Transient Tension Development Induced by Silver Ion in Ca\(^{2+}\)-channel Blocked Skeletal Muscle Fibers

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Summary Ag\(^{+}\) produced two different types of transient tension development in single frog toe muscle fibers in which the Ca\(^{2+}\) channel had been blocked by pretreatment with 2 mM Co\(^{2+}\). These contractions were never observed in detubulated fibers, indicating that the Ag\(^{+}\)-induced contraction is produced through Ca\(^{2+}\) channels located on the T-tubular membrane.

Key Words: Ag\(^{+}\)-induced tension, Ca\(^{2+}\) channel, T-tubular membrane.

Depolarization of the T-tubular membrane following excitation of sarcolemma leads to a release of Ca\(^{2+}\) stored in the sarcoplasmic reticulum (SR) and muscle contraction is initiated. The mechanism involved in signal transmission from the T-tubular membrane to the SR is poorly understood, although there are several hypotheses in existence (for review see Winegrad, 1982). Abramson and his colleagues (Abramson et al., 1983) presented attractive data showing that release of Ca\(^{2+}\) from the SR vesicles was initiated either by binding of heavy metal to the sulfhydryl groups or by oxidation of the sulfhydryl groups to form disulfides. This evidence suggests the possible occurrence of chemical reaction(s) on the T-tubular membrane during activation of fibers. To assess the possible occurrence of such reaction in intact muscle fibers, we studied the binding of heavy metal ions to the sulfhydryl groups on the T-tubular membrane and the effect on the contractile behaviour of intact fibers.

Single twitch fibers were isolated from the toe muscle of bull-frog (Rana catesbeiana, m. flexor brevis digit I) in Ringer's solution (pH 7.0) composed of NaCl (115 mM), KCl (2.5 mM), Na\(_2\)HPO\(_4\) (2.15 mM), NaH\(_2\)PO\(_4\) (0.85 mM), CaCl\(_2\) (1.8 mM) and left to equilibrate for at least 20 min. The intact fiber was mounted in a glass-bottomed chamber through which the tissue was exposed to a He-Ne laser to adjust the sarcomere length to 2.6 \(\mu\)m, as described previously (Oba and Hotta, 1983a, b). Both tendons of the fiber were wrapped in small foil clips.

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One end was clipped to the glass chamber and the other to a semiconductor transducer (Toyota, SP-5-12). An intact single fiber was stimulated along its entire length with a pair of Pt wires (1 msec duration) to elicit the twitch tension. The silver ion, a potent inhibitor of SH groups, was used to modify the SH groups on the membrane. Ag⁺ binds with Cl⁻ forming precipitates of AgCl. Therefore, we replaced the usual phosphate buffer with Cl⁻-free MOPS buffer (NaNO₃, 115 mM; KNO₃, 2.5 mM; MOPS, 10 mM; pH 7.0) after mounting the tissue in a glass chamber. The contractile response was stored in the digital memory (Iwatsu, DM 305) and displayed on a conventional recorder. The experiments were performed at room temperature (22-24°C).

As shown in Fig. 1 a and b, the maximum twitch tension of a fiber immersed in 0-Ca²⁺ MOPS solution was about 2.7-fold that in normal Ringer’s solution (309.6±23.8 mg and 114.3±26.6 mg, mean±S.E.M., N=7, respectively). This is consistent with the results reported by other investigators who noted a potentiation of twitch tension by replacing Cl⁻ with NO₃⁻ (Sandow et al., 1965) and by

\[ \text{Fig. 1. The Ag⁺-induced tension development (d-iv and e) in frog toe muscle fiber with Ca²⁺ channels blocked by 2 mM Co(NO₃)₂ for 10 min. a, b, and c represent twitch tensions induced by an electrical stimulation (1 msec duration) in Ringer’s solution (d-i), 0-Ca²⁺ MOPS buffer (d-ii) and 2 mM Co(NO₃)₂, 0-Ca²⁺ MOPS buffer (d-iii), respectively. A rapid exchange of the 2 mM Co(NO₃)₂ solution to a 5 µM AgNO₃, 0-Ca²⁺ MOPS buffer caused two different types of tension development (d-iv). e represents the amplified figure of d-iv with expansion of the time scale. After reversion to resting tension, application of 0.5 mM external Ca²⁺ to the same fiber led to a development of irreversible tension (d-v).} \]
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Lowering the concentration or raising the temperature of the external medium (CAPUTO and GIMENETZ, 1967; FRANK, 1978). The maximum tension was not influenced by the presence of 2 mM Co²⁺ in the medium (Fig. 1 c, 283.6±14.3 mg, N=7).

After exposure of the fiber to 2 mM Co²⁺ (Ca²⁺-channel blocker, STEFANI and CHIARANDINI, 1982) for 10 min, a rapid exchange of the solution for 0-Ca²⁺ MOPS buffer containing 5 µM AgNO₃ led to spontaneous activation of the fiber and resulted in development of two different types of tension shown in Fig. 1 d and e; repetitive twitch-like tensions and the following single large tension development. The first appearance of tension occurred 1 to 2 sec after application of Ag⁺. The amplitude of maximum tension in the first of several contractions was almost the same as that of the normal twitch tension observed in phosphate Ringer’s solution. Thereafter, the maximum tension linearly decreased. The cessation of this tension was followed by a second development of tension which reached a peak at 12.4±0.6 sec (N=5) after onset of the tension.

The fiber did not respond to electrical stimulation during and after exposure to Ag⁺. The other heavy metal ion, Hg²⁺, reportedly causes an irreversible depolarization of muscle membrane (JUANG, 1976; MIYAMOTO, 1983). In the case of Ag⁺, depolarization occurred very slowly (10% per min or less); therefore, loss of electrical responsiveness and appearance of twitch-like contraction by treat-
ment of fiber with Ag⁺ could not be attributed to the depolarization of the mem-
brane. Our preliminary results demonstrate that Ag⁺ alone, even in high con-
centration (100 µM), never induces muscle contraction in a medium containing
very low Ca²⁺ (less than 0.1 mM). Therefore, it is unlikely that the binding of
Ag⁺ with the SH group itself on the T-tubular membrane, transmits information
to the SR membrane for the release of stored Ca²⁺ and that Ag⁺ enters the cell
to act directly on the SR membrane. After Ag⁺ exposure for at least 3 min, the
fiber contracted irreversibly in response to 0.5 mM external Ca²⁺ (Figs. 1d and
2e).

The fiber detubulated by 400 mM glycerol (EISENBERG et al., 1971) showed
quite different behaviour to Ag⁺ exposure (Fig. 2e). Following detubulation,
the fiber did not respond to 5 µM Ag⁺, while a large irreversible tension did develop
in 0.5 mM Ca²⁺ MOPS buffer (Fig. 2e-v). These results show that repetitive
twitch-like contractions and the following large development of tension are in-
duced through Ca²⁺ channels (SH group related) located on the T-tubular mem-
brane, since these phenomena occurred only in Ca²⁺-channel blocked, T-tubule
intact fibers.

The Ag⁺-induced tension development observed here leads to the hypothesis
that Ca²⁺ on the T-tubular membrane immobilized by Co²⁺ can be readily and
rapidly mobilized by replacement of Co²⁺ with Ag⁺ and that the information for
the release of Ca²⁺ by the SR can be transmitted from T-tubules to the SR by
opening the Ca²⁺ channels on the membrane. This hypothesis is supported from
acquired data that a decrease of bound Ca²⁺ on the T-tubular membrane results
in a decrease of the amplitude of the Ag⁺-induced contraction (in preparation).

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