. Igaku Shoin, Tokyo, and Elsevier Publ. Co., Amsterdam, pp. 169-176.
- NAKAJIMA, S., IWASAKI, S., and OBATA, K. (1962) Delayed rectification and anomalous rectification in frog's skeletal muscle membrane. *J. Gen. Physiol.*, **46**: 97-115.
- NARAHASHI, T., DEGUCHI, T., URAKAWA, N., and OHKUBO, Y. (1960) Stabilization and rectification of muscle fiber membrane by tetrodotoxin. *Am. J. Physiol.*, **198**: 934-938.
- NASTUK, W. L. and HODGKIN, A. L. (1950) The electrical activity of single muscle fibers. *J. Cell. and Comp. Physiol.*, **35**: 39-73.
- TAYLOR, R. E. (1959) Effect of procaine on electrical properties of squid axon membrane. *Am. J. Physiol.*, **196**: 1071-1078.