Highly Cooperative and Hysteretic Response of the Skeletal Muscle Ryanodine Receptor to Changes in Proton Concentrations

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ABSTRACT Ryanodine receptors are key molecules in excitation-contraction coupling of skeletal muscle. They form the pore of the calcium release channel, which is regulated by Ca and ATP. Multiple proton titration sites are involved in controlling the different open states of the channel, as indicated by the following: i) the channel had a biphasic response to changes in proton concentrations around neutral pH; ii) the activities of the channel were inhibited by acidic pHs in a highly cooperative manner; and iii) the channel exhibited pronounced hysteresis to changes in pH. Four distinct conductance states can be identified in the single ryanodine-activated calcium release channel. The distribution of the multiple conductance states depends on the level of [Ca], ATP, and pH in the recording solution. The data are consistent with the multimeric structure of the skeletal muscle ryanodine receptor.

INTRODUCTION

The dihydropyridine receptor of the transverse tubule (TT) membrane and the ryanodine receptor of the sarcoplasmic reticulum (SR) membrane are the two major proteins involved in excitation-contraction (E-C) coupling of skeletal muscle (Fleischer and Inui, 1989; Numa et al., 1990; Rios et al., 1992; McPherson and Campbell, 1993). The ryanodine receptor is a single polypeptide of ~560 kDa that normally exists in a homotetrameric structure (Inui et al., 1987; Imagawa et al., 1987; Lai et al., 1988), which contains two functional domains: a C-terminal hydrophobic domain that forms the conduction pore of the Ca release pathway, and a large hydrophilic domain that spans the junctional gap between the TT and SR membranes (Takeshima et al., 1989, Wagenknecht et al., 1989; Zorzato et al., 1990). The dihydropyridine receptors sense the electrical excitation signal across the TT membrane, thereby triggering the opening of the Ca release channel that allows for contraction of the skeletal muscle (Rios et al., 1991). Although the ryanodine receptor has been purified and cloned, very little is known on the structure of the calcium release channel, particularly the number of the 560 kDa polypeptides that are involved, and the interactions among these polypeptides, in forming the functional channel.

The Ca release channel is a large nonselective pore with many properties that resemble a ligand-gated channel (Smith et al., 1985, 1988; Ma et al., 1988; Ma, 1993). The activity of the channel reconstituted into lipid bilayers is controlled by myoplasmic Ca concentration through activation and inactivation mechanisms. Ca in the nanomolar to micromolar concentration range activates the channel, whereas in the micromolar to millimolar concentration range it inhibits the channel. Millimolar ATP in the myoplasmic solution enhances the channel activity without altering the Ca-dependent regulation of the channel (Zhou et al., 1994).

The skeletal muscle Ca release channel is sensitive to changes in proton concentrations. Slightly acidic pHs (pH 6–7) inhibit the channel activity (Ma et al., 1988; Rousseau and Pinkos, 1990). The mechanism of inhibition, however, is not well understood. Studies carried out in this paper are focused on the following three questions. First, are the effects of pH reversible? Second, what are the cooperative interactions among Ca, ATP, and pH on the Ca release channel? Third, how does pH affect the multiple conductance states of the channel?

MATERIALS AND METHODS

Heavy SR membranes were isolated from the rabbit skeletal muscle following the procedure of Meissner (1984). Briefly, vesicles containing the Ca release channel were recovered from the 35–40% region of sucrose gradients that contained membranes sedimenting at 2,600–35,000 × *g*. The membrane vesicles were stored at −80°C at a concentration of 3–5 mg protein/ml. Five different preparations were used in the present studies.

Planar bilayers were formed across an aperture of 200 μm diameter with a mixture of phosphatidylethanolamine : phosphatidylserine : cholesterol (6 : 6 : 1); the lipids were dissolved in decane at a concentration of 40 mg lipid/ml decane. The SR vesicles (1–3 μl) were added to the *cis* solution. The recording solution contained symmetrical 200 mM Cs-gluconate. pH was buffered with 10 mM HEPES, and Ca was buffered with 1 mM EGTA. The initial free [Ca] in the *cis* solution was 6 μM; the concentration can be increased (or decreased) by adding calibrated amount of $CaCl_2$ (or EGTA). The free [Ca] in the *trans* solution was always 6 μM (measured with a Ca sensitive electrode). Changes in pH were achieved by adding calibrated amount of CsOH or HCl to both *cis* and *trans* solutions. To achieve equilibrium or pseudo-equilibrium open probability of the channel, the measurements were always started 3–5 min after each change in pH. The experiments were performed at room temperature (22–24°C).

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 accounts for less than 5% of the total experiments, were not used in the present study.

Single channel currents were measured with an Axopatch 200A amplifier. A pulse protocol was used, that started from a holding potential of 0 mV to different test voltages. The acquisition started 250 ms after the pulse,

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to avoid the capacitance transient. 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 the pClamp software. Bilayers with multiple channels were excluded from the analysis.

RESULTS

Ca and ATP dependence of the skeletal muscle Ca release channel

To measure the Ca release channel activity from the heavy SR vesicles, the following recording solution was used: 200 mM Cs-gluconate, 10 mM HEPES-TRIS (pH 7.5), 6 μM free [Ca] (buffered with 1 mM EGTA). The use of Cs as the current carrier allows for buffering of the free [Ca] to any desired level. In addition, Cs eliminates the K channel activities that are present in the SR membranes. The large anion gluconate do not permeate through the Cl channel present in the SR vesicles.

The reconstituted Ca release channel from rabbit skeletal muscle had a linear conductance of 508 ± 26 pS in 200 mM symmetrical Cs (data not shown). The open probability ranged from 0.008 ± 0.002 (mean ± SE) at 6 μM [Ca] to a maximum P_{max} = 0.584 ± 0.042 at 100 μM $[Ca]_i$ and 2 mM [ATP] (n = 42). Addition of 1 μM ryanodine resulted in the characteristic changes of reduced conductance (255 ± 17 pS) and increased mean open lifetime of the channel (Fig. 1 *A*).

The channel had a bell-shaped dependence on myoplasmic (*cis*) [Ca] (Fig. 1 *B*). Movements of Cs currents through the channel (from the luminal SR to the myoplasmic side at −50 mV test potential) had affinities for Ca activation and inactivation of 20 and 300 μM, respectively. Addition of 1 mM ATP to the myoplasmic solution increased the maximum open probability of the channel (P_{max} = 48%), without altering the Ca-dependent activation and inactivation properties of the channel (Fig. 1 *C*).

pH regulation of the native Ca release channel

To study the pH regulation of the Ca release channel, we started with a condition that gave the maximum open probability of the channel (100 μM Ca and 2 mM ATP, see Fig. 1 *C*). The channel had a complicated response to changes in proton concentrations (Fig. 2). Starting from pH 7.5, we consistently saw an increase in channel open probability in either acidic or alkaline directions. Two clear peaks can be identified at pH 7.2 and 8.5 (Fig. 2 *C*, *open circles*). Further decrease of pH from 7.2 to 5.6 resulted in a monotonic decrease in channel activity. Half maximal inhibition of the channel occurred at pK = 6.5, with a Hill coefficient of n_H = 3–4.

Open time histogram analysis revealed two different gating states associated with the Ca release channel recorded in 100 μM Ca and 2 mM ATP (Fig. 3). At a test potential of −50 mV, the two open states had mean lifetimes of approximately τ_{O1} = 0.40 ms and τ_{O2} = 2.8 ms. The relative occurrence of τ_{O2} ($y_{O2}/y_{O1}+y_{O2}$) varies with pH in the so-

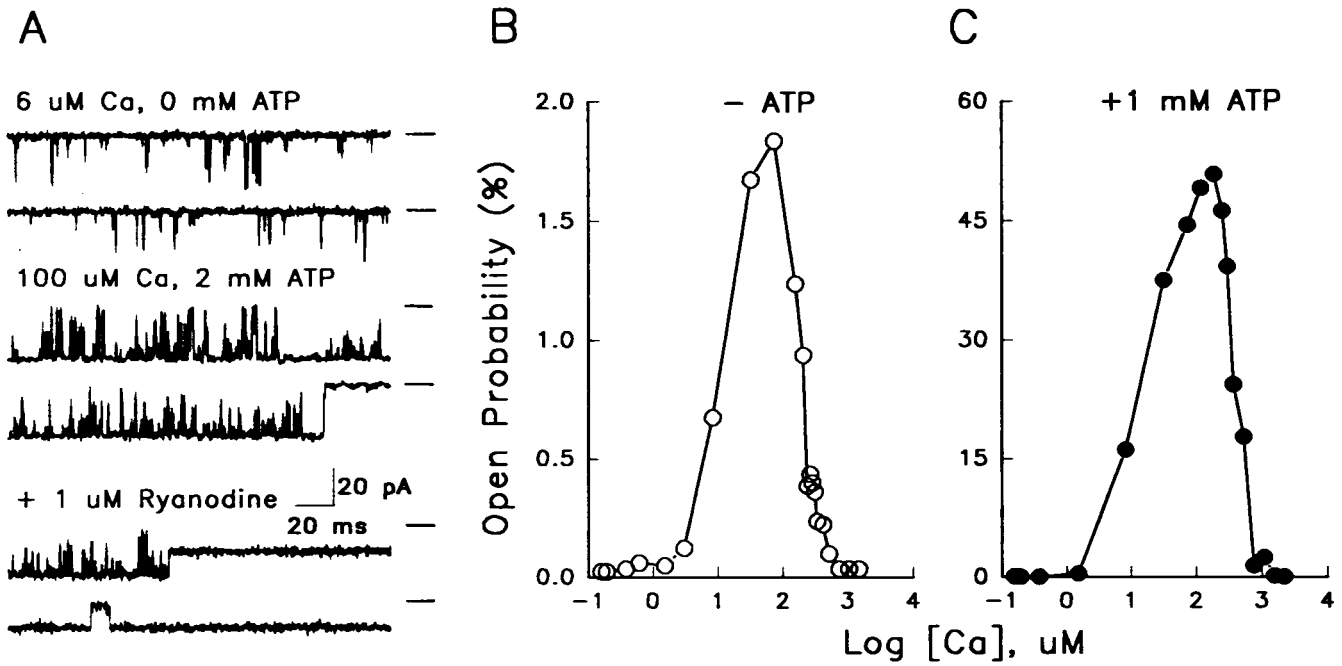

FIGURE 1 Regulation by Ca and ATP of the rabbit skeletal muscle Ca release channel. (*A*) Selected single channel currents at −50 mV. The currents were measured with 200 mM "symmetrical" Cs-gluconate, 10 mM HEPES-TRIS (pH 7.5). Records were taken from one complete experiment (Bilayer #92D30). The starting [Ca] in the *cis* solution was 6 μM (*top* traces), which was increased to 100 μM after addition of 2 mM ATP (tris salt) to the *cis* solution (*middle* traces). Four minutes after adding 1 μM ryanodine to both solutions, the characteristic changes from high conductance state with fast open-close transitions to low conductance state with slow open-close transitions were observed (*bottom* traces). Marks at the right of each trace correspond to the baseline current. Channel open probability as a function of myoplasmic Ca in the absence of ATP (*B*), and in the presence of 1 mM ATP (*C*). *B* contained data from four experiments. *C* contained data from two experiments.

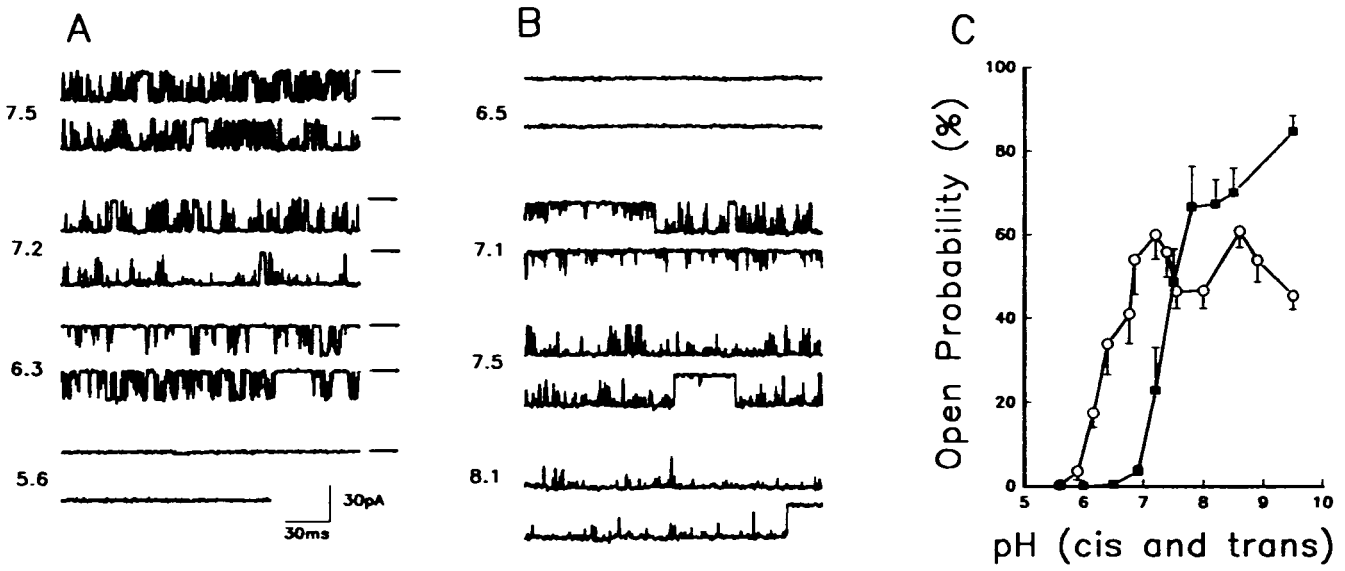

FIGURE 2 pH dependence of the native Ca release channel at 100 μM Ca and 2 mM ATP. (*A*) Acidic titration and (*B*) alkaline recovery of the same channel (Bilayer #93113). Selected single channel records at the given pH value were obtained at a test potential of −50 mV (*cis* minus *trans*). The *cis*-myoplasmic solution contained 100 μM free [Ca] and 2 mM ATP. Changes in pH were achieved by adding calibrated amount of CsOH or HCl to both *cis* and *trans* solutions. (*C*) Channel open probability versus pH. The acidic titration (○) and alkaline recovery (■) curves were constructed with 13 experiments. The individual data points were average of at least four experiments. The vertical bars represent the SE of the mean.

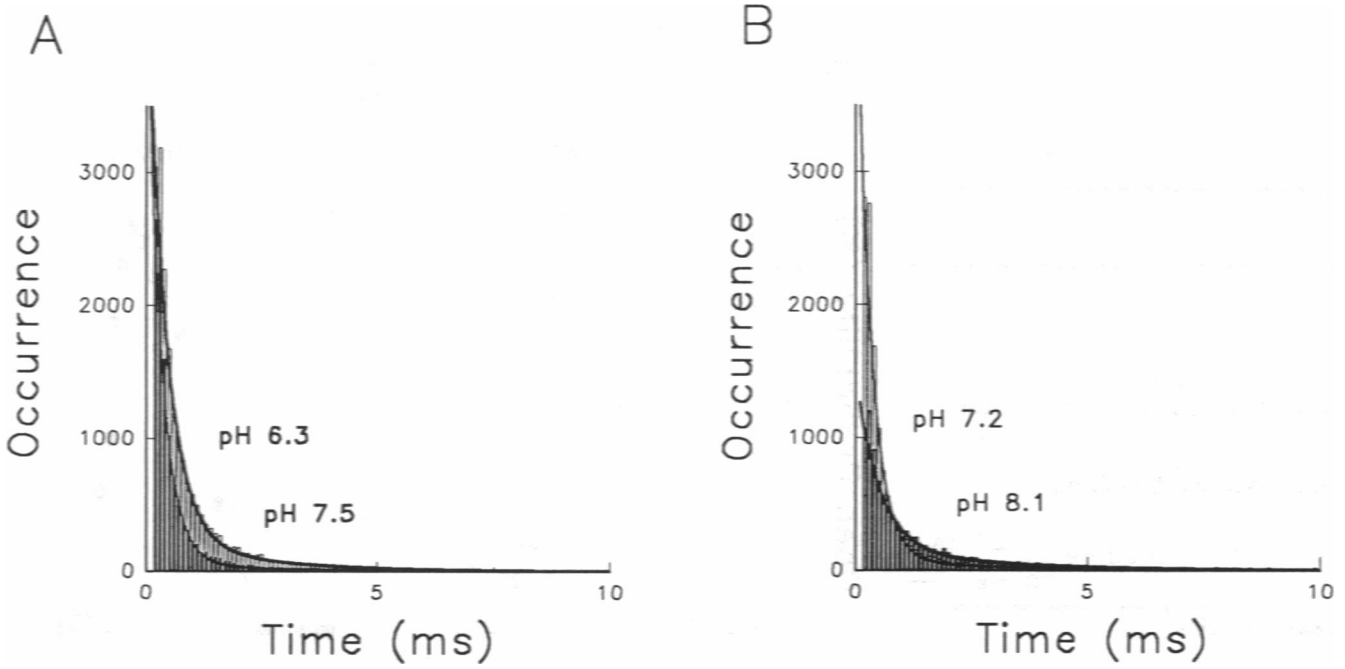

FIGURE 3 Open time histogram analysis at −50 mV. Open events were calculated at −50 mV test potential, from four separate experiments (Bilayer #93113, #M3113, #93714, and #A3714). The histograms of the reference channels at pH 7.5 and 6.3 (*A*) were constructed with 19632 and 11520 open events, respectively. The histograms of the recovered channel at pH 7.2 and 8.1 contained 10318 and 10094 events. The smooth lines represent the best fits according to the following equation:

$$y = y_{O1}/\tau_{O1}\exp(-t/\tau_{O1}) + y_{O2}/\tau_{O2}\exp(-t/\tau_{O2}).$$

The parameters were: τ_{O1} = 0.40 ms, τ_{O2} = 2.20 ms, y_{O1}/y_{O2} = 1848/556 at pH 7.5; τ_{O1} = 0.31 ms, τ_{O2} = 2.67 ms, y_{O1}/y_{O2} = 1422/149 at pH 6.3 for the reference channel. τ_{O1} = 0.28 ms, τ_{O2} = 2.52 ms, y_{O1}/y_{O2} = 1151/236 at pH 7.2; τ_{O1} = 0.50 ms, τ_{O2} = 2.91 ms, y_{O1}/y_{O2} = 673/491 at pH 8.1 for the recovered channel.

lution; $y_{O2}/y_{O1}+y_{O2}$ was 0.23 and 0.10 at pH 7.5 and 6.3, respectively (Fig. 3 *A*).

The effects of pH on the native Ca release channel exhibited a pronounced hysteresis (Fig. 2 *B*). To recover the channel that was closed at pH 5.6, more alkaline pH was required. The channels started reopening only at pH = 6.8, with maximal recovery at pH 8–9. The recovered channel seemed to have lost the proton regulatory sites manifested by the minimum at pH 7.5, because no clear minimum was found in the alkaline recovery curve (Fig. 2 *C*, *filled squares*). Significant differences can be seen in the distribution of the open lifetimes. At pH 7.2, the recovered channel had τ_{O1} = 0.28 ms and τ_{O2} = 2.5 ms with a ratio of $y_{O2}/y_{O1}+y_{O2}$ = 0.17; the occurrence of τ_{O2} was more frequent than that of the channel before the treatment of low pH (comparing the control channel at pH 6.3 with the recovered channel at pH 7.1, which had similar open probabilities). At pH 8.1, the mean open lifetimes of the recovered channel remained unchanged (τ_{O1} = 0.50 ms and τ_{O2} = 2.9 ms); however, the relative occurrence of the longer open state (τ_{O2}) increased significantly. The ratio of $y_{O2}/y_{O1}+y_{O2}$ was 0.42 at pH 8.1.

The observed hysteresis of the Ca release channel did not seem to depend on the presence of ATP in the myoplasmic solution. Fig. 4 shows the pH titration experiments that were carried out at 20 μM Ca and 0 mM ATP. The average open probability of the channel under this condition was 0.025 ± 0.007 (n = 7) at pH 7.6, it became essentially zero at pH 5.4. It is clear that recovery of the channel requires more alkaline pHs. The dose responses of the alkaline recovery (*filled circles*) and acidic titration was separated by approximately 1 pH unit (Fig. 4 *B*).

We also did similar experiments at lower Ca concentration in the presence of ATP (6 μM Ca, 2 mM ATP). At 6 μM Ca and 2 mM ATP, the average open probability of the channel was 0.057 ± 0.010 (n = 8) at pH 7.6. The acidic titration of the channel under this condition had a pK of 6.3. Recovery of the channel at alkaline pHs was tested in three experiments, in which meaningful channel activity was only observed at pH 6.8 or higher. Thus, it appears that the pH-dependent hysteresis of the Ca release channel do not dependent on the myoplasmic concentration of Ca.

Hysteresis dependence of the ryanodine-activated channel

Ryanodine is a plant alkaloid that produces irreversible contracture in skeletal muscle by acting specifically on the Ca release channel (Jendon and Fairhurst, 1969; Rousseau et al., 1987). The binding of ryanodine resulted in a reduction of the single channel conductance by ~50% (from 508 ± 26 to 255 ± 17 pS) (Fig. 1 *A*).

The open conductance state of the ryanodine activated channel lost its essential Ca dependence of activation. In the presence of 1 μM ryanodine, the channel remained fully open at 50 nM [Ca] (myoplasmic) (Fig. 5 *A*), unlike the native channel (in the absence of ryanodine), which was completely closed at $[Ca]_i$ < 100 nM (Fig. 1 *B*). Moreover,

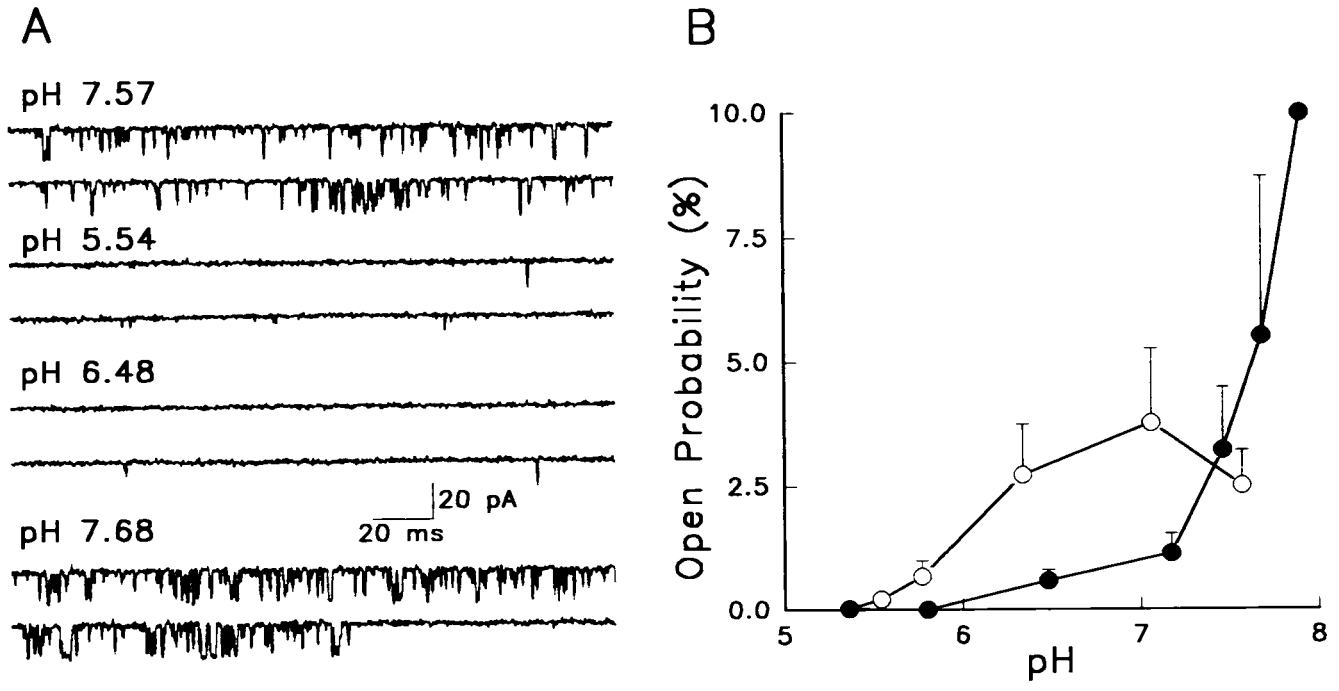

FIGURE 4 pH dependence of the native Ca release channel at 20 μM Ca. (*A*) Selected single channel records were taken from one complete experiment (Bilayer #93629). The test voltage was −50 mV. The *cis*-myoplasmic solution contained 20 μM free [Ca], 0 mM ATP. (*B*) Channel open probability versus pH. The acidic titration (○) contained average over seven experiments, and the alkaline recovery (■) contained average over three complete experiments. The vertical bars represent the SEM.

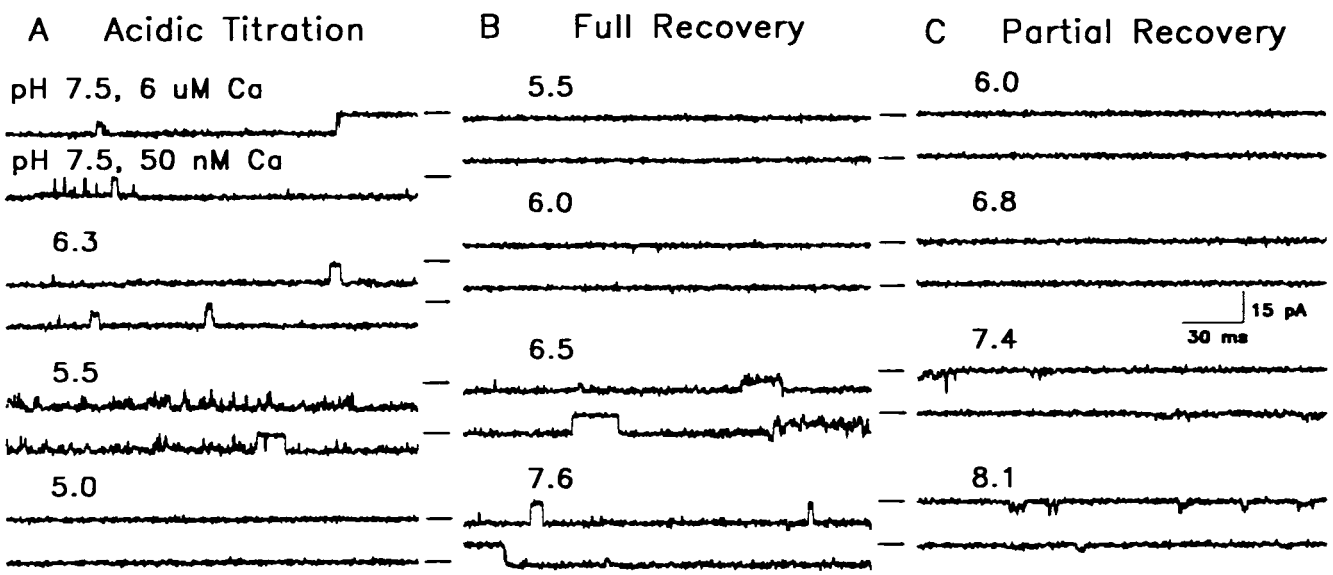

FIGURE 5 pH dependence of the ryanodine-activated Ca release channel. Upon successful incorporation of a single Ca release channel in the lipid bilayer, 1 μM ryanodine was added to both solutions. The ryanodine activated channel had a characteristic long open lifetime, with a dominant conductance state of 225 ± 17 pS (200 mM Cs current carrier). All records were taken at a test potential of −50 mV. (*A*) Acidic titration of the ryanodine activated channel. Upper trace at pH 7.5 were measured at a free [Ca] of 6 μM. All subsequent records were taken after addition of 1 mM EGTA to the *cis* solution, which reduced the free [Ca] to 50 nM. The measurements at each adjacent pH were separated by 5 min to allow for establishment of steady-state effect (Bilayer #93610). (*B*) Full recovery of the ryanodine activated channel. Single channel currents were taken from the same experiment as *A*. The records were representative of three out of seven complete experiments at 50 nM [Ca], and two out of seven experiments at 2 mM ATP, 6 μM Ca. (*C*) Partial recovery of the ryanodine activated channel. The records were taken from a separate experiment at 20 μM free [Ca] (Bilayer #92D18). They represent 11 out of 11 experiments performed at 6–20 μM Ca, three out of seven experiments performed at 50 nM Ca, and five out of seven experiments at 2 mM ATP, 6 μM Ca.

pH regulation of the ryanodine activated channel was shifted in the acidic direction, and the response became much steeper (Fig. 5). Reduction of pH from 7.5 to 5.5 had nearly no effect on the open probability of the channel. Complete and sudden closure of the channel occurred within a narrow window of pH, ranging from pH 4.8 to 5.2 depending on the concentration of Ca and ATP in the myoplasmic solution (Fig. 6). The large SDs in the dose response curves (Fig. 6) were caused by the instability of the channel near the critical point of channel break down.

The ryanodine-activated channel also exhibited hysteretic response to changes in pH (Fig. 6). With only Ca present in the myoplasmic solution, complete inhibition of the channel occurred at pH = 5.16 ± 0.10 (Fig. 6 *A*) and 5.34 ± 0.10 (Fig. 6 *B*) at 50 nM $[Ca]_i$ and 6 μM $[Ca]_i$, respectively. The addition of ATP shifted the titration curve towards a more acidic direction (pH = 4.86 ± 0.11, Fig. 6 *C*). To recover the channel, significantly higher pHs were always required. It is interesting that not all of the recovered channels had the same property as the original channel before the treatment of low pH. Most frequently, a partial recovery of channels with lower conductance states was observed (Fig. 5 *C*). This occurred in 11 out of 11 complete experiments at 6 μM $[Ca]_i$. The recovery of full conductance channel (225 pS) was only observed at low Ca (50 nM, Fig. 5 *B*) (3 out of 7 complete experiments). The addition of 2 mM ATP brought to some full recovery of channel activity at 6 μM Ca (2 out of 7 experiments) (Fig. 6 *C*). In other times, the recovered channel had low open probability, which is almost indistinguishable from the native channel. The partial recovery of channel activity is unlikely to be caused by dissociation of ryanodine from the channel, because it was routinely observed at 50 nM Ca: three out of seven experiments; in addition, three other bilayers showed full recovery.

These seemingly complicated results suggest the following possibilities: a) high proton concentrations altered the interactions within the ryanodine receptor complex, which led to the appearance of partial channel openings; b) there were cooperative interactions among Ca, ATP, and pH in regulating the ryanodine receptor complex. Micromolar [Ca] might favor the disassembly, whereas millimolar [ATP] might contribute to the formation of the intact channel complex.

Multiple conductance states of the ryanodine-activated channel

The analysis of the multiple conductance states of the single Ca release channel provided additional insights into the regulation of the Ca release channel by Ca, ATP, and pH. Subconductance levels were frequently observed as transition states in the native channel (Ma et al., 1988; Liu et al., 1989; Ma, 1993) (data not shown) and could be clearly seen in the ryanodine activated channel. At least four sub-conductance states could be identified, which appeared to be approximately equally spaced (Fig. 7). In the range from pH 7.5 to

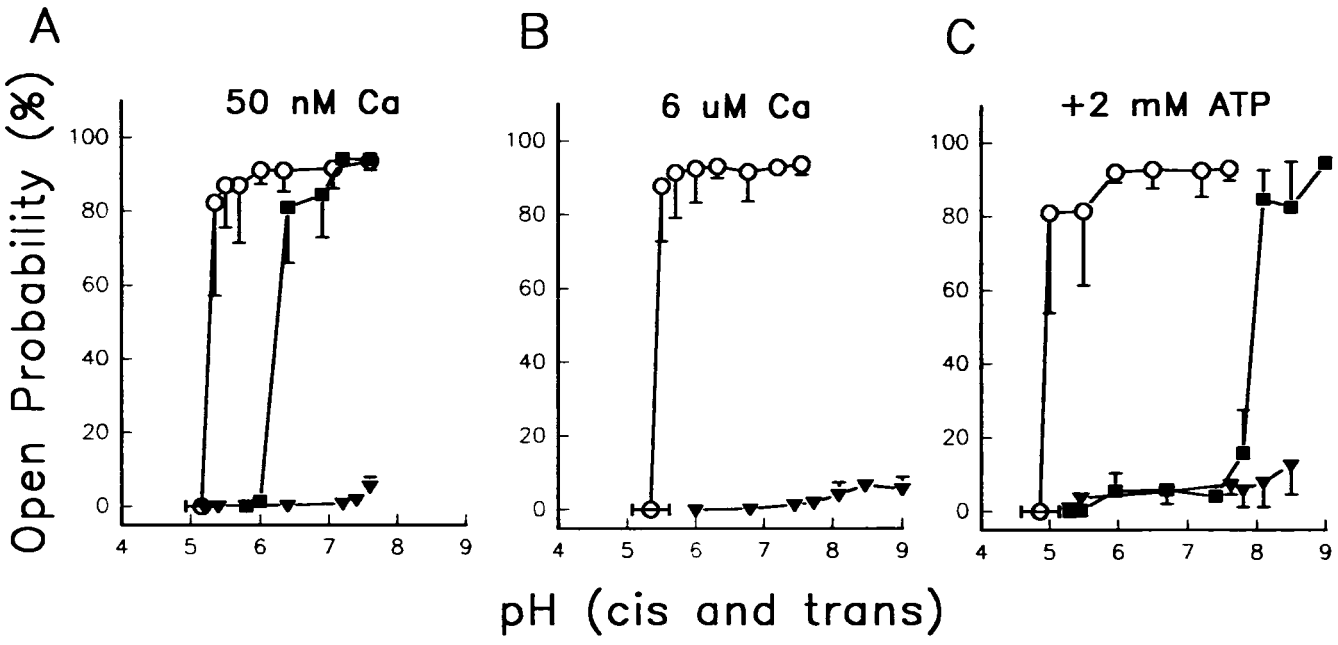

FIGURE 6 Effects of Ca and ATP on the hysteresis behavior of the ryanodine activated Ca release channel. (*A*) P_o vs. pH at 50 nM Ca. The acidic titration curve contained data from nine experiments. The full recovery (■) was observed in three out of seven experiments. The partial recovery (▼) was observed in three out of seven experiments. One experiment did not show any recovery. Vertical bars represent one SD from the mean. (*B*) P_o vs. pH at 6–20 μM [Ca]. The dose response curve represents the average over 11 experiments. Note that only partial recovery of channel activity was observed. (*C*) P_o vs. pH at 6 μM Ca, 2 mM ATP. The curve was constructed with seven bilayer experiments. Two channels showed full recovery (■), the other five showed partial recovery (▼).

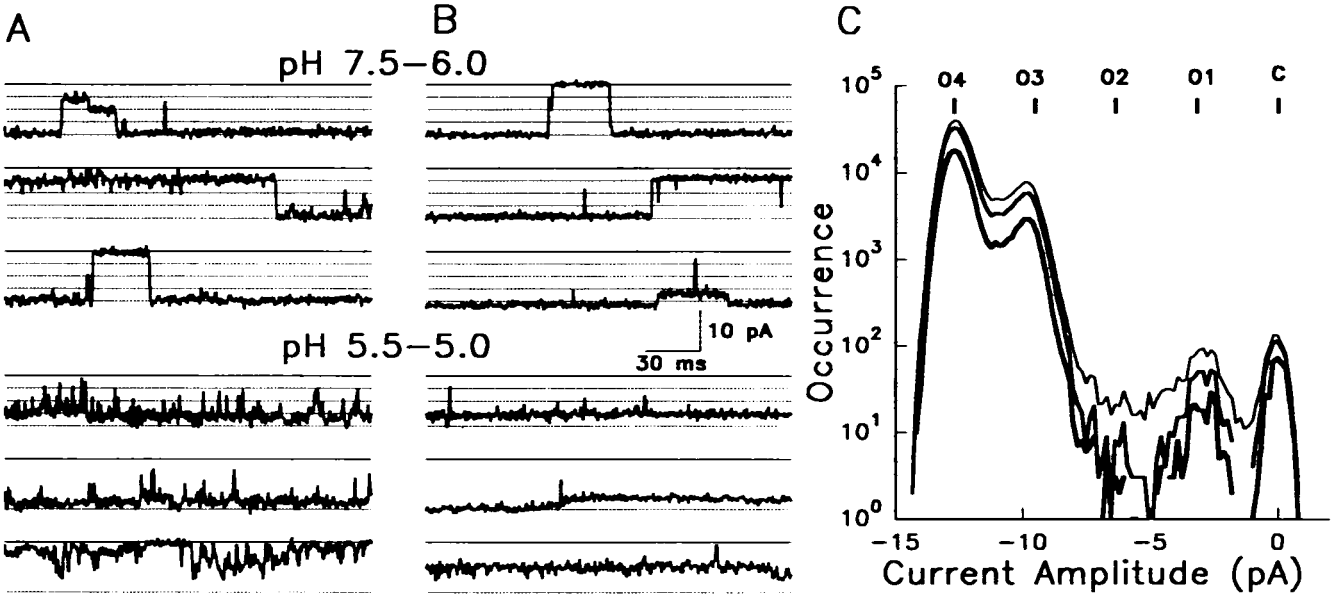

FIGURE 7 Multiple conductance states of the ryanodine activated channel. The representative traces were selected from the ryanodine activated channel (1 μM ryanodine) at −50 mV test potential. The records were digitally filtered at 1 kHz for better signal resolution. The equally spaced dashed lines correspond to the four individual conductance levels of the channel (O1, O2, O3, and O4). Channels measured without ATP (*A*) and with ATP (2 mM) (*B*) present in the myoplasmic solution. Note the fast conversions within the sub-conductance states (*A*) and frequent appearance of lower conductance states (*B*) at pH 5.5–5.0. (*C*) Low variance analysis of sub-conductance states. The amplitude histograms were constructed from 12 consecutive data files with a total of 192 episodes (each 200 ms, −50 mV test pulse) that span pH from 7.0 to 6.0 (Bilayer #92D23). The thin line contains all data points, and the thicker lines correspond to data points selected for lower variances, using a window of 15 points (1.5 ms). The algorithm for mean variance analysis was written by Dr. Steven W. Jones.

6.0, the channel had a dominant conductance of 225 pS, although infrequent transitions among the lower conductance levels could be observed. From the mean variance analysis of the amplitude histogram (Patlak, 1993), five distinct peaks can be identified (C, O1, O2, O3, and O4) (Fig. 7 *C*). Although the two lower levels (O1 and O2) seemed to correspond to 1/4 and 1/2 of the full conductance level (O4), the location of O3 is significantly higher than 3/4 of the full level.

Near the critical point of channel closure (pH 5.5–5.0), frequent conversions among sub-conductance states were detected. In the absence of ATP, the conversions were fast (Fig. 7 *A*). ATP seems to stabilize the lower conductance levels (3/4 and 1/2) of the channel, as shown in Fig. 7 *B*. The intermediate conductance states were usually unstable with noisy open currents. Close examination of the sub-conductance states revealed some unusual slow conversions within the adjacent conductance levels (Fig. 8). These transitions took as long as several milliseconds, unlike the normal fast opening and closing transitions. The slow type of transitions were present from high to low conductance level (O4→O3, for example), as well as from low to high conductance level (O2→O3, for example). Note that the last transition into the closed state of the channel was not as sharp as the normal closing events (Fig. 8 *e*).

The observation of multiple conductance states in a single Ca release channel suggests the presence of multiple quasi-stable configurations in the ryanodine receptor.

DISCUSSION

In the present study, we showed that the skeletal muscle ryanodine receptor had an hysteretic response to changes in proton concentrations. The complicated dose response of channel open probability as function of pH suggests that multiple proton titration sites could be involved in controlling the open conformation of the Ca release channel. The open state of the channel under the four different conditions: before and after treatment of low pH in the absence of ryanodine, and before and after treatment of low pH in the presence of ryanodine, had different responses to changes in pH. This indicated that the affinities of the proton titration sites might be governed by the conformation state of the ryanodine receptor complex.

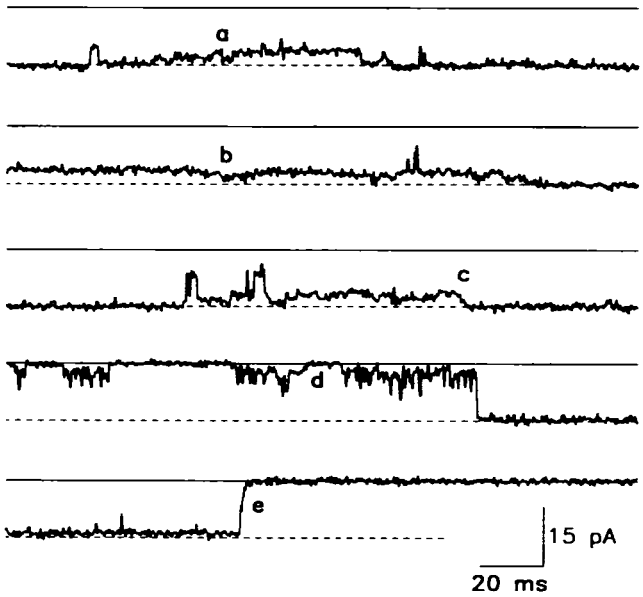

FIGURE 8 Slow transitions within the sub-conductance states of the ryanodine activated channel. The selected traces were representative of the many slow transitions frequently encountered. (*a*) Transition O4→O3→O_2. (*b*) O3→O4. (*c*) O_2→O4. (*d*) O1→C. The bottom trace represents the last transition (*e*) into the closed state of the channel. The records were digitally filtered at 1 kHz. Test pulse was −50 mV.

The early work of Meissner and Henderson (1987) demonstrated direct effects of protons on the rapid release of Ca from the SR vesicle. Previous studies with the pH regulation of the Ca release channel were focused mainly on the response to acidic titration. Using the purified ryanodine receptor, Ma et al. (1988) showed that the channels were sensitive to proton inhibition with a pK value of 7.2. Rousseau and Pinkos (1990) showed that the native Ca release channel had a pK of ~7.2, with Ca as the current carrier. Our data with the native channel differed significantly from the purified ryanodine receptor in that pH inhibition of the native channel occurred at more acidic pHs (pK = 6.5, Fig. 2 *C*). It is interesting, however, that the alkaline recovery curve of the native channel (Figs. 2 *C* and 4 *B*) compared well with the acidic titration of the purified ryanodine receptor (Ma et al., 1988). It is possible that some modulatory proteins interact with the ryanodine receptor, which could account for the difference. Proteins on the junctional SR membrane, such as triadin (Caswell et al., 1991), calsequestrin (Ikemoto et al., 1989), and FK506 binding protein (Jayaraman et al., 1992), might all play such a role. At present, we do not know to what extent the interaction of these putative regulatory proteins with the ryanodine receptor could account for the observed pH-dependent hysteresis of the native Ca release channel.

At least part of the pH-dependent hysteresis of the skeletal muscle Ca release channel is an intrinsic property of the ryanodine receptor protein, because a similar phenomenon has been observed previously with the immunoaffinity-purified ryanodine receptor (Ma et al., 1990). The highly cooperative action of pH on the ryanodine-activated channel indicated drastic changes in the channel structure at low pH. The difference between the native channel (without binding to ryanodine) and the ryanodine activated channel is rather striking. After treatment with low pH, the native channel always recovered fully at alkaline pHs, whereas the ryanodine activated channel was more likely to recover partially. These results could be accounted for by a more rigid structure of the channel in the presence of ryanodine (Caroll et al., 1991; Pessah and Zimanyi, 1991).

The actions of Ca, ATP, and pH on the skeletal muscle Ca release channel have different mechanisms. Myoplasmic Ca is the essential regulator of channel activity. The affinities of Ca activation and inactivation of the channel do not depend on the presence of ATP. The binding of ATP can shift the equilibrium between the last transition step that leads to the open state of the channel. The action of pH on the channel

cannot be as specific as that of Ca and ATP, because it involves multiple titration sites. However, the correlation between low pH and sub-conductance states of the channel suggests that protons provide a disintegrating force for dissociation of the ryanodine receptor complex.

The identified four distinct sub-conductance states in the ryanodine-activated channel are consistent with the tetrameric structure of the ryanodine receptor complex (Wagenknecht et al., 1989). It is interesting that the four sub-conductance levels of the channel do not always space equally. This might indicate cooperative interactions among the 560 kDa ryanodine receptor monomers within the channel complex (Lai et al., 1991; Buck et al., 1992). Understanding the hysteresis that characterizes regulation of the single calcium release channel should provide important clues about the structure and function of the ryanodine receptor in excitation-contraction coupling of skeletal muscle.

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