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Burns 32 (2006) 52–59

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Supramembrane potential-induced electroconformational changes in sodium channel proteins: A potential mechanism involved in electric injury

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Abstract

Effects of imposed large supraphysiological transmembrane potential (TP) pulses on channel proteins, particularly on the voltage-gated Na channels, were investigated. Voltage clamp techniques were used to deliver both shock and stimulation pulses, and to monitor changes in the channel functions. Our experimental results indicated that more than one 4 ms duration TP shock of −450 mV resulted in electroconformational denature of voltage-gated Na channels. This resulted in functional reductions in muscle cells' excitability. We quantified the TP shock-induced decrease in the Na channel currents, compared the pre- and post-shocked Na channel currents' voltage dependency, and studied the reversibility of the electroconformationally denatured ion channel proteins. These observations are particularly relevant to the problem of explaining the neuromuscular damage following high voltage electrical shock injuries despite no evidence of a thermal injury component.

Keywords: Voltage-gated sodium channel; Electroconformational change; Electroporation; Electrical injury; Ion channels

1. Introduction

The use of a controlled electric field has been broadly utilized as a tool in many areas of basic biological science and medical practice. For example, pulsed electric fields have been successfully employed in molecular biology to induce the formation of reversible pores or pore-like structures in cell membranes in order to facilitate cell fusion or gene transfection [1–4]. The use of an electric field to pace, fibrillate or defibrillate the heart has been well established as a common clinical practice [5–7]. Recently, electroporation techniques have been applied to skin in order to facilitate percutaneous drug delivery [8,9]. Meanwhile, accidental high intensity electric field-induced injury continues to present problems in clinical diagnosis and patient treatment. Better understanding of the underlying mechanisms involved in electrical injury will significantly improve capabilities of patient management and the development of therapeutic treatment.

The electrical impedance of cell membrane is about six to eight orders of magnitude higher than that of electrolytes in cytoplasmic and extracellular fluids. When living cells are exposed to an external electric field with a frequency equivalent to that found in power-lines, cell membranes suffer major voltage drops induced by this electric field. Because cell dimensions are in the order of hundreds or thousands of times larger than the thickness of the cell membrane, the external field-induced field-strength in the cell membrane is hundreds to thousands of times higher than the strength of the applied field [10,11]. An applied electric field with a field-strength in tens or hundreds of volts/cm may generate a field-strength of millions of volts/cm in the cell membrane. In all probability, such super high field-strength will induce damage to the membrane phospholipid bilayer and its embedded membrane proteins.

Electroporation recently has been postulated as an important mechanism involved in electrical injury [12,13]. Electropores are thought to be formed in the lipid

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0305-4179/$30.00
doi:10.1016/j.burns.2005.08.008

bilayer resulting from the extremely high induced transmembrane potential (TP) in skeletal muscle and nerves [14,15]. These microlesions in the cell membranes can cause leakage of the cellular metabolic substrates and consequent ionic exchange across the cell membranes [16,17]. Electroporation-induced loss of intracellular ATP molecules and ionic concentration gradients triggers the ATP-fused ion pumps. This accelerates the consumption of ATP, resulting in a rapid exhaustion of the supply of ATP molecules. The cells will then progress to biochemical arrest and eventually to necrosis.

In addition to membrane electroporation, we must consider how an external electrical field-induced supramembrane potential can affect membrane proteins. Tsong and Teissie studied the high intensity electric field-induced leakage on erythrocyte cells, and postulated the leakage as field-induced electroporation on the Na/K ATPase molecules [18]. However, the proteins' functional changes were not confronted. We suggested a modified double Vaseline-gap voltage clamp technique to study supramembrane potential-induced cell membrane damage [17]. We previously reported that an intensive electrical field can cause electroconformational changes in the delayed rectifier K^+ channel proteins resulting in dysfunction of peripheral nerves and skeletal muscles without any visible alteration in tissue appearance [19].

Field-induced electroconformational damages to the delayed rectifier K^+ channels and electroporation of the cell membrane adequately explains the phenomenon of membrane resting potential-depolarization in electrically injured nerves and muscles. However, they failed to explain the shock field-induced reduction in the membrane action potential. An in vivo study on rat skeletal muscles showed diminution in the magnitude of the membrane action potential after a high intensity electrical shock [20]. To understand these phenomena, it is necessary to study the effects of an intensive electrical field on the voltage-gated Na channels, a major determining factor in generation of the action potential.

Our recent studies have focussed on understanding the high intensity electric field-induced damage in the voltage-gated Na channels. Here we report voltage clamp studies of the response of muscle cell membranes to short duration supraphysiological TPs. We examine the shock field-induced reduction in the channel currents, and study changes in the channel's voltage-dependence, the channel *I–V* curve and channel conductance, finally discussing the reversibility of the damage to these channel proteins.

2. Material and methods

2.1. Skeletal muscle fiber preparation

The protocol of single fiber preparation has been adapted from previous studies [21–24] and used in our laboratory with some improvements [17,19]. Briefly, skeletal twitch muscles, *semitendinosus* and *illus*, were dissected and removed from frog (*Rana temporaria* or *Rana Pipiens)* hindlimb. A single fiber was hand-dissected and mounted in a custom-made chamber filled with relaxation solution. The fiber was held by clips at each end. The fiber was partitioned into three electrically isolated pools by two Vaseline seals. The width of the two partitions and the width of the central pool were 100 μm and 300 μm, respectively. The fiber was stretched up to a sarcomere length of 3 μm. The purpose of this stretching is to avoid fiber contraction during electrical stimulation.

The fiber segments in the two end pools were treated with a 0.2% saponin solution for 2 min, then, washed out with internal solution, resulting in electrical and ionic permeabilization of the two fiber segments. The three pools were then connected to a voltage clamp by six agar bridges and three Ag/AgCl pellets. Resistance of these agar bridges and pellets was less than 1 kΩ. Both stimulation pulses and shock pulses were delivered through a computer-controlled voltage clamp (Dagon TEV 2000). Data were sampled with an Axon data acquisition board (AC 1200) and stored in a hard disk for further analysis.

2.2. Composition of experimental solutions

Compositions of each solution used in maintaining the isolated muscle fibers are as follows:

- Relaxing solution: 120 mM, K-glutamate; 1 mM, $MgSO_4$; 0.1 mM EGTA; 5 mM, PIPES.
- External solution: 120 mM, NaCl; 5.4 mM, KCl; 4 mM, MOPS; 1.8 mM, $MgCl_2$; 2 mM, $BaCl_2$; 0.2 mM, $CdCl_2$; 1 mM, CsCl.
- Internal solution: 45.5 mM, Cs-glutamate; 5 mM, Cs_2-PIPES; 20 mM, Cs_2-EGTA; 6.8 mM, $MgSO_4$; 5 mM, glucose; 5.5 mM, Na_2-ATP; 20 mM, Tris–Creatine phosphate

Using voltage clamp techniques, the relatively large Na channel currents passing through the series resistance of the circuit will inevitably generate voltage drops on this resistor. The lower the elicited channel current, the less voltage drop observed on the resistance. To reduce the voltage drop in order to accurately determine the membrane potential, ionic concentration gradients in the external and internal solutions have been adjusted appropriately.

In this study, all of the experiments were performed at room temperature, 24 °C. Previously, we conducted experiments at both 4 °C and 24 °C, and compared their results. Indeed, the channel currents are larger at 24 °C than at 4 °C. However, the purpose of these experiments is to investigate the electric shock-induced effects in the Na channels, the temperature-induced changes in channel functions is trivial in comparison to that induced by the electric shock.

2.3. Electrical shock and function recording

The voltage clamp configuration is an improved double Vaseline-gap voltage clamp [17]. In contrast to the traditional configuration, the two end pools were connected and a positive feedback circuit was designed to compensate for the voltage drops on the series resistance underneath the two Vaseline partitions. The membrane holding potential was −90 mV. Two groups of pulses were employed in this study. Stimulation pulses were a sequence of 10 ms pulses ranging from 34 mV to 130 mV that held the membrane potential from −56 mV to 40 mV. Shock pulses held the cell membrane at a supraphysiological membrane potential of −450 mV. The duration of shock pulses was 4 ms in order to mimic that of a power-line frequency electrical shock. A 4 ms duration pulse and a half cycle sinusoidal current at commercial (60 Hz) power frequency with the same RMS value have the same energy. Before the application of a supraphysiological shock pulse, the sequence of stimulation pulses was applied to the cell membranes and the evoked Na channel currents were recorded. After this, the 4 ms supraphysiological potential pulse was delivered to the cell in order to electrically shock the cell membrane. The responding transmembrane currents were simultaneously monitored during the shock in order to identify the occurrence of electroporation. Right after the pulsed shock, the sequence of stimulation pulses was reapplied to the membrane to study the shock-induced effects on the channel currents. The *P*/*N* method was used to subtract linear currents including linear capacitance and linear leakage currents. In reference to the stimulation pulses, *N* is always chosen as four, and for the shock pulses, *N* is bigger in order to avoid pre-pulse-induced membrane electroporation.

3. Results

3.1. Supraphysiologic TP reduced the Na channel currents

To identify the voltage-gated Na channel currents, the K ions inside the cells were substituted with Cs in order to reduce the outward current carried by K ions. One millimolar 3,4-diaminopyridine (DAP), a blocker of the delayed rectifier K channels was also added to the external solution in the central pool. Because the kinetics of the Na channel currents (rising in an order of a few hundred μs) is much faster than that of the delayed rectifier K channel currents (rising in an order of a few ms), the little remaining K channel currents did not affect the accuracy of measurement of the Na channel peak currents.

A sequence of 17 10-ms duration stimulation pulses holding the membrane potential at a value ranging from −56 mV to 40 mV was applied to the cells. The magnitude step between two consecutive pulses through the sequence was 6 mV. The evoked transmembrane currents responding to each stimulation pulse were recorded. After subtraction of the linear leakage currents and the membrane capacitance currents, the Na channel currents were resolved as shown in Fig. 1.

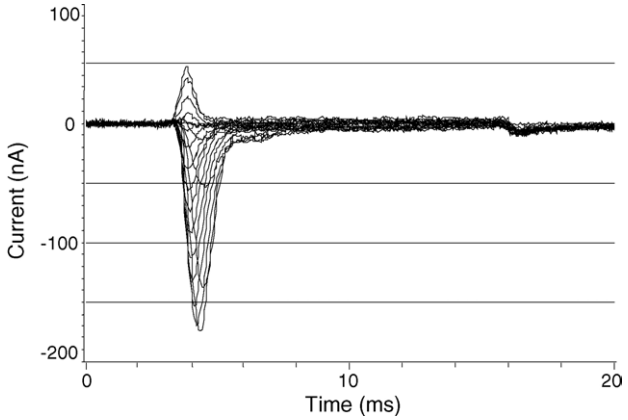

Fig. 1. Na channel currents recorded from frog skeletal muscle fibers using double Vaseline-gap voltage clamp techniques. The membrane holding potential was −90 mV. A sequence of 20 10 ms stimulation pulses held the membrane at a potential ranging from −55 mV to 40 mV. The magnitude step between two consecutive pulses through the sequence was 6 mV. After subtracting the linear capacitance and leakage currents recorded during the sub-stimulation pre-pulses, the Na channel currents were obtained and are shown in the figure.

The fiber was then shocked by two 4-ms pulses at a supramembrane potential of −450 mV with a time interval of separation of 5 s. The shock pulses were delivered through the voltage clamp and the responding transmembrane current was simultaneously recorded during the shock pulse. The raw data of the transmembrane currents corresponding to the first shock pulse is shown in the upper panel of Fig. 2. After the transient capacitance current, the following monotonically increasing current is the shock pulse-induced nonlinear leakage current, the membrane electroporation current [17]. Using the *P*/4 method, the linear resistive leakage and capacitance currents were removed. The remaining nonlinear leakage currents represent the electroporation current [15], as shown in the lower panel of Fig. 2.

After the cell membrane was shocked by two 4 ms supraphysiological membrane potentials, the fiber was relaxed until the holding current was observed to have fully recovered. The same stimulation pulse sequence was again applied to the cell membrane. With the same *P*/4 method used in the control, the Na channel currents were resolved as shown in Fig. 3. These post-shocked Na channel currents are clearly much smaller than the pre-shocked channel currents.

By comparing the Na channel currents recorded before and after the electrical shock shown in Figs. 1 and 3, we can see that the two 4-ms, −450 mV shock pulses significantly reduce the peak value of the Na channel currents. For this fiber, the peak values of the Na channel current were 170 nA and 95 nA before and after the electric shock, respectively. This corresponds to a 45% reduction in the Na channel

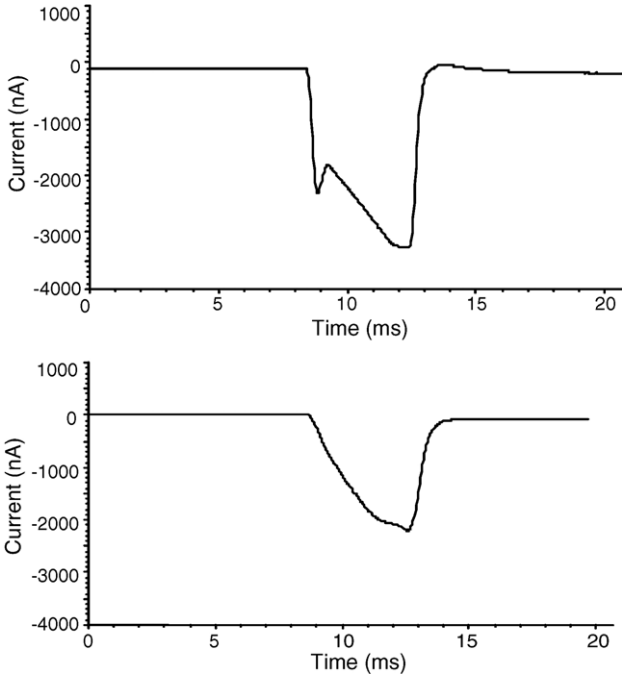

Fig. 2. Transmembrane current recorded during the supraphysiological membrane potential-shock. The shock pulse is 4 ms with a supramembrane potential of −450 mV. In the upper panel, the transient peak current responding to the rising phase of the shock pulse is a capacitance current with an exponential decay followed by a monotonically increasing electroporation current. In the lower panel, the isolated electroporation current obtained by subtracting the capacitive and linear leakage current using *P/N* method is shown.

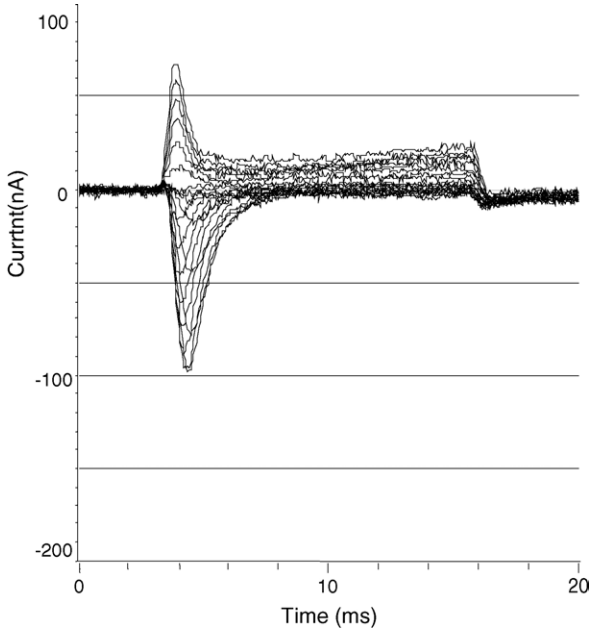

Fig. 3. Supramembrane potential-induced reduction of the voltage-gated Na channel currents. After the cell membrane was shocked by two 4 ms pulses of −450 mV supraphysiological membrane potential, the same stimulation pulse sequence was again applied to the cell membrane. With the same method used in the control, the Na channel currents were obtained as shown here. The channel peak currents show a significant reduction after the shock.

currents after the membrane was shocked by the two 4-ms pulse of −450 mV. Intuitively, our first impression was that a 45% reduction in the Na channel currents indicated that two 4-ms duration, −450 mV pulsed shocks could damage 45% of the channel proteins, either 45% of the channel population was fully damaged or each channel protein was individually reduced in functionality by about 45%. In fact, this is not true. This 45% channel current reduction is a global effect of the shock pulses on the cell membrane including the channel proteins' damage. To distinguish the proteins' damage, we need to study the channels' *I–V* curve.

3.2. Altered Na channels' current–voltage relationship

The peak values of the pre- and post-shocked Na channel currents shown in Figs. 1 and 3 were plotted as functions of the membrane potential of the stimulation pulses, shown in Fig. 4. The open squares represent the peak values of the Na channel currents recorded before the electrical shock and the upwards pointing triangles represent the channel currents after application of the electrical shock. Channel currents plotted as a function of the stimulation pulses reflect the dependence of the current on the membrane potential. Several differences in the two current–voltage relationships of the pre- and post-shocked Na channels could be noticed, indicating the shock pulse induced changes in the cell membrane, which includes changes in the lipid bilayer, membrane proteins and ionic concentration gradients.

By comparing the *I–V* curves of the Na channels before and after the electric shock, we found that the post-shocked *I–V* curve is different to that of the pre-shocked *I–V* curve in three aspects. First, the post-shocked Na channel currents are much smaller than the pre-shocked channel currents in almost the full potential range. The current reduction can be easily resolved by comparison of the channel current traces shown in Figs. 1 and 3. Secondly, the slops of the two *I–V* curves are different. After the two −450 mV shock pulses, the slope of the Na channel *I–V* curve was reduced. Finally, the cross-point between the *I–V* curve and the abscissa is shifted in the negative direction after the electric shock. Interestingly, the membrane potential that elicits the maximum Na channel currents remains the same. For both pre- and post-shocked Na channels, a membrane potential of between −20 mV and −30 mV generated the maximum channel current.

After the electric shock, the Na channel currents are significantly reduced. Two factors that determine the value of channel currents are the channel conductance, which reflects the characteristics of the channel proteins, and the driving force of the ionic concentration gradients, which reflects the corresponding environmental factors. We have previously found that the potential threshold for membrane electroporation in frog skeletal muscle fibers is between −250 mV and −300 mV, via measurement with a double

Vaseline-gap voltage clamp [17,25]. Tovar and Tung conducted experiments using a microelectrode whole cell voltage clamp on cardiac myocyte, and obtained similar results [25]. A shock pulse of −450 mV is much higher than the electroporation threshold, which will inevitably result in the generation of pores or pore-like structures in the cell membrane. These electropores in the cell membrane cause ionic leakage during the electric shock, which is shown as the huge inward electroporation current in Fig. 2. The consequent result is that the Na concentration gradient across the cell membrane, at least locally confined to the area of the cell membrane, is reduced due to membrane electroporation. As a result, the driving force for the Na channel currents is less than that in the control. To identify the ionic concentration gradient change, we should first consider the changes in the Na equilibrium potential induced by the electric shock.

The cross-point of the Na channel *I–V* curve with the abscissa represents the Na equilibrium potential. At the equilibrium potential, the electric driving force due to the applied membrane potential and the chemical force due to ionic concentration gradient are balance so that at this point there is no Na channel current. The physiological Na equilibrium potential in frog skeletal muscle fibers is about +60 mV. In our experiments, both external and internal solutions were designed to reduce the magnitude of Na channel currents in order to reduce the current-induced voltage drop due to the series resistance. Based on the compositions of our experimental solutions, the estimated Na equilibrium potential for this fiber is about 35 mV, which is consistent with the cross-point shown in the pre-shocked *I–V* curve. After the electric shock, the Na channel equilibrium potential is shifted to the negative direction at 22 mV.

With any membrane potential, *V*, applied to the cell membrane, only the potential difference of $V - V_{equi}$ is the driving force for Na channel currents. For example, for the stimulation pulse held the membrane potential at −19 mV, which is responding to the largest channel currents in Fig. 4, the driving forces for the pre- and post-shocked channel currents are not the same of −19 mV. Instead, they are (−19 − 35 = −54 mV) and (−19 − 22 = −41 mV), respectively, for the pre- and post-shock.

Slopes of the Na channel *I–V* curves represent the channel conductance. Any conformational damages in channel proteins induced by the electric shock will result in channel functional reductions, or decrease in the channel conductance. Channel conductance reduction can be represented by a decrease in the slope of the *I–V* curve. For the control, the Na channel conductance, or the slope of the pre-shocked Na channel *I–V* curve is 168 nA/54 mV = 3.11 μS. After two 4 ms duration, −450 mV pulsed shocks, the slope of the *I–V* curve, or the channel conductance was reduced to 95 nA/41 mV = 2.26 μS, about 70% of that before the electric shock.

We have conducted more than 10 experiments (10 fibers) in study of this field-induced conformational change in the Na channels. All of these results consistently show a significant decrease in the channel conductance. A statistical analysis shows that after two 4 ms duration, −450 mV pulsed shocks, the mean decrease of the channel conductance is 27% with 12% standard deviation. The relative large standard deviation mainly results from variance in the fiber diameter. Due to the large Na channel current and the electroporation currents during the shock pulse, the currents may induce a large voltage drop on the current pathway, which is primarily the interior of the fiber. This voltage drop will affect the actual potential across the cell membrane. Even though, in experimental design, we have used our improved configuration of the voltage clamp and adjusted the ionic concentration to minimize channel currents, the effects may not be fully avoidable.

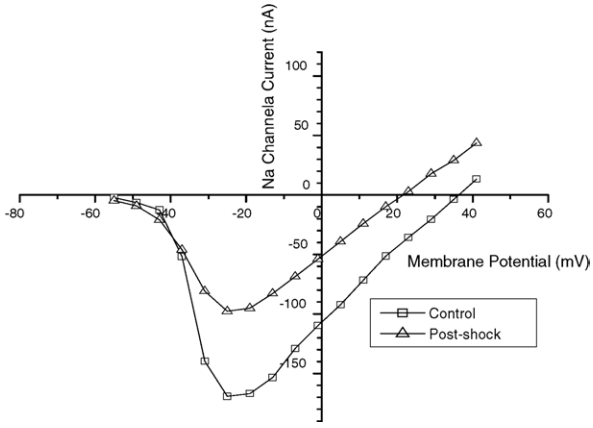

Fig. 4. The Na channel currents as a function of the membrane potentials before and after the electrical shock. The squares represent the Na channel currents before the electrical shock and the upwards pointing triangles represent the Na channel currents after the shock by two 4 ms pulses of −450 mV. Clearly, most of the post-shock Na channel currents responding to different stimulation pulses are reduced by the shock. However, the slopes of the two *I–V* curves for the pre- and post-shocked Na channel currents are similar for most membrane potentials except for the potentials close to the threshold of opening the Na channels.

3.3. Reversibility of Na channel denaturisation

The next question relevant to our study is whether this supramembrane potential-induced decrease in the Na channel currents is reversible. To answer this question, the same experiments described above were repeated. The sequence of stimulation pulses was applied to the cell membrane. Then, the fiber was shocked by two supramembrane potential pulses at −450 mV. The stimulation pulse sequence was applied to the cell membrane immediately after the pulsed shock. After 10 min relaxation and every 10 min thereafter, the stimulation pulse sequence was reapplied to the cell five times.

By comparing the Na channel currents recorded at different times, the results showed that the peak currents of the post-shocked Na channels remained at a reduced level. By plotting *I–V* curves at different stages, the electrical shock induced channel conductance reduction showed very

little, if any, spontaneous recovery up to 50 min relaxation. The reversibility of the electrical shock-induced damages in the Na channel was very poor.

4. Discussion

4.1. Reduction in the Na channel conductance

A comparison of the channel current traces shown in Figs. 1 and 3 provide strong evidence that two 4 ms supraphysiological membrane potential shock pulses at −450 mV can cause a significant decrease in the Na channel currents. By analysing the channel *I–V* curves, two 4-ms, −450 mV pulsed shocks can reduce the channel conductance by up to 30%. This dysfunction of the Na channel proteins in the cell membrane will certainly affect generation of the membrane action potential, in the magnitude, shape and latency period. Indeed, the results from in vivo studies of the action potential on rat skeletal muscles showed that a high intensity electric field can reduce the magnitude of the membrane action potentials and increase their latency period [20].

This study utilizing isolated adult muscle fiber study with voltage clamp techniques to directly measure changes in the channel currents and channel conductance provides cellular level evidence that a high intensity electrical field may damage the voltage-gated Na channels. It is necessary to point out that electric shock-induced reductions in the magnitude of membrane action potential and changes in action potentials' characteristics are due to two distinct reasons: Na channel protein damage resulting in channel conductance reduction, and membrane electroporation resulting in a decrease in the ionic concentration gradient. Because the membrane potential threshold for electroporation (−250 mV to −300 mV) is much smaller than our shock pulses (−450 mV), it is impossible to avoid the effects of membrane electroporation.

4.2. Mechanism involved in protein's electroconformational changes

Approximately 40% of the cell membrane, by weight, consists of proteins functioning as ion channels, transporters and signal receptors. The applied supramembrane potential will inevitably affect these membrane proteins. We have proposed a new mechanism involved in electrical injury, membrane proteins electroconformational changes (damages). In this study, we showed strong evidence of supraphysiological membrane potential induced reductions in the Na channel conductance, which implies conformational denaturisation. The results together with our previous studies of the K channel provides strong evidence of electroconformational changes in the membrane proteins.

Detailed mechanisms involved in membrane protein denaturisation remain unknown. Without using a molecular probe, it is impossible to precisely localize the damage in amino acids or subgroups. One possible explanation is that a large transmembrane current could cause a size reduction of the narrowest pores of the ionic pathway in channel proteins. However, this possibility does not seem reasonable. Channel conductance is directly related to the size of the narrowest pore of the channel. It is difficult to imagine that an external field-induced current passing through the narrowest pore could reduce the pore size. In fact, the large transmembrane current may cause local thermal damage to the narrowest pore, resulting in ionic leakage.

According to physical law, an electric field can only exert a force on charged particles, and the force magnitude is proportional to the field-strength. An intensive electric field, even though briefly applied to the cells, may cause structural changes in the membrane proteins. The amino acids containing charged particles experience a large electrical force, which may cause those particles to be locally moved in the direction of the external electrical field, or cause the equivalent dipole moments to be reoriented more or less parallel to the external electrical field.

One possible location of electroconformational changes is at the narrowest pore of the channel proteins. It has been shown that more than half of the amino acids are polarized in the S4 domains of the Na channel [26], which is postulated to consist of the narrowest pores of the channels. The mechanism of shock-induced decrease in the ionic selectivity of the delayed rectifier K channels has been considered as electroconformational changes in the vulnerable subgroups at the narrowest pore of the channel proteins [19].

Meanwhile, the amino acids or subgroups in channel proteins functioning as voltage sensors may also be vulnerable to the high intensity electrical field. These voltage sensors are clearly sensitive to changes in the membrane potential. A high intensity electric field may break down some chemical bonds or disable some movable particles in these amino acids functioning as gating system, resulting in a loss of their capability to open the channels. This assumption is consistent with our experimental results of the supraphysologic TP potential-induced reduction in the Na channel currents and the channel conductance.

4.3. Reversibility of Na channel conformational denaturisation

It has been observed that electropores on the cell membrane induced by a high intensity electric field can be divided into two categories in terms of reversibility: transient and stable. For the transient pores, there exist two groups with different recovery time scales: those sealing in microseconds to milliseconds and those taking minutes to close. Comparatively, our experimental results show that the reversibility of the high intensity electrical field-induced reduction of the Na channel currents is far less than the reversibility of membrane electroporation.

4.4. Relevance to electrical shock induced tissue damage

Nerves and muscles use electrical potentials to convey information. A rapid signaling procedure, the action potential conveys signals rapidly and efficiently over long distances. During the action potential, due to the opening and closing of ion channels, especially the Na and K channels, the membrane potential is quickly reversed and then returns to nearly the original resting potential, all within a few milliseconds. Features of the action potential such as its magnitude, shape, frequency, latency time and propagation speed often determine the conveyed information. For example, shapes of the action potentials in nerves, skeletal muscle, and cardiac muscle differ significantly, and action potential frequency is often used to code signals. All of these features of the action potential depend on the functions of the Na and K channels, especially the Na channels, including their ionic selectivity, channel conductance, and their kinetic time course for the channel to open and close. Functions for these Na and K channels are critical for many cells, especially for excitable cells.

The ion channels' electrical susceptibility strongly suggests that these membrane proteins may be structurally damaged and functionally disabled during an exposure to high voltage electrical fields. Our previous studies showed that the supramembrane potential may induce K channel conformational damage, including ionic selectivity reduction and decrease in the channel conductance. These changes may cause membrane resting potential-depolarization, and change the shape and duration of the action potentials. In this study we further showed that the maximum Na channel currents and their conductance may also be reduced. Because Na channel proteins are the major resource in cells to generate an action potential, their conformational damages may inevitably result in an alteration in the action potential. This study explains the cellular mechanism of the in vivo study in rats that electric shock reduced the magnitude of the action potential in muscles and sciatic nerves [20]. In addition, the Na channel functional reduction may cause other characteristic changes in the action potential. In neuromuscular junctions, the higher the magnitude and frequency of the action potential that propagates into a nerve terminal, the higher the rate of transmitter secretion. In excitation contraction coupling, the higher the frequency and magnitude of the action potential, the higher the tone and the stronger the fiber contraction. Any decrease in action potential magnitude and frequency may result in dysfunction in those neuromuscular junctions and consequentially the E–C coupling.

In clinical situations, we often see electrically injured patients whose injured limbs or organs show significant reductions but their functions and muscles around the current pathway shows no sign of tissue damage. Common symptoms of an electrically shocked patient include shaking of the limbs, over sensitivity, and loss of loading capability. These neuromuscular symptoms may be partially explained by the functional reduction in the ion channels, especially the Na channels, the main determinant of an action potential.

The reason for there being no significant change in tissue appearance is because electroconformational damage in the membrane proteins is mainly resulting from the high voltage of the shock field. There is very little, if any, current needed to alter the protein structure. This is significantly different from thermal damage, and even membrane electroporation. Industrial workers electrocuted by high voltage field, and people shocked by lightning are some typical examples.

In summary, the experimental results presented in this paper provide direct evidence of the shock field-induced reduction in the Na channel functions. This will affect generation of the membrane action potential resulting in alteration of the action potential's characteristics. Action potentials are used by nerves, muscles and many excitable cells to code information and to convey signals. As a result, functions of these cells and tissue may be reduced. Results from this study in Na channels together with our previous study in K channels strongly suggest a new underlying mechanism involved in electrical injury: an intensive, brief electric shock may cause electroconformational damage to membrane proteins, especially voltage-dependent membrane proteins. For ion channels, these conformational damages may affect the signal conveyed in excitable cells, resulting in functional reduction in those cells and tissues.

Acknowledgements

This study is supported by the NIH grant 2R01. GM50785 (W.C.), NSF grant PHY 0515787 (W.C.) NIH grant GM61101 (RCL) and R01 GM64757 (RCL) and The Electric Power Research Institute (RP WO-2914 and RP WO-9038) (RCL).

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