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Recovery of Action Potentials and Twitches after K-contractures in Frog Skeletal Muscle

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ABSTRACT—To give information about intracellular Ca\(^{2+}\) translocation during and after K-contractures in vertebrate skeletal muscle fibers, we examined recovery of action potentials and Twitches after interruption and spontaneous relaxation of K-contractures at low temperature (3\(^\circ\)C) that greatly reduced the rate of Ca\(^{2+}\) reuptake by the sarcoplasmic reticulum. On membrane repolarization interrupting K-contractures, the amplitude of both action potentials and Twitches recovered quickly, while the falling phase of action potential was markedly slowed at first to prolong its refractory period, so that repetitive stimulation (20 Hz) did not produce a complete tetanus. Meanwhile, on membrane repolarization after spontaneous relaxation of K-contractures, the action potentials were markedly reduced in amplitude and prolonged in duration at first, also resulting in prolonged refractory period. These results are discussed in connection with Ca\(^{2+}\) absorption to the surface and transverse tubule membranes, producing changes in action potential kinetics.

Key words: frog skeletal muscle fiber, K-contracture, intracellular Ca\(^{2+}\) translocation, sarcoplasmic reticulum, transvers tubules

INTRODUCTION

When the surface membrane of vertebrate skeletal muscle fibers is depolarized above mechanical threshold by elevating external K ion concentration, the fibers develop K-contracture tension caused by the release of Ca\(^{2+}\) from the terminal cisternae (TC) of the sarcoplasmic reticulum (SR) (Ebashi and Endo, 1968). The K-contractures relax spontaneously in the high-K solution, and after repolarization of the fiber membrane the fibers restore their ability to contract in response to electrical stimulation and to develop K-contracture tension in response to membrane depolarization (Hodgkin and Horowicz, 1960). The spontaneous relaxation of K-contractures is thought to result from the Ca\(^{2+}\) reuptake by the SR component other than the TC, while the contractile repriming after membrane repolarization is explained to be due to the movement of Ca\(^{2+}\) back to the TC (Winegrad, 1965a, b; Suzuki et al., 1990). At room temperatures, the contractile repriming after K-contractures takes place in 60 s (Hodgkin and Horowicz, 1960), and only the amplitude of Twitches has been used to examine the time course of repriming (Hodgkin and Horowicz, 1960; Caputo, 1972a, b); it is difficult to study how recovery of action potential is related with the contractile repriming due to the rapid repriming process.

Caputo (1972a, b) has shown that, at low temperature, the rate of both the SR Ca\(^{2+}\) release and the SR Ca\(^{2+}\) reuptake are greatly slowed, so that both the duration of K-contractures and the rate of repriming process is markedly prolonged, providing an opportunity to study the processes underlying the spontaneous relaxation of K-contractures and the repriming after membrane repolarization in more detail. In the present experiments, we examined recovery of action potentials and Twitches after interruption and spontaneous relaxation of K-contractures at low temperature. The results obtained strongly suggest that, during K-contractures, the Ca\(^{2+}\) released from the SR may first be absorbed to the membrane of the transverse tubules (T-tubules) and then to the surface membrane, in addition to the Ca\(^{2+}\) binding to troponin C on the thin filament and to parvalbumin in the myoplasm.

MATERIALS AND METHODS

Single muscle fibers (diameter, 90–150 \(\mu\)m, slack length 7–11 mm) were isolated from the semitendinosus muscles of the frog...
Rana japonica, and mounted horizontally in an experimental chamber (2 ml) filled with the standard experimental solution containing (mM): 115 NaCl, 2.5 KCl, 1.8 CaCl\textsubscript{2} and 10 Tris-malate (pH 7.2). One end of the fiber was fixed in position, while the other end was connected to tension transducer (AE801, Senso-Nor) to record isometric tension. The fiber was kept at slack length, and was made to contract with the contracture solution containing (mM): 190 KCl, 1.8 CaCl\textsubscript{2} and 10 Tris-malate (pH 7.2). In some experiments Cl\textsuperscript{–} in the contracture solution was replaced by SO\textsubscript{4}\textsuperscript{2–} with similar results. Solutions in the chamber were exchanged with the water vacuum suction tube in 1s. The fiber was also stimulated to contract with single or repetitive supramaximal 1ms current pulses (20 Hz) given through a pair of Pt wire electrodes. Temperature of the solutions was kept at 3°C with a thermoelectric device.

For recording membrane potentials, conventional microelectrodes connected to a high impedance amplifier were used. To facilitate impalement of the fiber, a small piece of lucite was placed underneath the fiber region where the impalement was made. Tension changes were recorded with an ink-writing oscillograph, while membrane potential changes were recorded with an oscilloscope (type 5000, Tektronix). In each type of experiment described in this paper, 8–10 different muscle fibers were used with similar results.

RESULTS

Recovery of action potentials and twitches after interruption of K-contractures

At low temperature (3°C), the duration of K-contractures in the contracture solution with 190 mM-K was 60–70 s, being more than 10 times longer than that at room temperature (Caputo 1972a). In the present experiments, K-contractures were interrupted by suddenly returning the fiber to the standard solution with 2.5 mM-K at 25–30 s after the onset of contracture tension, so that the time integral of ten-

![Fig. 1. K-contractures in a single frog skeletal muscle fiber at 3°C. (A) Uninterrupted K-contracture. (B) The first K-contracture was interrupted at 20s after its onset, and the second K-contracture was produced after a period of 90 s.](https://bioone.org/journals/Zoological-Science)

![Fig. 2. Recovery of action potential after K-contracture interruption. (A) Control action potential. (B) Action potential at 2 min after contracture interruption. (C) Time course of recovery of action potential amplitude and half width. In Figs. 2 and 4, data points with vertical bar at the right indicates mean ± SD values of 10 control action potentials obtained from 10 different fibers.](https://bioone.org/journals/Zoological-Science)

![Fig. 3. Recovery of twitch and tetanus amplitude after K-contracture interruption. (A) Twitch and unfused tetanus in response to repetitive stimulation (20Hz) at 2 min after contracture interruption. (B) Twitch and fused tetanus at 10 min after contracture interruption. In both A and B, time of repetitive stimulation is shown at the bottom. (C) Time course of recovery of twitch and tetanus amplitude after contracture interruption.](https://bioone.org/journals/Zoological-Science)
Recovery of Action Potential and Twitch

The tension was about 50% of that in the uninterrupted contracture (Fig. 1). On returning the fiber to the standard solution, the contracture tension relaxed in a few seconds and the resting membrane potential was quickly repolarized to 80–100 mV in 1–2 min.

Since repeated insertion of microelectrodes without giving damage to the fiber was very difficult, we recorded action potentials only up to 4 times from one and the same fiber during the early stage of recovery after the interruption of K-contracture. At 2 min after the contracture interruption, the action potentials almost recovered its normal amplitude, while their falling phase was greatly slowed (Fig. 2A, B). The action potential recovered its normal time course in 8–10 min (Fig. 2C).

On the other hand, the amplitude of isometric twitch was about 80–90% of the normal value at 2–4 min after the contracture interruption. And their relaxation phase was not exponential in shape, but convex upwards (Fig. 3A). Due to the prolonged refractory period of action potential resulting from its prolonged falling phase, repetitive stimulation (20 Hz) could produce action potentials only at intervals of 150 ms, i.e., one action potential was produced for each series of 3 current pulses. As the result, twitches were also produced at intervals of 150 ms, only building up an unfused tetanus in the early stage of recovery (Fig. 3A). A complete tetanus with the normal amplitude was obtained at 10–15 min after the interruption of K-contracture (Fig. 3B). A typical time course of recovery of twitch and tetanus amplitude is shown in Fig. 3C.

**Recovery of action potentials and twitches after spontaneous relaxation of K-contractures**

When the fibers were returned to the standard solution after the completion of spontaneous relaxation in the contracture solution, the resting membrane potential was also repolarized to the normal value of 80–100 mV in 1–2 min, while the action potentials were at first markedly reduced in amplitude and prolonged in duration (Fig. 4A), and slowly recovered its normal amplitude and duration in 60 min (Fig. 4B).

Meanwhile, no detectable twitches were produced for the first 2–3 min after returning the relaxed fiber to the standard solution, and twitches produced after this period of mechanical refractoriness were at first markedly reduced in amplitude and shortened in duration, and repetitive stimulation produced only an unfused tetanus with markedly reduced amplitude (Fig. 5A), due also to the prolonged refractory period of action potential. Both twitches and tetani slowly recovered their normal amplitude and time course in 60 min (Fig. 5B, C, D).

**Fig. 4.** Early stage of recovery of action potential after spontaneous relaxation of K-contracture. (A) Action potential at 2 min after returning the relaxed fiber to the standard solution. (B) Time course of recovery of action potential amplitude and half width.

**Fig. 5.** Recovery of twitch and tetanus after spontaneous relaxation of K-contracture. Records A, B and C were obtained at 10, 30, and 60 min after returning the relaxed fiber to the standard solution, respectively. Time course of recovery of twitch and tetanus amplitude is shown in D.
**DISCUSSION**

In the present experiments, we have succeeded in studying recovery of action potentials, twitches and tetani in single vertebrate muscle fibers after the interruption and the spontaneous relaxation of K-contracts, taking the advantage of slowing these processes at low temperature. When K-contracts were interrupted by returning the fibers to the standard solution, the fiber surface membrane was repolarized quickly, and the action potentials recovered their normal amplitude in 4 min (Fig. 2). Due to the prolonged refractory period of action potential resulting from the marked slowing of the falling phase, the twitches at first showed convex-shaped relaxation phase, and did not fuse to build up complete tetanus (Fig. 3). The recovery in amplitude of twitches and tetani indicates that the action potentials recover their normal amplitude and time course in 10–15 min.

In frog skeletal muscle fiber, the Na-channels are located at the surface membrane, while the K channels, responsible for the delayed rectification and responsible for the normal falling phase of action potential, are located at the membrane of the T-tubules (Freygang et al., 1964a, b), forming transverse network at the level of Z-band in each sarcomere.

As the T-tubules constitute triadic junctions with the TC (Porter and Pallade, 1957), their close proximity suggests that, during K-contracts, the Ca$^{2+}$ released from the TC not only binds to troponin C on the thin filament to produce contractile tension, but is also absorbed at the T-tubule membrane and exerts its stabilizing action (Lüttgau, 1963) to decrease the delayed rectifier conductance, thus causing the marked slowing of the action potential falling phase. After membrane repolarization interrupting K-contracts, the Ca$^{2+}$ absorbed at the T-tubule membrane would be detached in 10–15 min as indicated by the recovery of twitches and tetani (Fig. 4).

It has been well-established that the T-tubule membrane depolarization causes the movement of the dihydropyridine receptor at the T-tubule membrane, which in turn opens the Ca$^{2+}$ releasing channel of the ryanodine receptor at the TC membrane (Gordon and Yates, 1992). It seems possible that the Ca$^{2+}$ absorbed at the T-tubule membrane may also exert its stabilizing action on the dihydropyridine receptor, and inactivates Ca$^{2+}$ release from the TC, thus contributing to the spontaneous relaxation of K-contracts.

On returning the fiber to standard solution after the spontaneous relaxation of K-contracts, the surface membrane was also repolarized quickly in 2 min, while the action potentials were at first markedly reduced in amplitude and prolonged in duration (Fig. 4), and slowly recovered their normal amplitude and duration in 60 min as indicated by the recovery in amplitude of twitches and tetani (Fig. 5). The very slow recovery of action potentials, twitches and tetani after the spontaneous relaxation of K-contracts suggests that a large amount of Ca$^{2+}$ released during K-contracts may also be absorbed at the surface membrane to stabilize the Na-channels, thus markedly reducing the amplitude and duration of action potential.

The contractile repriming after the spontaneous relaxation of K-contracts much slower than that after the interruption of K-contracts (Figs. 4 and 5) may be accounted for in the following way. At the completion of spontaneous relaxation of K-contracts in the contracting solution, the Ca$^{2+}$ is completely detached from troponin C on the thin filament, while the Ca$^{2+}$ remaining in the myoplasm may largely be bound to parvalubumin, a Ca-binding protein occurring in the myoplasm in frog skeletal muscle fibers (Gillis, 1985). On membrane repolarization, the Ca$^{2+}$ absorbed at the T-tubule and the surface membranes is slowly detached first by being taken up by parvalubumin in the myoplasm, and then taken up into the SR. The Ca$^{2+}$ translocation from the surface membrane into the SR may be expected to be very slow, since the surface membrane is distant form the majority of the SR network located deep in the fiber interior.

To summarize, the present study strongly suggests that the contractile repriming after the interruption and the spontaneous relaxation of K-contracts is associated with the membrane potential-dependent absorption of Ca$^{2+}$ to, and its detachment from, the T-tubule and the surface membranes. The same explanation would apply for the rapid contractile repriming after K-contracts at room temperature (Hodgkin and Horowicz, 1960), because the recovery of twitch amplitude shows a time course similar to that in the present study, though the rate is about 60 times faster at room temperature (20°C) than at low temperature (3°C).

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