Desensitization of the Skeletal Muscle Ryanodine Receptor: Evidence for Heterogeneity of Calcium Release Channels

Jianjie Ma
Department of Physiology and Biophysics, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106 USA

ABSTRACT Ca release channels from the junctional sarcoplasmic reticulum (SR) membranes of rabbit skeletal muscle were incorporated into the lipid bilayer membrane, and the inactivation kinetics of the channel were studied at large membrane potentials. The channels conducting Cs currents exhibited a characteristic desensitization that is both ligand and voltage dependent: 1) with a test pulse to −100 mV (myoplasmic minus luminal SR), the channel inactivated with a time constant of 3.9 s; 2) the inactivation had an asymmetric voltage dependence; it was only observed at voltages more negative than −80 mV; and 3) repetitive tests to −100 mV usually led to immobilization of the channel, which could be recovered by a conditioning pulse to positive voltages. The apparent desensitization was seen in approximately 50% of the experiments, with both the native Ca release channel (in the absence of ryanodine) and the ryanodine-activated channel (1 μM ryanodine). The native Ca release channels revealed heterogeneous gating with regard to activation by ATP and binding to ryanodine. Most channels had high affinity to ATP activation (average open probability (p_o) = 0.55, 2 mM ATP, 100 μM Ca), whereas a small portion of channels had low affinity to ATP activation (p_o = 0.11, 2 mM ATP, 100 μM Ca), and some channels bound ryanodine faster (<2 min), whereas others bound much slower (>20 min). The faster ryanodine-binding channels always desensitized at large negative voltages, whereas those that bound slowly did not show apparent desensitization. The heterogeneity of the reconstituted Ca release channels is likely due to the regulatory roles of other junctional SR membrane proteins on the Ca release channel.

INTRODUCTION

Skeletal muscle contracts when the myoplasmic Ca concentration increases from the resting level of 50–150 nM to micromolar ranges. This increase is a consequence of opening of Ca release channels in the sarcoplasmic reticulum (SR) membrane (Smith et al., 1985), controlled by depolarization of the transverse tubule membrane, a process referred to as excitation-contraction coupling (Fleischer and Inui, 1989; Rios et al., 1991). The Ca release channel is a large conductance pore, formed by the multimeric structure of the ryanodine receptor (Wagenknecht et al., 1989). The channel, in isolation, is controlled by physiologically relevant ligands (Smith et al., 1988; Lai et al., 1988; Coronado et al., 1994). Myoplasmic [Ca] regulates opening of the channel through activation and inactivation mechanisms, millimolar concentrations of ATP enhance the channel activity, and slightly acidic pH inhibits the channel open probability (Ma and Zhao, 1994). The Ca release channel has two other properties resembling a ligand-gated channel: the channel allows permeation of both divalent cations (Ca, Mg) and monovalent cations (K, Na) (Smith et al., 1988), and open states of the channel exhibit multiple conductance levels (Ma et al., 1988; Liu et al., 1989; Ma and Zhao, 1994).

One of the characteristic properties of ligand-gated channels is desensitization. For example, the acetylcholine receptor channels open and then close when high concentrations of acetylcholine binds to the receptor (Katz and Thesleff, 1957). The cation channel formed by the ATP receptor desensitizes at large membrane potentials (Friel and Bean, 1988). It is not known, however, whether the skeletal muscle Ca release channels will maintain their normal open-close transitions at large membrane potentials.

Ryanodine receptors in skeletal muscle are highly clustered in the junctional region of the SR membrane, where many other proteins reside, such as calsequestrin, triadin, and FK506 binding protein. Triadin is a 95-kDa glycoprotein that co-precipitates with both the dihydropyridine receptor and the ryanodine receptor (Caswell et al., 1991), which presumably functions as the linker that joins the transverse tubule voltage sensor (dihydropyridine receptor) with the SR Ca release channel. Calsequestrin is a low affinity, high capacity Ca binding protein in the lumen of the SR; its main function is to buffer the Ca inside the SR. Calsequestrin has been shown to alter the rate of Ca release flux from the SR (Ikemoto et al., 1989). FK506 binding protein is the immunophilin in the skeletal muscle (Jayaraman et al., 1992), which co-purifies with the ryanodine receptor. The FK506 binding protein has been shown to alter the multiple conductance states of the Ca release channel (Brillantes et al., 1994). These junctional proteins had modulatory roles in controlling opening of the Ca release channel and thus may participate in the process of excitation-contraction coupling in skeletal muscle.

The present studies were focused on the gating properties of the isolated rabbit skeletal muscle Ca release channel from the junctional SR membranes, particularly at large membrane potentials. The experiments were performed to analyze the desensitization properties that are confined to a portion

Received for publication 10 August 1994 and in final form 28 November 1994.

Address reprint requests to Dr. Jianjie Ma, Department of Physiology and Biophysics, Case Western Reserve School of Medicine, 10900 Euclid Ave., Cleveland, OH 44106. Tel.: 216-368-2684; Fax: 216-368-5586; E-mail: jxm63@po.cwru.edu.

0006-3495/95/03/893/07 $2.00

of the Ca release channels. Heterogeneous behavior of the individual Ca release channels was identified in the present study, which provided indirect evidence for the regulation of the Ca release channel by other junctional SR membrane proteins.

MATERIALS AND METHODS

Isolation of junctional SR membranes from rabbit skeletal muscle

Junctional SR membranes were isolated from rabbit skeletal muscle following the procedure of Meissner (1984). Briefly, skeletal muscle was homogenized in 100 mM NaCl, 2 mM EDTA, 0.1 mM EGTA, and 5 mM Tris-maleate (pH 6.8). Microsome vesicles were obtained after sequential centrifugation at 2,600 × *g* and 35,000 × *g*. The microsome vesicles were loaded onto discontinuous sucrose gradients and the junctional SR membranes were recovered from the 35–40% region of the gradients. The junctional SR membrane vesicles were stored at −70°C at a concentration of 3–5 mg of protein/ml. Four different preparations were used in the present studies.

Planar bilayer reconstitution of skeletal muscle Ca release channel

Lipid bilayer membranes were formed across an aperture of 200 μm diameter with a mixture of phosphatidylethanolamine, phosphatidylserine, and cholesterol (6:6:1); the lipids were dissolved in decane at a concentration of 40 mg of lipid per ml of decane (Ma and Zhao, 1994). The SR vesicles (1–3 μl) were added to the *cis* solution. The recording solution contained symmetrical 200 mM cesium gluconate and 10 mM HEPES-Tris (pH 7.4). The concentration of Ca was buffered with 1 mM EGTA. The free [Ca] was 100 μM in the *cis* solution, and 6–10 μM in the *trans* solution (measured with a Ca-sensitive electrode). The experiments were carried out at room temperature (22–24°C).

Two sets of experiments were performed in the present study. With the native Ca release channel, the concentrations of ATP and free Ca in the *cis* solution were varied to study their effects on the gating of the channel. With the ryanodine-activated channel (1 μM ryanodine), different voltage pulses were applied to study the voltage- and time-dependent gating of the channel.

Orientation of the channel in the lipid bilayer was always *cis*-myoplasmic, *trans*-luminal SR, as determined by the sensitivity of the channel to *cis* Ca and ATP. Those channels with opposite orientation, which account for less than 5% of the total experiments, were not used in the present study.

Analysis of single channel data

Single channel currents were measured with an Axopatch 200A amplifier. Data acquisition was achieved by Digidata 1200 A/D - D/A board (Axon Instruments, Foster City, CA) in combination with a 486 computer. Test pulses of various amplitudes and durations were applied from a holding potential of 0 mV. The linear capacitative current and electrostrictive current were subtracted from the total current by the control, an average of the null sweeps (Ma et al., 1991). The records were filtered at a cutoff frequency of 2 kHz and digitized at a rate of 10 points/ms. The analyses of single channel data were performed with pClamp software.

RESULTS

Inactivation of ryanodine-activated Ca release channel at negative potentials

The first experiment that demonstrated inactivation of the Ca release channel is shown in Fig. 1. Currents through a single

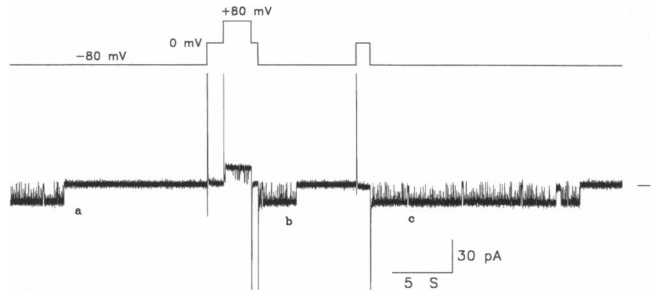

FIGURE 1 Inactivation of the ryanodine-activated Ca release channel at −80 mV. The continuous trace represents currents through a single ryanodine-activated Ca release channel (bilayer 93422). The recording solution contained symmetrical 200 mM cesium gluconate, 10 mM HEPES-Tris (pH 7.5), 1 mM Ca-EGTA (free [Ca] = 6–10 μM), plus 1 μM ryanodine. The *cis* (myoplasmic) solution contained, in addition, 2 mM ATP (Tris salt). The pulse protocol is shown at the top. Polarity of the applied voltage was defined as *cis* minus *trans*. (*a*) Inactivation of the channel at −80 mV. (*b*) Recovery of the channel by a conditioning pulse to +80 mV. (*c*) Recovery of the channel by a conditioning pulse to 0 mV.

Ca release channel in the presence of 1 μM ryanodine were measured in 200 mM symmetrical cesium gluconate. The continuous trace was collected 3 s after switching the test pulse from 0 to −80 mV. The ryanodine-activated channel had a single channel current of 17 pA at −80 mV, which was approximately 50% of the unitary conductance seen with the native Ca release channel (506 pS) (Ma and Zhao, 1994). Two phenomena could be clearly identified: first, there was a consistent decline in channel activity at a sustained negative voltage of −80 mV (*a*); second, the inactivated channel (at −80 mV) could be recovered by a conditioning pulse to +80 (*b*) or 0 (*c*) mV.

Other experiments showed that the observed inactivation was a property of the Ca release channel at large negative membrane potentials. For test potentials of −50 mV or less, no apparent decay of channel activity occurred during the 10-s test pulse (Fig. 2*A*). Actually, persistent channel activity could be measured at −50 mV for 25 min or longer (data not shown). The channel opened continuously without inactivation during the 10-s pulse to large positive voltages (+100

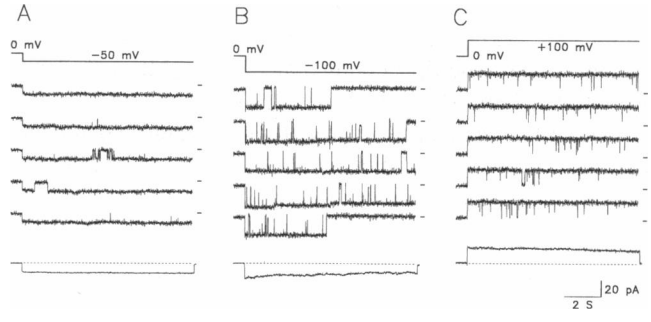

FIGURE 2 Voltage-dependent inactivation of the ryanodine-activated channel. Selected consecutive single channel records through a ryanodine-activated channel with test pulses to −50 mV (*A*), −100 mV (*B*), and +100 mV (*C*) (bilayer 93N23). The corresponding ensemble averages at different voltages are shown in the bottom traces. Marks at the right of each trace correspond to the baseline current.

mV; Fig. 2*C*), whereas a clear inactivation of the channel was measured at −100 mV (Fig. 2*B*). Thus, inactivation of the ryanodine-activated channel had an asymmetric voltage dependence.

To study the time course of inactivation, repetitive test pulses to −100 mV were applied to a single ryanodine-activated channel in the bilayer. A representative experiment is shown in Fig. 3. At the beginning of the test pulse, the channel behaved normally; it opened and closed with voltage and time. However, after 7–10 episodes, the channel failed to respond and appeared to become immobilized. The normal openings of the channel disappeared, which changed to infrequent openings with a small conductance state (Fig. 3*C*). The residue small conductance state associated with desensitization of the Ca release channel was observed in many experiments (n = 7).

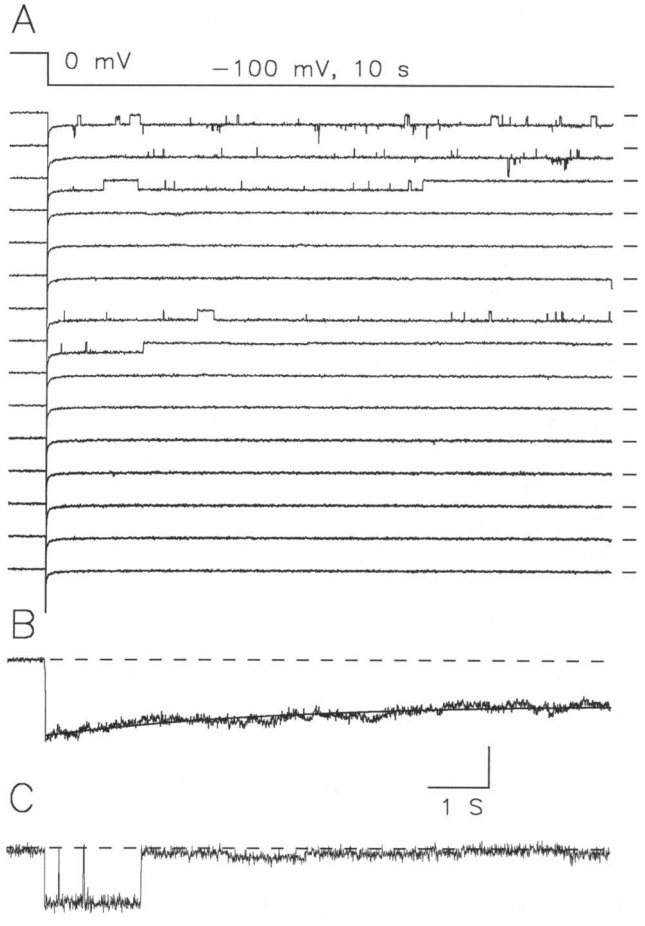

FIGURE 3 Immobilization of the Ca release channel with repetitive test pulses to −100 mV. (*A*) The episodes were collected with test pulses to −100 mV (10-s duration), which were separated by 10 s at 0 mV holding potential (bilayer 93813). The channel became immobilized starting from the seventh episode, and the normal openings changed to infrequent openings with lower conductance states (episodes 11 and 12). (*B*) Ensemble average of repeated episodes at −100 mV. A total of 154 episodes were pooled from eight experiments. The solid line represents the best fit exponential decay function, $y = y_o\, x \exp(-t/\tau) + b$, where $y_o = -17.9 \pm 0.92$ pA (mean ± SE), $\tau = 3.9 \pm 0.4$ s, and $b = -19.0 \pm 0.4$ pA. (*C*) The trace was taken from episode 8 of *A*, after subtraction of capacitative currents. The current scale was 100 pA for *A* and 20 pA for *B* and *C*.

The time constant of channel inactivation at −100 mV could be obtained through an ensemble average of repeated episodes that contained channel activity (Fig. 3*B*). The solid line represents the best fit exponential function. The channel decayed with a time constant of 3.9 s, from full conductance level to ~55% of the open level at the end of the 10-s pulse (Fig. 3*B*).

The completely immobilized channel did not open with a test pulse of −50 mV. However, it could be recovered by a conditioning pulse to positive voltages. The traces shown in Fig. 4 are continuations of the same channel in Fig. 3. After a brief delay of 1–2 s, the channel started reopening at both +80 mV and −100 mV. It is interesting that alternate test pulses between positive and negative voltages prevented channel immobilization. Under such alternate test pulses, the channel remained active for as long as the bilayer lasted. This suggests that the accumulative effect of large negative voltage on the apparent inactivation of the channel probably reflects some slow desensitization process of the ryanodine receptor.

The apparent inactivation at large negative voltages was observed in ~55% (39 of 72 experiments) of the ryanodine-activated channel. Of those 39 experiments that exhibited inactivation, 21 of the channels showed immobilization after repetitive test pulses to large negative voltages.

Desensitization of native Ca release channel

The observed desensitization is an intrinsic property of the ryanodine receptor (not due to the binding of ryanodine), for it was also seen with the native Ca release channel. The records shown in Fig. 5 were taken from a single Ca release channel in the absence of ryanodine, at 100 μM myoplasmic Ca (Fig. 5*A*) and after addition of 2 mM myoplasmic ATP (Fig. 5*B*). It is clear that the channel in the presence of ATP exhibits apparent inactivation at −80 mV.

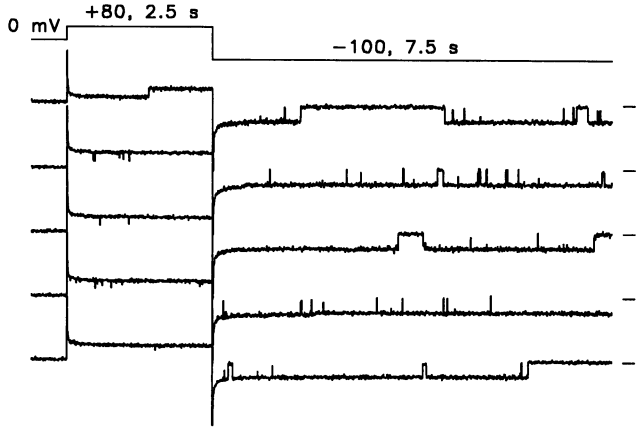

FIGURE 4 Recovery of the immobilized channel by conditioning pulses. The traces were taken approximately 8 s after the end of Fig. 3. The channel started reopening after a brief delay (1–2 s, first episode) at +80 mV. Subsequent test pulses between +80 mV and −100 mV maintained stable channel activity in the bilayer.

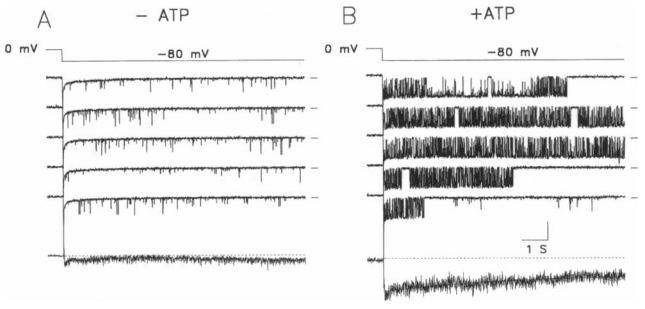

FIGURE 5 Desensitization of the native Ca release channel. The records were taken from the same channel at 100 μM free Ca (*A*) and after addition of 2 mM ATP to the myoplasmic solution (*B*) (bilayer 94207). The bottom traces were ensemble averages of five complete experiments. The solid line is the best fit exponential decay function, $y = -5.63\ x\ \exp(-t/4.94(s)) - 5.38$ (pA). The vertical bar corresponds to a 30 pA current for the single channel traces, and 6 pA for the ensemble averages.

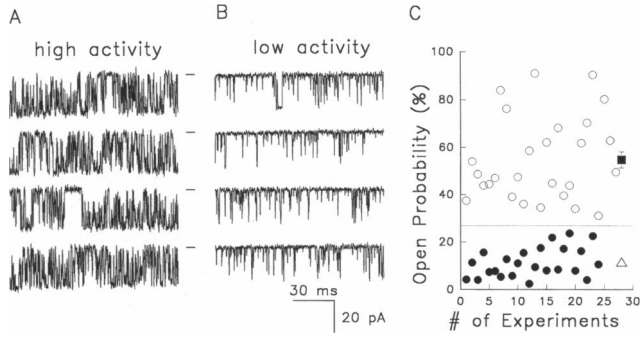

FIGURE 6 Different sensitivity of the native channel to ATP activation. Selected single channel records from a native Ca release channel at −50 mV test potential. (*A*) A high activity channel (bilayer 94D20) (*B*) A low activity channel (bilayer 94D14). The currents were measured under the identical condition of 100 μM Ca and 2 mM ATP present in the *cis* solution. Before the addition of 2 mM ATP, both channels had approximately the same *po* (1–2%). The average open probabilities for the individual experiments are plotted in *C*. Each point represents the averaged *po* over 32–80 episodes (each episode had a duration of 200 ms). The arbitrary dotted line separates the high activity mode (○) from the low activity mode (●) of the channel. The square symbol (■) represents the averaged *po* (54.9 ± 3.4) for the high activity mode ($n = 27$), and the triangle symbol (△) represents the averaged *po* (11.3 ± 1.3) for the low activity mode ($n = 24$; the standard error falls within the size of the symbol). Bilayers with multiple channels were excluded from the analysis.

The isolated native Ca release channel had a weak, but significant, voltage dependence such that the channel had higher open probability when currents moved from the myoplasmic side to the luminal SR side at positive potentials (Sitsapesan and Williams, 1994). The skeletal muscle Ca release channel had an average open probability of 1.1% at a −50 mV test potential in the presence of 100 μM myoplasmic Ca. A significantly higher open probability of 3.6% was measured at +50 mV under identical conditions.

Desensitization of the native Ca release channel differed from the ryanodine-activated channel in that it required strictly the presence of ATP in the myoplasmic solution. Without ATP, desensitization was never observed in the native channel ($n = 16$). However, the ryanodine-activated channel did not require ATP for desensitization ($n = 26$ with ATP; $n = 13$ without ATP).

Desensitization of the native channel is similar to the ryanodine-activated channel in that they both require a large amplitude of test potentials. The time course of inactivation of the native Ca release channel (with ATP) is also similar to that of the ryanodine-activated channel. The average open probability of the channel declined from 50 to 25% with a time constant of 4.9 s (Fig. 5*B*).

Different sensitivity of channel to ATP activation

The reconstituted Ca release channels from rabbit skeletal muscle exhibited multiple gating modes with regard to ATP activation. Fig. 6*A* shows a high activity channel, which was observed in the majority of the experiments. The channel had an average open probability of 54.9 ± 3.4% at 2 mM ATP and 100 μM Ca. Fig. 6*B* shows a low activity channel, which was observed in multiple instances ($n = 24$). This is indeed a Ca release channel with the right orientation (*cis*-myoplasmic, *trans*-luminal SR) in the bilayer, because it responded accordingly to addition of Ca or EGTA to the *cis* solution, and it could also bind to ryanodine. The difference is that ATP had a significantly lower effect on the channel. The average open probability at 100 μM Ca was increased from 1.1% to only 11.3 ± 1.3% upon addition of 2 mM ATP.

The histogram shown in Fig. 6*C* represents the channel open probability at −50 mV for the 51 individual experiments at 100 μM Ca and 2 mM ATP. It is clear that there were two groups or modes of channel openings: a high activity mode with an average open probability of ~55% and a low activity mode with an average open probability of ~12%.

Conversion from the low activity mode to the high activity mode was never observed. In a few experiments, the channels showed an irreversible and abrupt change from high activity to low activity in the later stage of the experiment. This suggests the following two possibilities: there were either two functionally different populations of Ca release channels or the different gating modes of the channel were due to the role of some putative modulatory proteins.

Fast and slow kinetic effects of ryanodine

To further understand the heterogeneity of the gating properties of the Ca release channel, the kinetic effect of ryanodine on the channel was studied. Fig. 7 shows the sequence of events that occurred upon addition of 1 μM ryanodine to the channels at 100 μM Ca and 2 mM ATP. Fig. 7*A* was taken at +100 mV, starting 2 min after the addition of ryanodine. The fast effect of ryanodine could be clearly seen at the end of the first test pulse. After the binding of ryanodine, the channel changed its kinetics from fast gating to slow gating. The bilayer contained two channels (see episode 8, Fig. 7*A*). One was already bound, whereas the other had not bound to ryanodine.

The traces shown in Fig. 7*B* were collected 9 min after those shown in *A*. It was clear that the ryanodine-activated

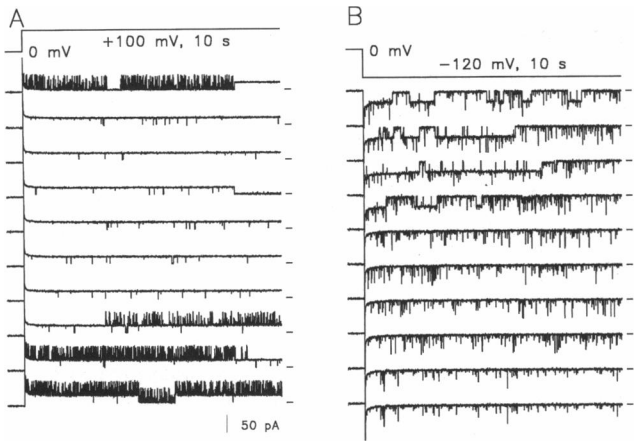

FIGURE 7 Fast and slow kinetic effects of ryanodine. (*A*) Consecutive episodes were collected 2 min after the addition of 1 μM ryanodine to both *cis* and *trans* solutions. There were two channels in the bilayer (see episode 8). Ryanodine bound to the first channel during the first episode, which modified the channel gating kinetics (bilayer 93810). (*B*) The episodes were collected 9 min after *A*. The ryanodine-bound channel desensitized with voltage, which became immobilized after the fifth episode. The channel that had not bound to ryanodine stayed open continuously.

channel desensitized with voltage, and eventually it became immobilized, as the characteristic long open events of the ryanodine-activated channel disappeared after the fifth episode (Fig. 7*B*). However, the channel that had not bound to ryanodine remained open continuously. This channel had low open probability ($po = 9\%$), suggesting that it probably belonged to the low ATP affinity type of channel (Fig. 6*B*). Similar phenomena were observed in four other experiments.

The results suggest the following: there were two kinetic effects of ryanodine, fast and slow, and the faster ryanodine binding channels desensitized with voltage, whereas the slow binding channels did not desensitize.

DISCUSSION

The skeletal muscle Ca release channels are allosteric proteins of the ryanodine receptor, with many properties resembling ligand-gated channels. The present study identified a characteristic behavior of the Ca release channel, voltage- and ligand-dependent desensitization, which is common to many other ligand-gated channels. Desensitization of the skeletal muscle ryanodine receptor had the following three properties: 1) it required a large membrane potential, 2) repetitive test pulses led to immobilization of the channel, and 3) the phenomena were observed in only a portion of the channels (not all channels showed desensitization).

Previous studies with the Ca release channel were mostly performed at a potential range from −50 mV to +50 mV (Smith et al., 1986; Rousseau et al., 1988), probably due to the difficulty of maintaining stable channels at large membrane potentials. In the present study, the activities of the single Ca release channel were measured by using pulse protocols. By keeping the holding potential of the bilayer at 0 mV, the high voltage-induced steady state inactivation of the channel was avoided. Thus, it is possible to study the kinetics of channel inactivation and the time course of recovery after inactivation at different voltages.

Opening of the isolated native Ca release channel had a weak voltage dependence that favored the movement of cations into (rather than out of) the SR membrane, as the channel had higher open probabilities when the potential in the myoplasmic side is more positive than the luminal SR side (Ma et al., 1988; Sitsapesan and Williams, 1994). Desensitization of the Ca release channel was observed when current moved from the SR lumen to the myoplasmic side, which occurred predominantly at large negative voltages. This asymmetric voltage-dependent gating and voltage-dependent desensitization of the Ca release channel is consistent with the asymmetric structure of the ryanodine receptor with a large myoplasmic domain (foot structure) and a small membrane-spanning domain (channel pore-forming region) (Wagenknecht et al., 1989).

The correlation between repetitive test pulses and immobilization of the channel (Fig. 3) suggests that desensitization reflects a slow conformational change of the ryanodine receptor complex. The functional Ca release channel contained a tetrameric structure of the 560-kDa ryanodine receptor polypeptide. The adaptation of the Ca release channel at large negative potentials is likely due to an altered interaction among the monomers of the ryanodine receptor. This may account for the changes in the conductance state of the channel (from large to small) once the channel became immobilized (Fig. 3).

The ligand-dependent gating properties of the Ca release channel changed significantly upon binding to ryanodine (Buck et al., 1992; Ma, 1993). The ryanodine-activated channel had a reduced single channel conductance (508 pS for the native channel and 255 pS for the ryanodine-activated channel in 200 mM symmetrical Cs) (Ma and Zhao, 1994), which existed in a near permanent open configuration. In terms of desensitization, there was a clear difference between the native release channel and the ryanodine-activated channel. The native channel required the presence of ATP in the myoplasmic solution whereas the ryanodine-activated channel did not require ATP to enter the desensitization state. The open probability at which desensitization occurred is ~55% for the native channel and ~70% (the average *po* measured at −80 mV) for the ryanodine-activated channel. As the main effect of ATP was to enhance the maximum open probability of the channel without having a significant effect on the Ca-dependent regulation of the channel (Ma and Zhao, 1994), this indicated that desensitization occurred mainly through the open state of the channel.

Binding of ryanodine to the ryanodine receptor had a close correlation with the open configuration of the Ca release channel. Conditions that favor opening of the channel, such as micromolar Ca and millimolar ATP, also favor interaction of ryanodine with the channel (Inui et al., 1987; Mitchalak et al., 1988; Chu et al., 1990; Pessah and Zimanyi, 1991). The distinct fast and slow kinetic effects of ryanodine on the separate channels in the same bilayer (Fig. 7) suggested that

the two channels were either structurally different or the conformation states of the two channels were different. As high densities of ryanodine receptors are localized in the junctional region of the SR membrane, it is likely that the Ca release channels may interact with each other. As a consequence, binding of ryanodine to the first channel could alter the conformation of the neighboring channels, thus slowing down the process of further ryanodine binding.

The Ca release channels obtained from the isolated junctional SR membranes exhibited heterogeneity in gating with regard to activation by ATP. The observed different open behavior of the Ca release channel in response to ATP activation could be due to the following four possibilities: 1) there were different populations of Ca release channels in rabbit skeletal muscle; 2) the channel had different sensitivity to Ca activation (Bull and Marengo, 1993); 3) there was a phosphorylation-dephosphorylation-mediated effect on the channel; or 4) the apparent variation in channel open probability was a result of the interaction between the ryanodine receptor and certain regulatory proteins. The fact that there was occasional conversion from a high activity mode to a low activity mode of the channel indicated an involvement of other protein-mediated effects on the Ca release channel. The many different junctional SR membrane proteins, such as calsequestrin or triadin, could all interact with the ryanodine receptor, thus altering the gating properties of the Ca release channel.

In our previous studies with pH regulation of the Ca release channel (Ma and Zhao, 1994), it was shown that the channel had a highly cooperative and hysteretic response to changes in proton concentrations. The dose-response curves that describe the acidic titration and alkaline recovery of the channel were separated by ~1 pH unit. Of relevance to this study was the observation that there were two types of recovery of the ryanodine-activated channel after the treatment of low pH: complete recovery of full conductance states at nanomolar free Ca and partial recovery of subconductance states at micromolar free Ca. A possible explanation for the partial recovery of channel activity is that at low pH some regulatory proteins dissociated from the channel protein may alter the structure of the channel complex. On the basis of studies of Brillantes et al. (1994), it is possible that FK506 binding protein could be involved in this type of interaction.

The SR membrane is near 0 mV at rest and at most produces approximately a 7 mV potential change during the release of Ca (Somlyo et al., 1977). The observed desensitization of the individual Ca release channels in bilayer cannot be taken at face value, as it required large membrane potential changes. The desensitization phenomenon and heterogeneity in response to ATP and ryanodine could be largely due to the result of altered (or different) protein-protein interactions of the channel complex within the artificial lipid bilayer. One of the limitations of the bilayer reconstitution studies is the use of the symmetrical cesium gluconate (200 mM) as the current carrier through the Ca release channel, which is far from close to physiological conditions. It will be interesting to see how the channel would behave if a gradient of Ca (present in the *trans* solution) were used as the current carrier.

In summary, understanding the desensitization properties of the ryanodine receptor should lead to more insight into the structure-function relationship of the Ca release channel. Additional studies of the interaction between the junctional proteins and the ryanodine receptor are essential for understanding the regulation of the Ca release channel and the mechanism of excitation-contraction coupling in skeletal muscle.

Supported by grants from the National Institutes of Health (R29-AR42057) and Muscular Dystrophy Association. J. Ma is an Established Investigator of the American Heart Association.

REFERENCES

Brillantes, A. B., K. Ondrias, A. Scott, E. Kobrinsky, E. Ondriasova, M. C. Moschella, T. Jayaraman, M. Landers, B. E. Ehrlich, and A. R. Marks. 1994. Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell.* 77:513–523.

Buck, E., I. Zimanyi, J. J. Abramson, and I. N. Pessah. 1992. Ryanodine stabilizes multiple conformational states of the skeletal muscle calcium release channel. *J. Biol. Chem.* 267:23560–23567.

Bull, R., and J. Marengo. 1993. Sarcoplasmic reticulum release channels from frog skeletal muscle display two types of calcium dependence. *FEBS Lett.* 331:223–227.

Caswell, A. H., N. R. Brandt, J.-P. Brunschwig, and S. Purkerson. 1991. Localization and partial characterization of the oligomeric disulfide-linked molecular weight 95,000 protein (triadin) which binds the ryanodine and dihydropyridine receptors in skeletal muscle triadic vesicles. *Biochemistry.* 30:7507–7513.

Chu, A., M. Diaz-Munoz, M. J. Hawkes, K. Brush, and S. L. Hamilton. 1990. Ryanodine as a probe for the functional state of the skeletal muscle sarcoplasmic reticulum calcium release channel. *Mol. Pharmacol.* 37: 735–741.

Coronado, R., J. Morrissette, M. Sukhareva, and D. M. Vaughan. 1994. Structure and function of ryanodine receptors. *Am. J. Physiol.* 266: C1485–C1504.

Fleischer, S., and M. Inui. 1989. Biochemistry and biophysics of excitation-contraction coupling. *Annu. Rev. Biophys. Biophys. Chem.* 18:333–364.

Friel, D. D., and B. P. Bean. 1988. Two ATP-activated conductances in bullfrog atrial cells. *J. Gen. Physiol.* 91:1–27.

Ikemoto, N., M. Ronjat, L. G. Meszaros, and M. Koshita. 1989. Postulated role of calsequestrin in the regulation of calcium release from sarcoplasmic reticulum. *Biochemistry.* 28:6764–6771.

Inui, M., A. Saito, and S. Fleischer. 1987. Purification of the ryanodine receptor and identity with feet structures of junctional terminal cisternae of sarcoplasmic reticulum from fast skeletal muscle. *J. Biol. Chem.* 262: 1740–1747.

Jayaraman, T., A.-M. Brillantes, A. P. Timerman, S. Fleischer, H. Erdjument-Bromage, P. Tempst, and A. R. Marks. 1992. FK506 binding protein associated with the calcium release channel (ryanodine receptor). *J. Biol. Chem.* 267:9474–9477.

Katz, B., and S. Thesleff. 1957. A study of the 'desensitization' produced by acetylcholine at the motor end-plate. *J. Physiol.* 138:63–80.

Lai, F. A., H. Erickson, E. Rousseau, Q. Y. Liu, and G. Meissner. 1988. Purification and reconstitution of calcium release channel from skeletal muscle. *Nature.* 331:315–319.

Liu, Q. Y., A. Lai, E. Rousseau, R. V. Jones, and G. Meissner. 1989. Multiple conductance states of the purified calcium release channel complex from skeletal sarcoplasmic reticulum. *Biophys. J.* 55:415–424.

Ma, J. 1993. Block by ruthenium red of the ryanodine-activated calcium release channel of skeletal muscle. *J. Gen. Physiol.* 102:1031–1056.

Ma, J., M. Fill, C. M. Knudson, K. P. Campbell, and R. Coronado. 1988. Ryanodine receptor of skeletal muscle is a gap junction-type channel. *Science.* 242:99–102.

Ma, J., C. Mundina-Weilenmann, M. M. Hosey, and E. Rios. 1991. Dihydropyridine-sensitive skeletal muscle Ca channels in polarized planar bilayers. I. Kinetics and voltage dependence of gating. *Biophys. J.* 60:890–902.

Ma, J., and J. Y. Zhao. 1994. Highly cooperative and hysteretic response of the skeletal muscle ryanodine receptor to changes in proton concentrations. *Biophys. J.* 67:626–633.

Meissner, G. 1984. Adenine nucleotide stimulation of Ca-induced Ca release in sarcoplasmic reticulum. *J. Biol. Chem.* 259:2365–2374.

Mitchalak, M., Dupraz, P., and Shoshan-Barmatz, V. 1988. Ryanodine binding to sarcoplasmic reticulum membrane: comparison between cardiac and skeletal muscle. *Biochim. Biophys. Acta.* 939:587–594.

Pessah, I. N., and I. Zimanyi. 1991. Characterization of multiple [^{3}H]ryanodine binding sites on the Ca^{2+} release channel of sarcoplasmic reticulum from skeletal and cardiac muscle: evidence for a sequential mechanism in ryanodine action. *Mol. Pharmacol.* 39: 679–689.

Rios, E., J. Ma, and A. Gonzalez. 1991. The mechanical hypothesis of excitation-contraction (EC) coupling in skeletal muscle. *J. Muscle Res. Cell. Motil.* 12:127–135.

Rousseau, E., J. Ladine, Q.-Y. Liu, and G. Meissner. 1988. Activation of the Ca release channel of skeletal muscle SR by caffeine and related compounds. *Arch. Biochem. Biophys.* 267:75–86.

Sitsapesan, R., and A. J. Williams. 1994. Regulation of the gating of the sheep cardiac sarcoplasmic reticulum Ca-release channel by luminal Ca. *J. Membr. Biol.* 137:215–226.

Smith, J. S., R. Coronado, and G. Meissner. 1985. Sarcoplasmic reticulum contains adenine nucleotide-activated calcium channels. *Nature.* 316:446–449.

Smith, J. S., R. Coronado, and G. Meissner. 1986. Single channel measurements of the calcium release channel from skeletal muscle sarcoplasmic reticulum. *J. Gen. Physiol.* 88:573–588.

Smith, J. S., T. Imagawa, J. Ma, M. Fill, K. P. Campbell, and R. Coronado. 1988. Purified ryanodine receptor from rabbit skeletal muscle is the calcium-release channel of sarcoplasmic reticulum. *J. Gen. Physiol.* 92:1–26.

Somlyo, A. V., H. Shuman, and A. P. Somlyo. 1977. Elemental distribution in striated muscle and the effects of hypertonicity. *J. Cell Biol.* 74:828–857.

Wagenknecht, T., R. Grassucci, J. Frank, A. Saito, M. Inui, and S. Fleischer. 1989. Three-dimensional architecture of the calcium channel/foot structure of sarcoplasmic reticulum. *Nature.* 338:167–170.