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Evidence of electroconformational changes in membrane proteins: field-induced reductions in intra membrane nonlinear charge movement currents

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Abstract

Experimental results are presented to show that a pulsed, intensive membrane potential can reduce intra membrane, nonlinear charge movement currents, which are the voltage-sensors in the voltage-dependent membrane proteins and in the excitation–contraction coupling of skeletal muscle fibers. The results indicate a possible mechanism involved in electrical injury: dysfunctions of the voltage-dependent membrane proteins caused by electroconformational damages in their voltage-sensors.

Keywords: Electroconformational change; Membrane proteins; Electric field

1. Introduction

Electrically injured patients may lose their limb functions with little or without any visible alteration in their tissue appearance. When using a controlled electrical field for the purpose of treatments in rhythmic of cardiamyocytes, undesirable collateral effects can occur: arrhythmia, immediate refibrillation, and other signs of dysfunctions. The underlying mechanisms involved in these electrical injuries may include the nature of field-interactions with the membrane protein, especially, the voltage-dependent membrane proteins of living cells. The consequent results are the dysfunctions of the membrane proteins, and the cells [1].

One of the common features among the voltage-dependent membrane proteins is the function of their voltage-sensors, which can be measured as intra membrane, nonlinear charge movement currents. Our hypothesis is that an applied intensive electric field may damage these voltage-sensors because of their membrane potential vulnerability [2]. This paper presents our experimental results of supra-physiological membrane potential-induced changes in intra membrane charge movement currents. These results provide further evidence of electric field-induced conformational damages [2] in membrane proteins.

2. Methods

When exposing to an electric field, voltage sensors in many voltage-dependent membrane proteins may undertake conformational changes expressed as intra membrane movement of the charge particles, or reorientation of the equivalent dipole moments. The conformational changes can be monitored by measuring proteins' nonlinear charge movement currents using voltage clamp techniques.

In our experiments, an improved double Vaseline-gap voltage-clamp technique [3] was used. This technique allows us to deliver pulsed intensive electric shock to the cell membrane, and to accurately measure the shock-induced changes in the charge movement currents. A custom-modified voltage-clamp (Dagon voltage clamp TEV 200) with a compliance voltage source of 130 V was used in the experiments.

The protocol of isolation of charge movement currents has been routinely used in our laboratory [4] which followed from other laboratories [5]. Single fibers from frog skeletal muscle, *semitendinosus*, were hand-dissected and mounted in a custom-made chamber. Ag/AgCl pellets and agar bridges were used to reduce the junction poten-

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tials. To identify charge movement currents, channel ionic currents have been eliminated by applying various channel blockers, tetrodotoxin (TTX) for Na^+ channels, Co for Ca^{2+} channels, and by substituting K^+ ions with tetraethylammonia (TEA). Membrane linear capacitive currents were subtracted from the total currents by using the traditional P/4 method.

Membrane holding potential was −90 mV. To measure the charge movement currents, a sequence of nine stimulation pulses was used to alter the membrane potential from −65 to −25 mV with a time interval of half minute. About two orders in magnitude of charge movement currents are less than that of channel ionic currents. To increase signal/noise ratio, each stimulation pulse was repeatedly applied to the same fiber for 13 times. Averages of the recorded currents were defined as charge movement currents.

The supraphysiological shock pulse was −400 mV of 4-ms duration. The reason of using 4-ms duration for all of shock pulses is to avoid thermal effects, and to mimic the electric shock by a power line frequency. The purpose of choosing negative polarity is to eliminate remnant of channel currents.

3. Results

We have compared the intra membrane charge movement currents measured using our improved double Vaseline-gap voltage clamp technique with those measured using the traditional method, and found that their kinetics and magnitudes were very consistent. A group of measured charge movement currents responding to the stimulation pulse-sequence are shown in Fig. 1 as a control. There are a distinguishable fast component, Q_β (marked by a dot-line),

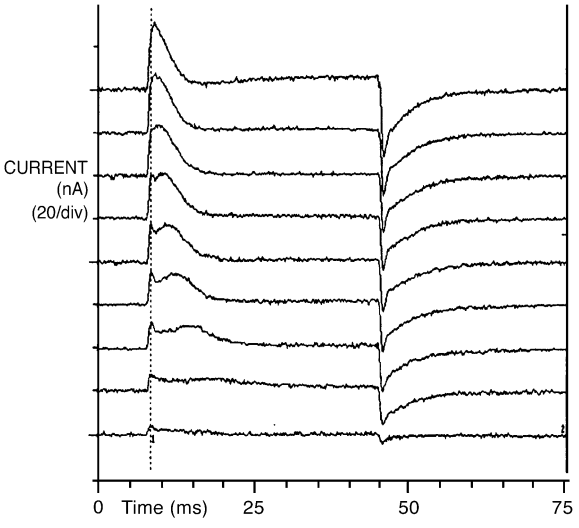

Fig. 1. Charge movement currents in response to a sequence of stimulation pulse holding the membrane potential from −65 to −25 mV. The dotted line shows the peaks of the fast components Q_β. The hump, slow components are Q_γ.

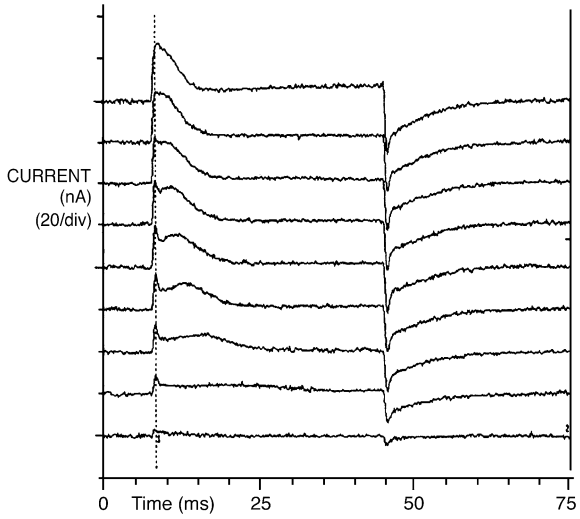

Fig. 2. Charge movement currents recorded after the fiber was shocked by a single 4-ms, −400 mV pulse.

and a slow hump component, Q_γ in response to the stimulation pulses. The equity of the amount of charged particles corresponding to the stimulation pulse and the discharged particles when membrane potential was returned to the holding potential has been checked by integrating the corresponding currents, respectively.

Then, the fibers were shocked by a pulsed, supraphysiological membrane potential delivered by the voltage clamp. When the shock pulse magnitude was less than −300 mV, very little reduction of the charge movement currents could be observed, nor the kinetics of the two components in the charge movement currents.

However, when the fiber was shocked by a −400 mV pulse, both the peak of charge movement currents and the total amount of moved charge particles decreased.

The charge movement currents shown in Fig. 2 were measured from the same fiber of Fig. 1 after shocked by a

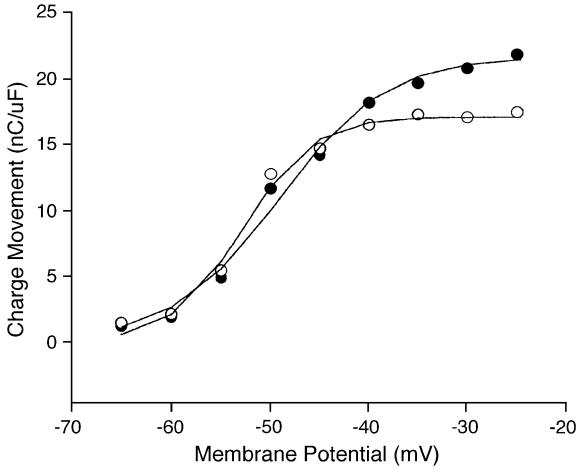

Fig. 3. The normalized, integrated total charge movement particles are plotted as a function of the stimulation pulses before and after the electric shock.

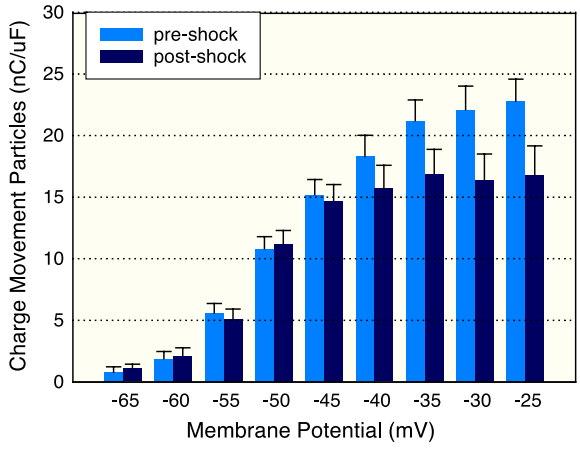

Fig. 4. A comparison of pre- and post-shocked charge movement currents. The results are based on five experiments.

4-ms, −400 mV pulse. Interestingly, the two components responded differently to the electric shock. The peak value of the post-shocked fast component, Q_β, showed no reduction. However, both the kinetics and the magnitudes of the slow component, Q_γ, were changed significantly after the electric shock. For the control traces, when the simulation pulses holding the membrane potential higher than −40 mV, the peak of the slow component, Q_γ was gradually over the peak of the fast component, Q_β, shown as the top four traces in Fig. 1. After the electric shock, all of the peak values of Q_γ became small, and never caught up the peak of Q_β. Moreover, the kinetics of Q_γ became slower after the electric shock. For example, the peak of the Q_γ corresponding to the stimulation of −45 mV in the control is about 3.2 ms delay from the peak of Q_β, where after the electric shock, the delay increases to 4.6 ms.

The total amount of charge movement particles in response to each stimulation pulse before and after the electric shock can be estimated by integrating the corresponding charge movement current trace [4]. The resultant charge movement particles are then normalized by the membrane capacitance, nC/μF, and are plotted as a function of the stimulation pulses, shown in Fig. 3. Five experiments have been performed. A histogram result is shown in Fig. 4. We found that total amount of moved charge particles were reduced after a single pulsed shock of 4-ms, −400 mV.

4. Discussion

Voltage-dependent membrane proteins are more vulnerable to an applied electrical field than other membrane proteins. That is because the voltage-sensor or the moveable charge particles in these membrane proteins is sensitive to the applied electric field. High intensity electric field may cause structural damages in those membrane proteins, especially in their moveable charged particles. Any changes in the number of movable charge particles, the equivalent moving distance or the mobility of each particle may affect the functions of the membrane proteins.

This report provides experimental results of intensive electric field-induced reductions in charge movement currents of membrane proteins in skeletal muscle fibers. Our results showed that the slow, hump component, Q_γ, especially for those elicited corresponding to the stimulation pulses over −40 mV is very sensitive to the electric shock. The peak magnitudes of the Q_γ were reduced and the delay time from the rising-phase of the stimulation pulse increased. Moreover, the total amount of movable charge particles in the membrane proteins was reduced by the supraphysiologically electrical shock. In contrast, the fast component, Q_β has little change in response to shock pulse.

The measured charge movements reflect the total amount of moveable charge particles and their mobility in the membrane proteins. Shock field-induced decrease in magnitude and increase in time-delay in charge movement currents indicate less number of charge particles involved in the voltage-sensor or less mobility of these charge particles.

These results imply that a 4-ms single shock pulse of −400 mV can induce somewhat conformational changes in the membrane proteins resulting in a reduction in both the total amount and the kinetics of their charge movement particles. These results may explain the shock field-induced dysfunctions of voltage-dependent membrane proteins.

It is necessary to point out that the charge movement currents in the transverse (T)-tubular membrane are involved in voltage-sensor in the excitation–contraction coupling of the skeletal muscle fibers [5]. Our experiments may further reveal a new mechanism underlying electric injury, especially for skeletal muscle tissues.

Acknowledgements

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References

[1] W. Chen, R.C. Lee, Altered ion channel conductance and ionic selectivity induced by large imposed membrane potential, Biophys. J. 67 (1994) 603–612.
[2] W. Chen, Supra-physiological membrane potential induced conformational changes in K+channel conducting system skeletal muscle fibers, Bioelectrochemistry 62 (2004) 47–56.
[3] W. Chen, R.C. Lee, An improved double vaseline gap voltage clamp to study electroporated skeletal muscle fibers, Biophys. J. 66 (1994) 700–709.
[4] C.S. Hui, W. Chen, Charge movement in cut twitch fibers of Rana temporaria containing 0.1 mM EGTA, J. Physiol. 503 (3) (1997) 563–570.
[5] L. Kovacs, E. Rios, M.F. Schneider, Measurement and modification of free Ca transients in frog skeletal muscle fibres by a metallochromic indicator, J. Physiol. 343 (1983) 161–196.