J. Membrane Biol. 130, 163–181 (1992)

The Journal of
Membrane Biology

A Calcium Conducting Channel Akin to a Calcium Pump

Jinsong Wang, John M. Tang, and Robert S. Eisenberg
Department of Physiology, Rush Medical College, Chicago, Illinois, 60612

Summary. Calcium conducting channels were studied in blebs of sarcoplasmic reticulum described by Stein & Palade (1988). The calcium channels had at least three conductance states (70 pS, 50 pS and 37 pS) and were weakly selective for calcium ions, with a permeability ratio Ca^{2+} to K^+ of about 3.4. The open probability of the channel was strongly voltage dependent, decreasing at positive membrane voltages. 10 μM ryanodine and 5 μM ruthenium red had no effect on this channel; neither did millimolar concentrations of ATP, Mg^{2+}, caffeine, and Ca^{2+}, implying that the calcium conducting channels are *not* ryanodine receptors. Several calcium pump inhibitors—namely, vanadate, AlF_4^-, reactive red 120, and cyclopiazonic acid—had obvious effects on the calcium conducting channels, suggesting that the calcium conducting channel of SR membrane blebs is some form of the SR calcium pump.

Key Words calcium pump · sarcoplasmic reticulum · calcium channel · ryanodine receptor

Introduction

The sarcoplasmic reticulum (SR) is a closed membranous system surrounding the myofibrils within skeletal muscle fibers, consisting of two parts, the terminal cisternae and the longitudinal reticulum, which, together with the T-tubule, are the structures responsible for excitation contraction coupling (E-C coupling). The calcium release channel, or ryanodine receptor, located in the terminal cisternae, is responsible for calcium release during E-C coupling (Rios & Pizarro, 1991). The membrane of the longitudinal reticulum contains K^+, Cl^-, H^+ channels (and lipid) but is packed nearly solid with calcium pump.

One of the many methods of studying the SR uses the patch clamp method to record currents through single channels in the "sarcoball" preparation of Stein and Palade (1988). After isolation and mechanical splitting of the single muscle fiber, contractures induced by millimolar concentration of calcium produce membrane blebs, semi-spheres of apparently empty membrane on the cut surface of the split muscle fiber. The origin of these blebs is a subject of discussion. Vivaudou and colleagues (1991) reported ATP sensitive K^+ channels on membrane blebs prepared by a similar method and suggested that these membrane blebs came from sarcolemma. The membrane blebs in our experiments could not come from the sarcolemma, which had been previously dissected away, although it is remotely possible that they came from T-tubules or other organelles.

Several channels have been studied in SR membrane blebs: K^+ channels (Stein, Nelson & Palade, 1989; Vivaudou, Arnoult & Villaz, 1989), Cl^- channels (Hals, Stein & Palade, 1989; Hals & Palade, 1990) and Ca^{2+} channels (Stein & Palade, 1988; Kwok & Best, 1990), thought to be ryanodine receptors. Kwok and Best (1990) report in abstract that micromolar ryanodine increases the open probability of the Ca^{2+} channels of their preparation.

In our experiments, Ca^{2+} conducting channels were *not* sensitive to ryanodine, ruthenium red, ATP, Mg^{2+}, or Ca^{2+}; they were sensitive to calcium pump inhibitors. These results suggest that the Ca^{2+} conducting channel is not the ryanodine receptor, but some form of the calcium pump.

Materials and Methods

Preparation of Sarcoplasmic Reticulum Membrane Blebs

The preparation of membrane blebs was inspired by the paper of Stein and Palade (1988). Semitendinosus muscles were isolated and removed from both legs of adult frogs *Rana catesbiana* and kept in physiological saline solution. One of the semitendinosus muscles was rinsed then bathed with relaxing solution after it had been fixed by insect pins to the bottom of a petri dish lined with Sylgard (Dow Corning, Midland, MI). A single muscle fiber was isolated with one end still attached to the muscle. Initially, two pair of tweezers were used to make a short split (0.5 mm) in the middle of the free end of the fiber. The split was then extended down most of the fiber by pulling apart the two split ends, rolling

the external sarcolemma into a cuff in the process. The fiber was not split all the way to its far end, but a short segment was left intact, yielding a "Y" shaped preparation, after the fiber was cut from the muscle. At this stage, no membrane blebs could be seen.

The "Y" shaped fiber was then pinned down at its bottom (i.e., the intact end) to a Sylgard disk which can be transferred to a dish on the microscope stage. The dish was filled with one of two solutions, depending on the experiment: one solution contained high Ca^{2+} and no caffeine; the other solution contained low Ca^{2+} and high caffeine. The K^+ channel was studied in preparations made with the caffeine solutions; the Ca^{2+} conducting channel was studied in preparations made with high Ca^{2+} solutions *not* containing caffeine. Either solution induced contractures after which membrane blebs formed spontaneously. Gigaseals could be obtained readily on the surface of blebs formed with either solution.

Single Channel Recording

Standard patch-clamp techniques (Hamill et al., 1981) were used to record channel activity with an Axopatch 1B amplifier (Axon Instruments, Foster City, CA) connected to a computer through an Axolab-1 interface. Voltage pulses were generated by using pCLAMP software (Axon Instruments). A pulse protocol consisting of 16 episodes with 2 sec on (applied membrane voltage) and 4 sec off (resting potential of 0 mV) was used. Most of the experiments were performed with a pipette perfusion system (Tang et al., 1990) which allows convenient comparison of the effects of ions or drugs applied to a particular channel molecule.

Data were filtered at 1 kHz, with an eight pole Bessel filter (model 902LPF; Frequency Devices, Haverhill, MA), and stored directly on a removable but high speed (Bernoulli) disk. Data were analyzed with pCLAMP. Usually, a histogram of current amplitudes was calculated first and fit separately with two Gaussian distributions, one for open and one for closed levels. Data was then converted into a file of event definitions (open, closed, and durations) by using a 50% threshold. A wide range of information could be obtained from these files with the analysis programs. Histograms of single channel current amplitude and open probability were used for the comparison of drugs before and after pipette perfusion.

Solutions

The physiological saline solution contained (in mM): 117 NaCl, 2.5 KCl, 1.8 $CaCl_2$, and 5 MOPS. The pH was adjusted to 7.2 and osmolality was 235 mOsm. The relaxing solution contained (in mM): 120 K Aspartate, 5 Na_2 creatine phosphate, 3 Na_2ATP, 3 $MgSO_4$, 0.0281 $CaCl_2$, 5 MOPS, and 0.1 $Tris_2EGTA$. The pH was 7.1 and osmolality was 240 mOsm. Calculations (Fabiato, 1988) suggest that the free Ca^{2+} concentration was 100 nM. Membrane blebs were produced by contractures induced by either "relaxing" solution plus 10 mM caffeine, or a Ca^{2+} recording solution, containing (in mM): 50 Ca $(Gluconate)_2$, 2.5 $CaCl_2$, 5 MOPS, and 100 sucrose. The pH was adjusted to 7.1 and osmolality was 220 mOsm. The K^+ recording solution contained (in mM): 95 K Gluconate, 5 KCl, 5 MOPS, 0.1 $Tris_2EGTA$ and 0.1113 $CaCl_2$. The pH was 7.2 and osmolality was 190 mOsm. Calculation suggested that the solution had 12.5 μM free Ca^{2+}. Hexamethonium (10 mM) was added to the K^+ recording solution if it was used to study Ca^{2+} conducting channel.

Results

Identification of K^+ and Ca^{2+} Channels

K^+ channels were not detected in membrane blebs prepared by 52.5 mM Ca^{2+} recording solution (133 recordings). K^+ channels were detected in 36 out of 72 patches in blebs prepared with 10 mM caffeine. In this procedure, the fiber was never exposed to a high concentration of Ca^{2+}: the fiber was dissected in relaxing solution and induced to contract in *relaxing* solution ($Ca^{2+} = 10^{-7}$ M) with 10 mM caffeine. Since K^+ ions can move through the Ca^{2+} conducting channel, the identity of the channel was verified at the end of the experiment by putting Ca^{2+} recording solution in the bath and seeing that the channel was selective for K^+.

Ca^{2+} selective channels could be observed in membrane blebs prepared with either high Ca^{2+} or caffeine induced contractures, although the probability of seeing a Ca^{2+} channel was much higher in Ca^{2+} prepared membrane blebs (>70%) than in caffeine prepared membrane blebs (49%).

K^+ Channels

The K^+ channel could only be observed in membrane blebs formed by contractures induced by caffeine. The K^+ channel had at least two conductance states: 82 ± 1.4 pS and 61 ± 0.8 pS (mean ± SE; $n = 5$) when currents were recorded in a symmetrical 100 mM K^+ recording solution (Fig. 1). The 61 pS state was the dominant state; the 82 pS state was seldom observed. The K^+ channel was selective for K^+ ions: a permeability ratio of $K^+/Ca^{2+} > 50$ was measured in bi-ionic conditions.

The K^+ channel could be blocked by 5 mM hexamethonium and 1 mM decamethonium in a voltage-dependent manner. The experiments were done in symmetrical 100 mM K^+ recording solution with drugs added to the cytoplasmic side of the channel (in the pipette). Channel conductance was obviously reduced by the drugs at positive (inside the pipette) voltages expected to push the drugs into the channel.

Multiple-Conductance States and Selectivity of the Ca^{2+} Channel

At least three conductance states could be identified in symmetrical 52.5 mM Ca^{2+} recording solution. For convenience, the largest was called the α state; the next largest was the most common state, called the β state; and the smallest was called the γ state. Transitions from α to β, β to γ and *vice versa* were observed. Although these three open states could be

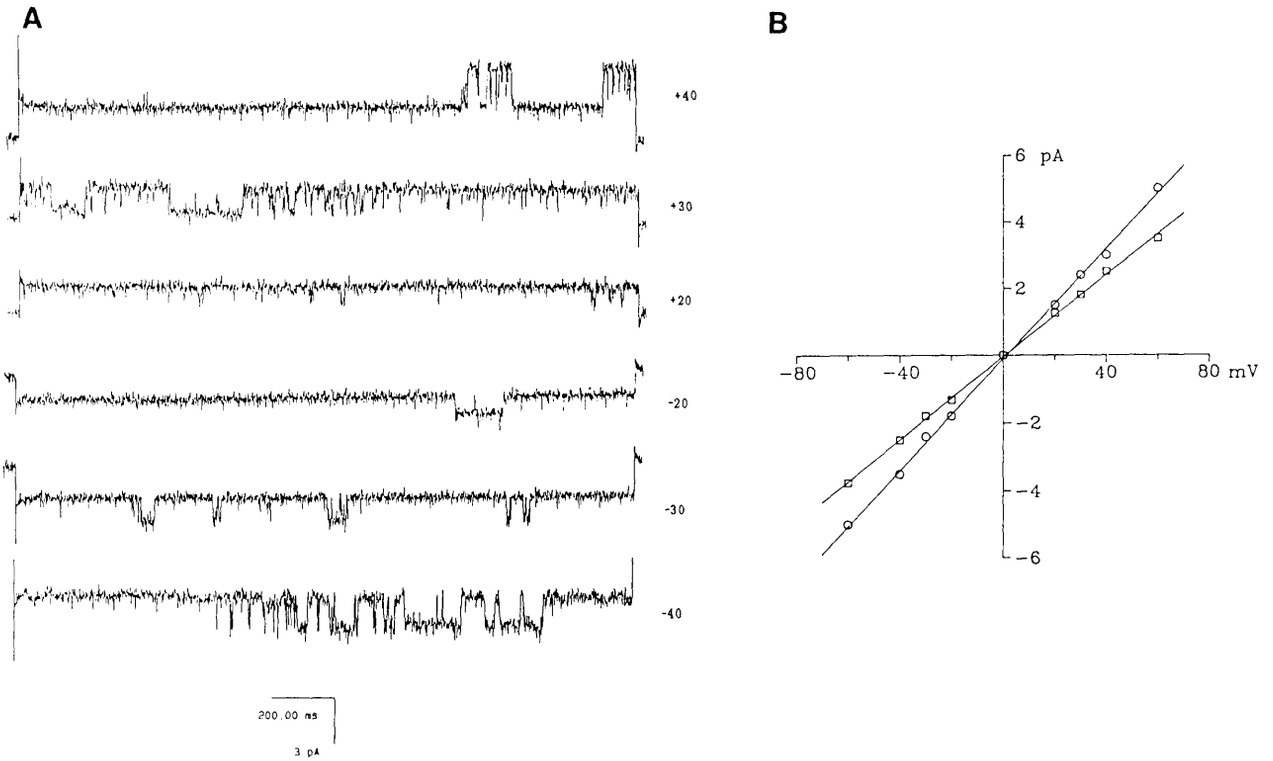

Fig. 1. SR K^+ selective channel. (*A*) Single channel current traces of the SR K^+ selective channel at various membrane voltages. The closed state and the dominant conducting state are shown. Upward deflection of the traces (at positive voltages) means outward current, which is current flow from cytoplasmic side to SR lumenal side. Recording was made in symmetrical 100 mM K^+ recording solution. Channel was recorded in an excised, inside-out configuration and filtered at 1 kHz. (*B*) Current-voltage relationship for both the full and sub-conductance states. Data points were mean value from five different patches and the solid lines were drawn by least-square regression. Slope conductance of the full opening state was 82 ± 1.4 pS, whereas the conductance of the dominant substate was 61 ± 0.8 pS. The *I-V* relationship was linear within the voltage range of recording.

identified convincingly, only the dominant β state was observed in most cases (Fig. 2). It would be interesting to compare the state diagrams compatible with the channel's kinetics to state diagrams of the Ca^{2+}-ATPase.

Current traces at various voltages and the current-voltage relationship for the three conductance states is shown in Fig. 2. All recordings were made in the symmetrical 52.5 mM Ca^{2+} recording solution. The slope conductances of the three open states are 69 ± 1.2 pS, 47 ± 1.2 pS, and 35 ± 0.6 pS (n = 8), as shown in Fig. 2. The current voltage relationship was linear in the membrane potential range ±80 mV.

The selectivity of the Ca^{2+} conducting channel was measured under bi-ionic conditions, with 52.5 mM Ca^{2+} in the bath and 100 mM K^+ in the pipette. Two conducting states can be observed: the α and β states. At zero holding potential, an inward current was seen, indicating that the channel is selective for Ca^{2+} ion (Fig. 3). The reversal potential was 21 ± 1.6 (n = 5) mV which yields the permeability ratio P_{Ca}/P_K = 3.37 calculated from the corrected constant field equation for the mixed divalent and monovalent ions (Lee & Tsien, 1984).

The conductance of the open state β, measured in Ca^{2+} or K^+ solutions, was used to calculate the conductance ratio (instead of the full open state α) because it is the most common state of the channel. The conductance of the β state in Ca^{2+} or K^+ solutions is 47 pS or 45 pS respectively, yielding the conductance ratio γ_{Ca}/γ_K = 1.04 (*result not shown*). This Ca^{2+} channel was weakly selective for Ca^{2+} *vs* K^+.

Voltage Dependence

The open probability of the Ca^{2+} conducting channel varies from one experiment to the next; however, the voltage dependence of the channel was qualitatively consistent. As shown in Fig. 4, the open probability peaked around zero membrane potential. At membrane potentials more positive than +60 mV, the channel was almost completely closed (except

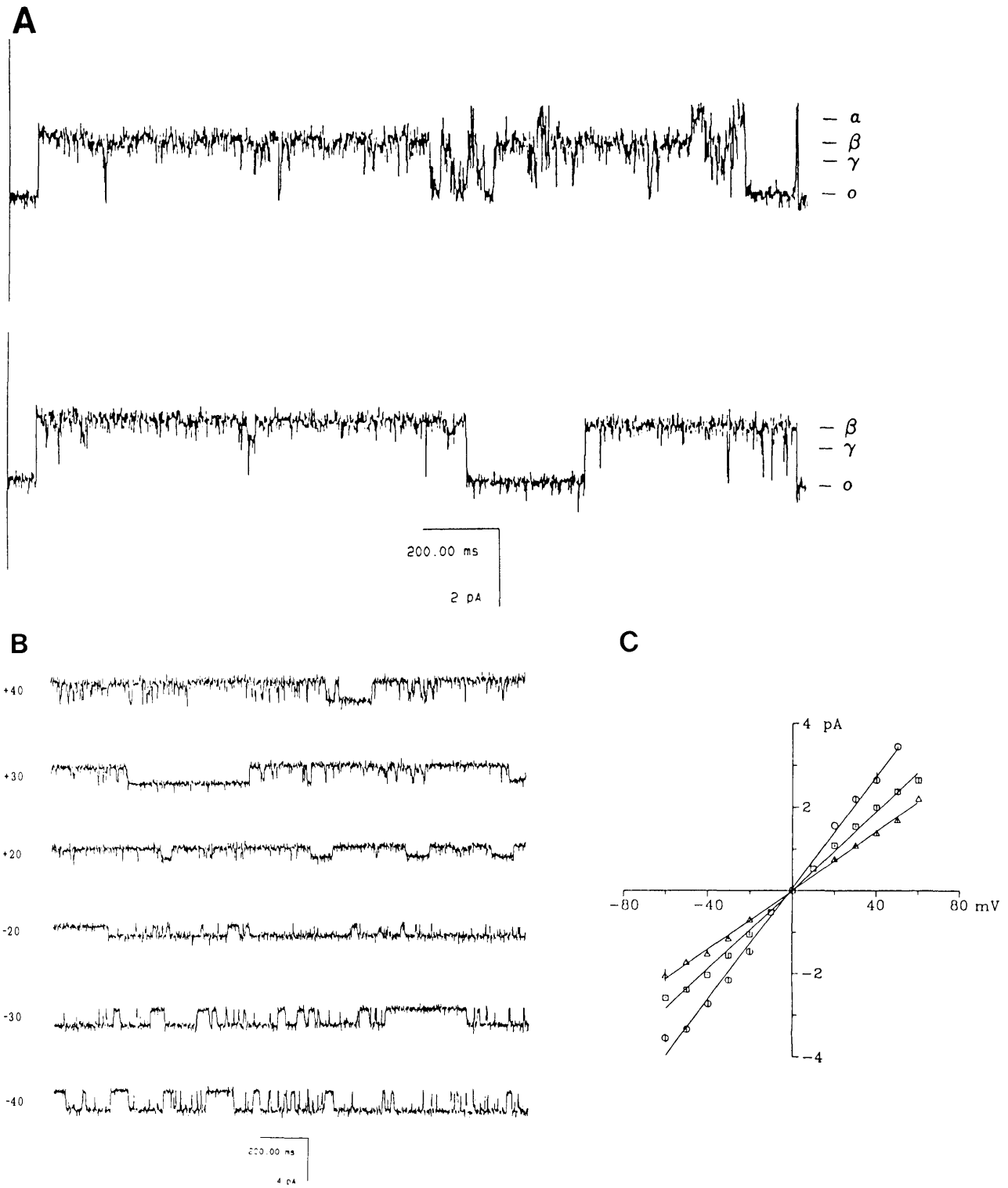

Fig. 2. SR Ca^{2+} conducting channel. (*A*) Single channel current traces showing the multi-conductance states of the Ca^{2+} conducting channel. At least three different conducting states were observed, the full conducting state (α), the dominant sub-conducting state (β), and the sub-conducting state (γ). The channel closed at close state (o). Recording was made in symmetrical 52.5 mM Ca^{2+} recording solution with the holding potential of +30 mV. (*B*) Single channel current traces at various membrane potentials of the Ca^{2+} conducting channel. Currents were recorded in symmetrical 52.5 mM Ca^{2+} solution. The β conductance state of the channel was mostly shown here since the other two states were seldom seen. (*C*) Current voltage relationship of the Ca^{2+} conducting channel. Recordings were done in symmetrical 52.5 mM Ca^{2+} solution. At least three conductance states were identified: 69 ± 1.2 pS (α), 47 ± 1.2 pS (β) and 36 ± 0.6 pS (γ) (n = 8).

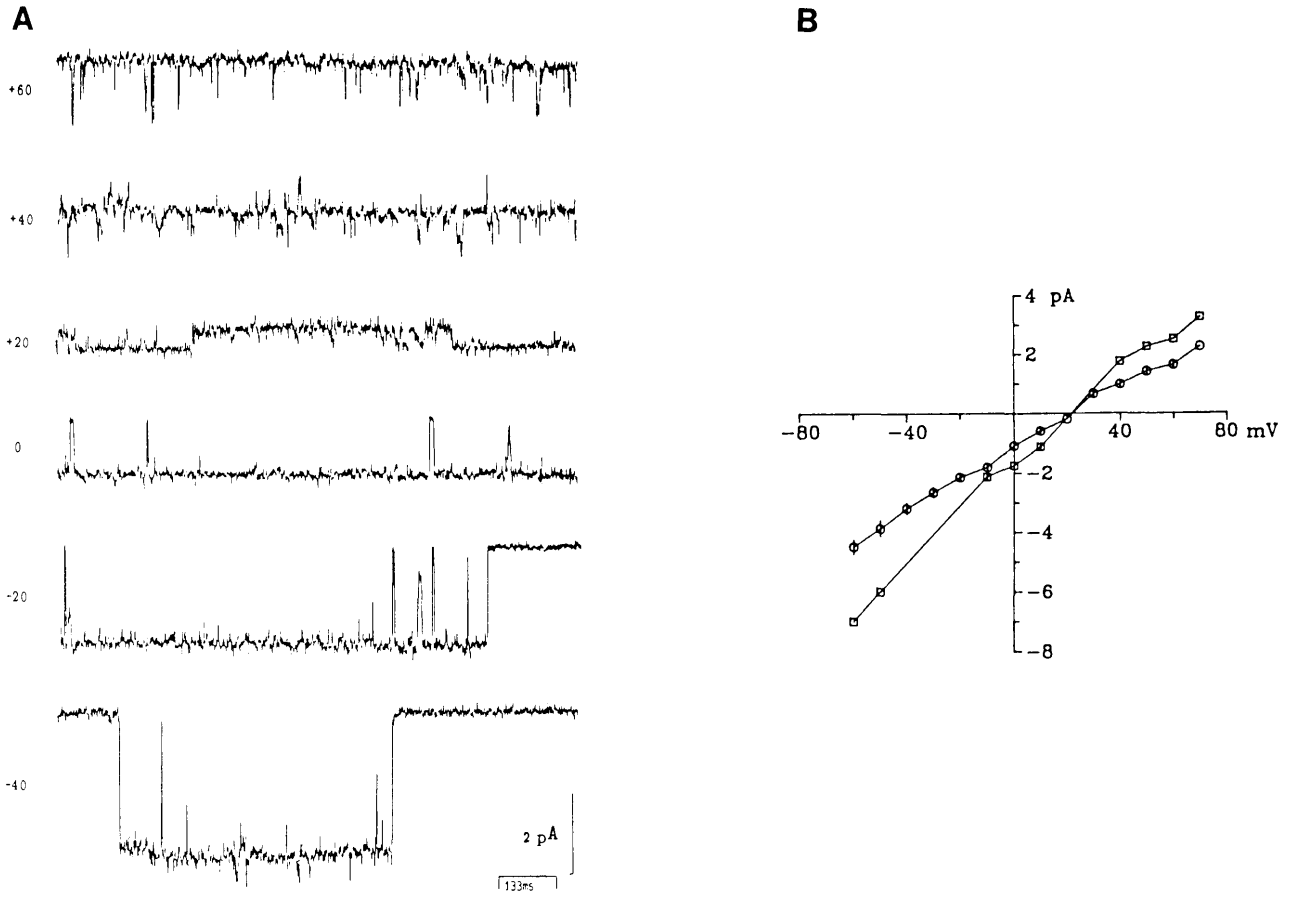

Fig. 3. Ca^{2+} conducting channel selectivity. (*A*) Single channel currents traces at various membrane voltages. Recording were done in bi-ionic condition. Bath solution contained 52.5 mM Ca^{2+} and pipette contained 100 mM K^+ solution. Downward deflection of current (inward current) was observed at 0 membrane potential indicating that the channel was selective for Ca^{2+}. (*B*) Current voltage relationship of the Ca^{2+} conducting channel. The reversal potential was measured to be +21 mV and the permeability ratio of Ca^{2+} *vs* K^+ was 3.37 calculated with the equation: $P(Ca)/P(K) = [K]_i/4[Ca]_o \{(1 + \exp FV/RT) \exp FV/RT\}$ (Lee & Tsien, 1984).

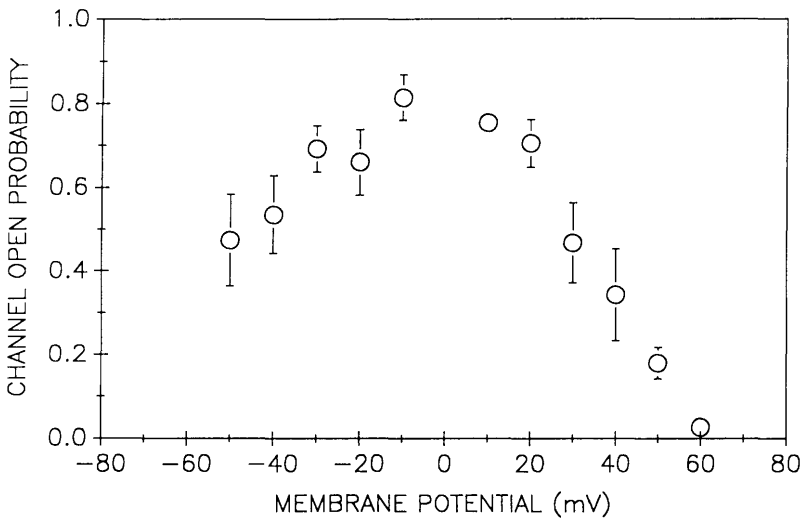

Fig. 4. The Ca^{2+} conducting channel open probability versus membrane potentials. The open probability of the dominant β conducting state was measured and used to represent the open probability of the channel. Currents were recorded in symmetrical 52.5 mM Ca^{2+} recording solution. The open probability of the channel peaked around −10 mV with the P_o value of 0.81 ± 0.05 (n = 5) and decreased dramatically to the lowest at +60 mV with the P_o of 0.03 ± 0.01. The open probability decreased only slightly at negative holding potentials.

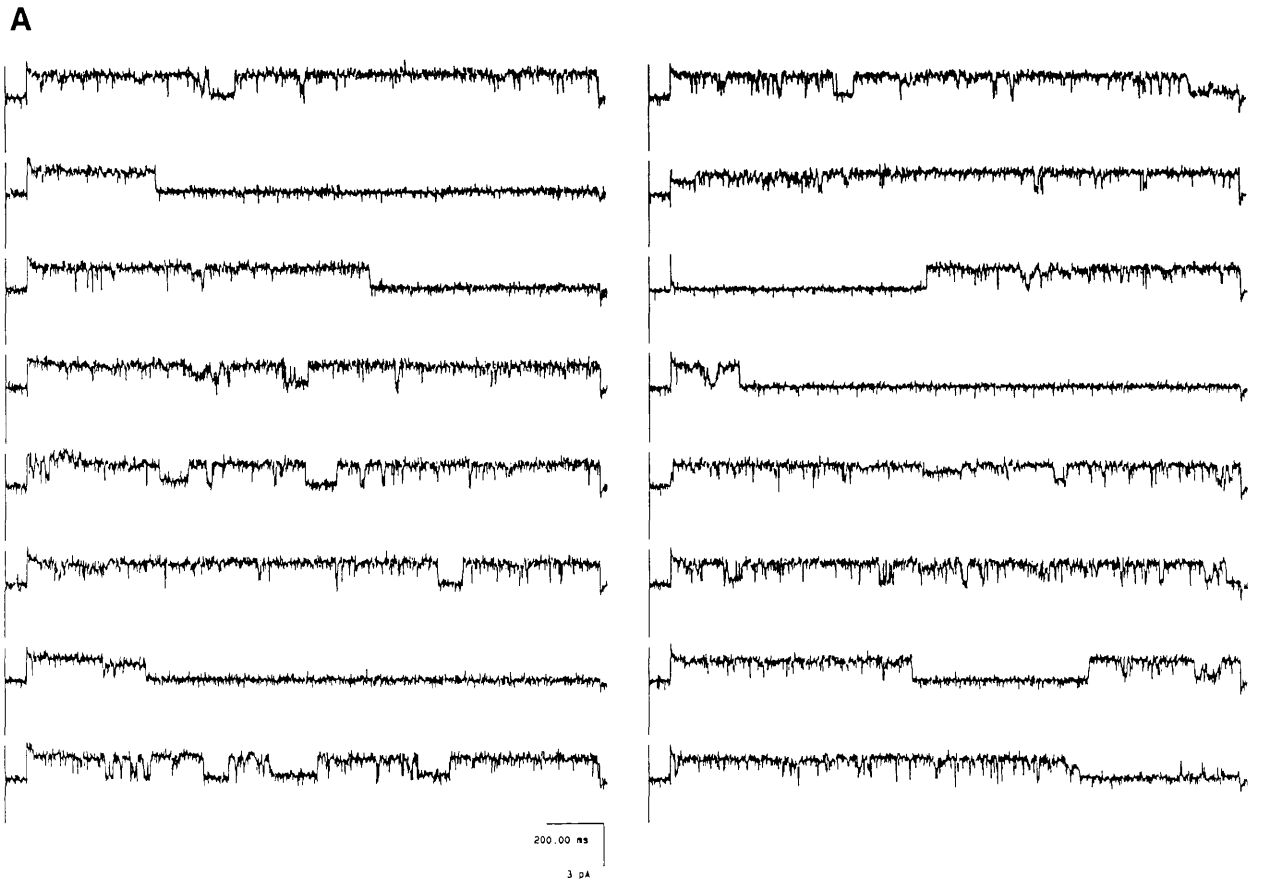

Fig. 5. The properties of Ca^{2+} conducting channel before and after the addition of 10 μM ryanodine. (*A*) Currents traces were recorded at +30 mV in symmetrical 52.5 mM Ca^{2+} solution and perfusion pipettes were used to change the pipette solution. Eight traces on the left were recorded before, and eight traces on the right were recorded after the addition of 10 μM ryanodine to the pipette. (*B*) Amplitude histogram shows no obvious change before (left) and after (right) the addition of ryanodine in terms of single channel open probability (0.45 *vs* 0.49) and single channel current amplitude (1.32 pA *vs* 1.4 pA). (*C*) The open lifetime histogram shows no obvious difference before (left) and after (right) the addition of ryanodine. The histograms are fitted with two exponentials. Before perfusion, τ_1 = 1.34 msec, τ_2 = 37.4 msec, and after perfusion, τ_1 = 1.95 msec, τ_2 = 33.78 msec.

during transients immediately after switching the potential to these levels). At negative membrane potentials, the channel was not as sensitive to voltage and the open probability decreased to about half the value at zero membrane potential. The largest P_o value was 0.81 ± 0.05 (n = 5) measured at −10 mV, and the smallest value of P_o was 0.03 ± 0.009, measured at +60 mV.

Effects of Ryanodine on the Ca^{2+} Channel

Ryanodine is a neutral alkaloid that specifically blocks the Ca^{2+} release channel of sarcoplasmic reticulum: nanomolar concentrations of ryanodine drives the release channel into a low conductance but permanently open state (Rousseau, Smith, & Meissner, 1987; Smith et al., 1988). The ryanodine effect is often used as the marker of the sarcoplasmic reticulum Ca^{2+} release channel.

Ryanodine effects on our Ca^{2+} channel were tested in symmetrical solutions containing 52.5 mM Ca^{2+}. Two different methods were used: in the first group of experiments, 10 μM ryanodine was already in the pipettes before seal formation; in the second group of experiments, a perfusion system (Tang et al., 1990) was used to apply the drug after a channel had been characterized in the control (i.e., drug free) 52.5 mM Ca^{2+} recording solution. The open probability, open lifetime, and conductance of the open state β were used as measures of drug effects. In the first group of experiments, results were compared to control experiments at the same voltage. No effect of ryanodine on the Ca^{2+} conducting channel was evident in either set of experiments (n = 6) (Fig. 5).

Effects of Ruthenium Red on Ca^{2+} Channel

Ruthenium red (1 μM) blocks the Ca^{2+} release channel in an all-or-none fashion from either cytoplasmic

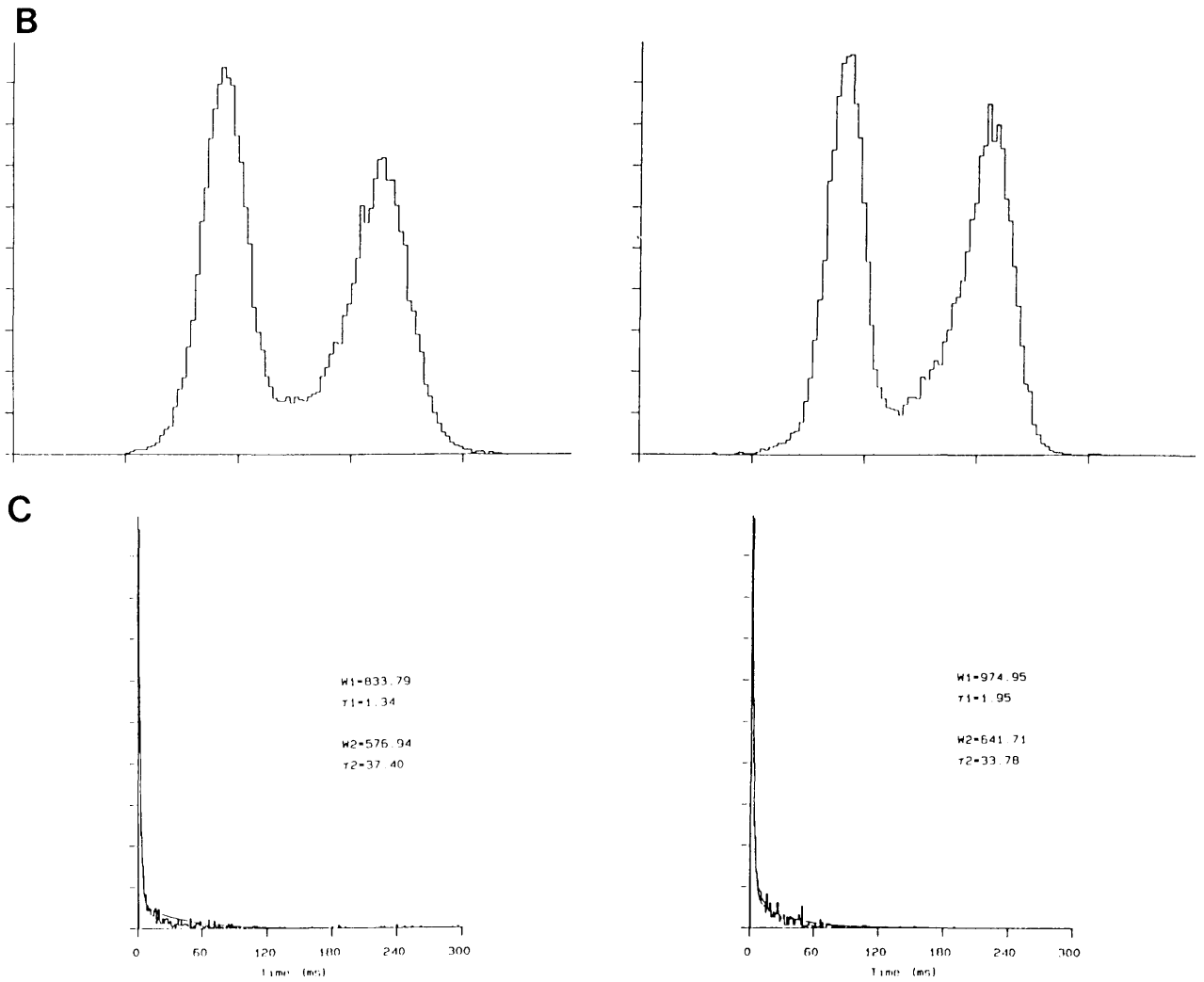

Fig. 5. (*Continued*).

or lumenal side (Smith, Coronado & Meissner, 1985; Smith et al., 1988).

The effects of ruthenium red were investigated in two ways, as just described, using pipettes previously filled with the drug or using pipette perfusion. Single channel currents were recorded in symmetrical 52.5 mM Ca^{2+} recording solution with an applied potential of +30 mV. Figure 6 shows that the drug has no significant effects on open probability, amplitude, or open lifetime of the channel (n = 6).

Effects of Ca^{2+} Pump Inhibitors on the Ca^{2+} Conducting Channel

Reactive red 120, a triazine dye, was applied since it can bind tightly, but not covalently, to the Ca^{2+}-ATP. Reactive red 120 inhibits the Ca^{2+}-ATPase with noncompetitive kinetics, suggesting that its site of action is distinct from both the active site and the putative regulatory site of the enzyme (Coll & Murphy, 1987). Xu and colleagues (1989) reported that reactive red 120 activates (not inhibits) the SR Ca^{2+} release channel.

Figure 7 shows single channel currents recorded in symmetrical 52.5 mM Ca^{2+} recording solution. The dominant open state β was used for analysis. After recording of current at an applied potential of +30 mV, the pipette was perfused with 5 μM reactive red 120 for about 1 min, and then another recording was made at the same holding potential. After 1 min of perfusion, the channel began to flicker; indeed, sometimes the individual transitions could not be resolved. The common long lasting component of the open state was not observed after the perfusion of the reactive red 120. Besides the flickering events, attenuation of the single channel conductance was also seen, probably due to fast flickering that cannot be resolved at our recording bandwidth

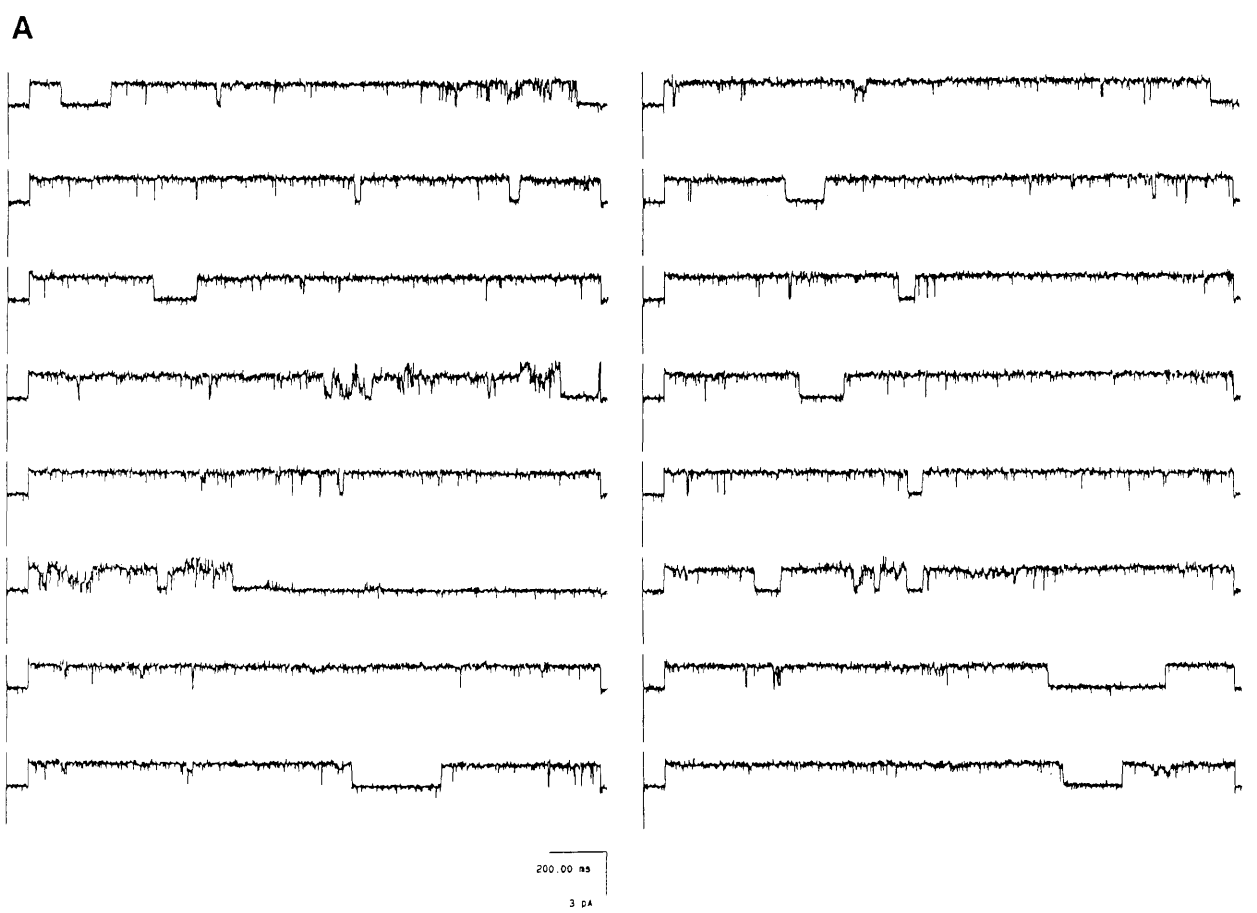

Fig. 6. The properties of Ca^{2+} conducting channel before and after the addition of 5 μM ruthenium red. (*A*) Currents were recorded in symmetrical 52.5 mM Ca^{2+} solution with perfusion pipettes. Holding potential was +30 mV. Left set of current traces was recorded before, and right set was recorded after the pipette perfusion of 5 μM ruthenium red. (*B*) Amplitude histogram shows no obvious change was detectable before (left) and after (right) the addition of ruthenium red in terms of single channel open probability (0.68 *vs* 0.71) and current amplitude (1.47 pA *vs* 1.44 pA). (*C*) The open lifetime histogram shows no obvious difference before (left) and after (right) the addition of ruthenium red. The open time histogram was fitted with 2 exponentials: τ_1 = 4.23 ms, τ_2 = 202 ms before and, τ_1 = 4.61 ms, τ_2 = 198 ms after the pipette perfusion of ruthenium red.

of 1 kHz. The inhibition of the channel by reactive red 120 was sometimes almost complete, with channel openings rarely observed (n = 5). Reactive red 120 has no obvious effect on this channel if applied from the lumenal side.

Cyclopiazonic acid, an indole tetramic acid metabolite of *Aspergillus* and *Penicillium*, is a mycotoxin toxic to muscle (Norred et al., 1985). Cyclopiazonic acid is a potent inhibitor of Ca^{2+} uptake and ATPase activity in rat skeletal muscle sarcoplasmic reticulum (Goeger et al., 1988). Cyclopiazonic acid has a fairly specific action on the sarcoplasmic reticulum Ca^{2+}-ATPase: it has no effect on kidney and brain Na^+/K^+-ATPase, gastric H^+/K^+-ATPase, mitochondrial F_1-ATPase, Ca^{2+}-ATPase of erythrocytes, or the Mg^{++}-activated ATPase of T-tubule and surface membranes of rat skeletal muscle (Seidler et al., 1989).

Figure 8 shows current traces recorded in symmetrical 52.5 mM recording Ca^{2+} solution. Perfusion pipettes were used to change the solution on the cytoplasmic side. Flickering events and reduction of amplitude can be seen clearly after the perfusion of pipette with 1 μM CPA (n = 5). Histograms of the data confirmed the observation. Sometimes, several minutes after the addition of cyclopiazonic acid, the inhibition was essentially complete, with channel openings occurring only infrequently. Application of cyclopiazonic acid from the lumenal side of the channel had no detectable effect.

Aluminofluorides are known to affect the activity of enzymatic systems, such as G-proteins and

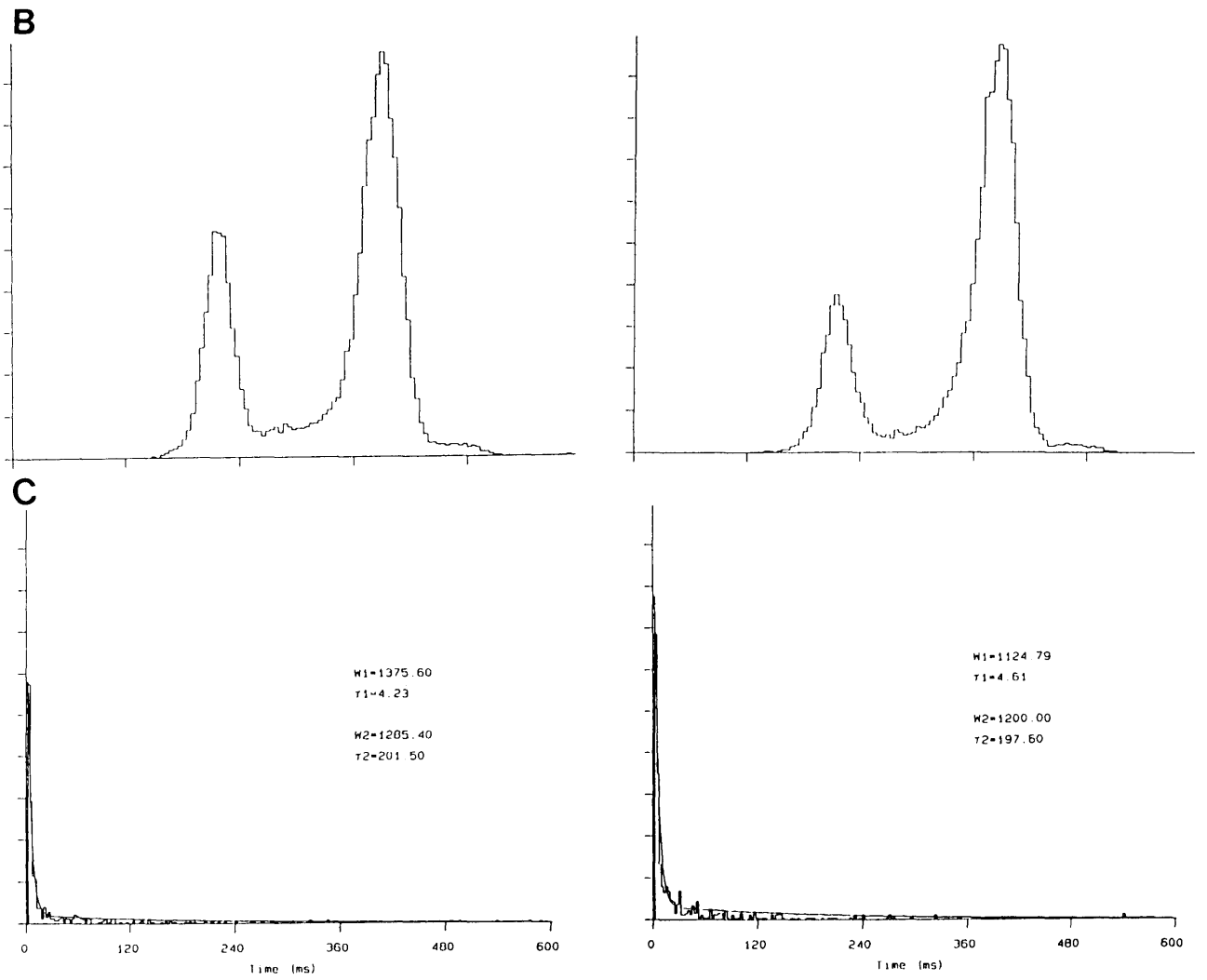

Fig. 6. (*Continued*).

Ca^{2+}-ATPase. They inhibit the sarcoplasmic reticulum Ca^{2+}-ATPase by acting as an analog of inorganic phosphate, binding to the E_2 form of the enzyme and preventing its return to the E_1 conformation (Troullier, Girarde & Dopant, 1991).

The experimental protocol for testing AlF_4^- effect was the same used with the other agents. Single channel currents were recorded in symmetrical 52.5 mM Ca^{2+} recording solution before and after the perfusion of AlF_4^-. The amplitude and the open probability of the dominant open state β at +30 mV were used as the measure of the effects. Figure 9 shows the current traces at +30 mV before and after the perfusion of 1 mM AlF_4^-. The effect of the AlF_4^- on the channel was somewhat different from the previous two agents. Although the flickering and decrease of open probability are obvious, the amplitude of the channel was left almost unaffected (n = 5). The amplitude histogram confirms the observation.

Vanadate is also a potent inhibitor of the sarcoplasmic reticulum Ca^{2+}-ATPase. Although the mechanism of its action is not clear, vanadate has been said to act as an analog of inorganic phosphate (Inesi et al., 1980; Dupont & Bennett, 1982).

The previous experimental protocol was once again employed to study the effect of vanadate. Since 52.5 mM Ca^{2+} recording solution affects the solubility of vanadate (precipitation was observed when 1 mM vanadate solution was added to the Ca^{2+} solution), recordings were made in symmetrical 100 mM K^+ solutions in which K^+ is expected to carry current through the channel that conducts predominantly Ca^{2+} in the 52.5 mM solution. Hexamethonium (10 mM) was added to the 100 mM K^+ recording

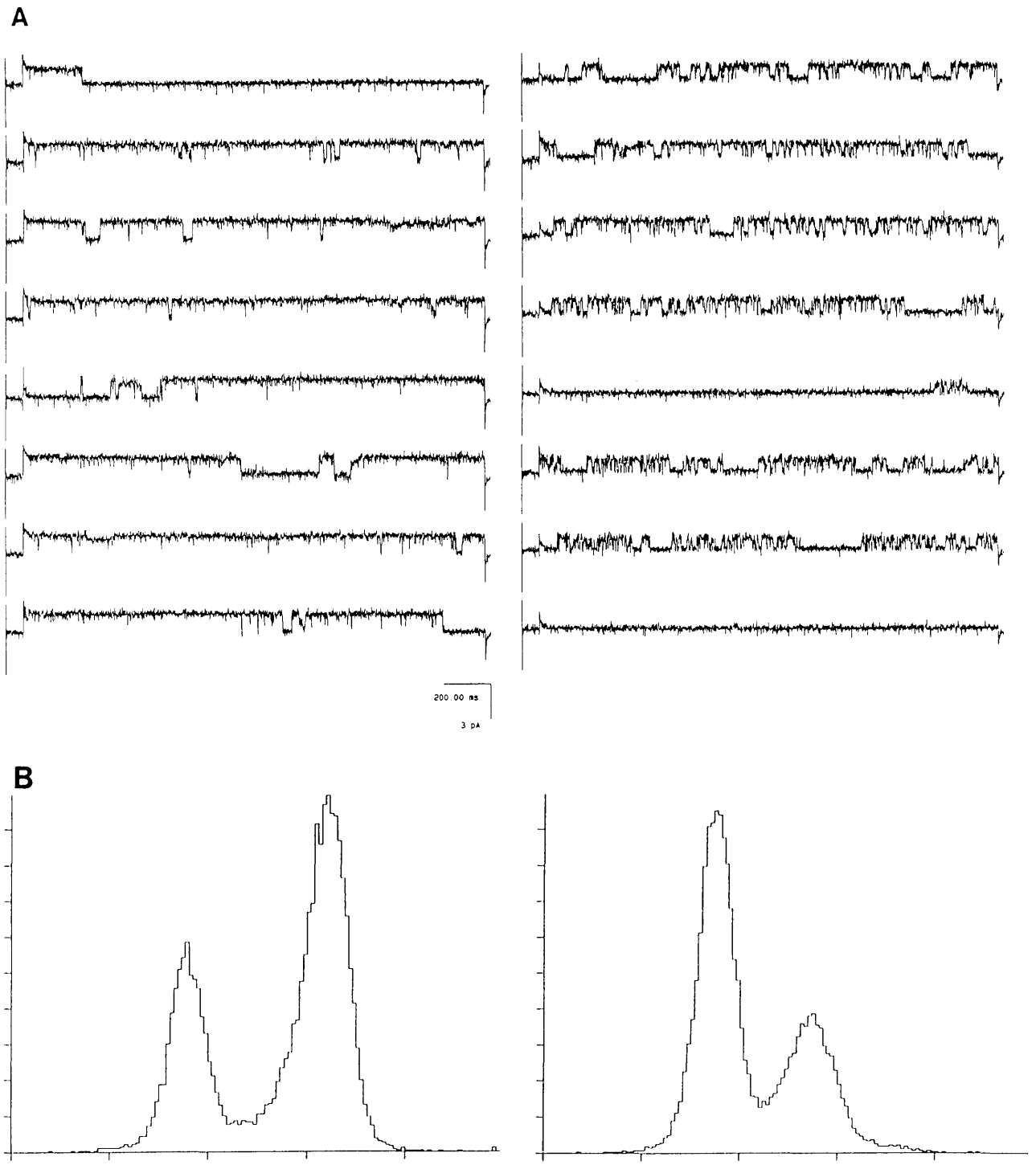

Fig. 7. Effects of 5 μM reactive red 120 (RR120) on the Ca^{2+} conducting channel. (*A*) Current traces were recorded in symmetrical 52.5 mM Ca^{2+} recording solution. Perfusion pipettes were used to change the solution on cytoplasmic side. Left eight traces and right eight traces were recorded at the holding potential of +30 mV before and after the addition of 5 μM RR120, respectively. Flickering events and reduction of amplitude could be seen clearly after the perfusion of pipette with 5 μM RR120. Data were filtered at 1 kHz. (*B*) Amplitude histogram of the Ca^{2+} conducting channel before (left) and after (right) the addition of RR120 from the same experiment. The open probability and the conductance of the channel were reduced from P_o = 0.67 and amplitude = 1.45 pA before to P_o = 0.36 and amplitude = 0.99 pA after the addition of RR120. (*C*) Open lifetime histogram shows the reduction of both τ_1 and τ_2, especially τ_2, by the RR120 (right). The τ_1 was reduced from 5.38 ms to 1.41 ms and τ_2 was reduced from 139 ms to 8.71 ms after the application of RR120. Please note the time scale is different in the two histograms.

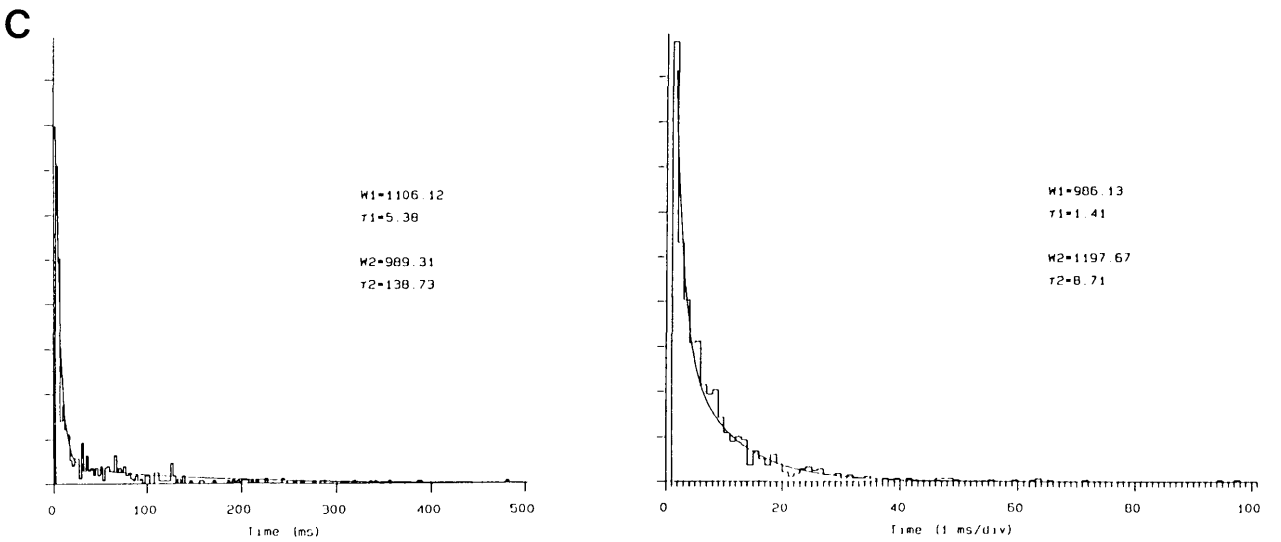

Fig. 7. (*Continued*).

solutions to block the K^+ selective channel of sarcoplasmic reticulum and prevent our confusing it with the Ca^{2+} conducting channel. The effects of vanadate on the Ca^{2+} channel were similar to those of AlF_4^-. Figure 10 shows current traces at holding potential of +30 mV before and after the perfusion of 1 mM vanadate. The amplitude was left unaffected but the open probability was dramatically reduced, so much that useful histograms of open lifetime could not be calculated from the few open times observed (n = 5).

Discussion

Membrane Blebs Come from Sarcoplasmic Reticulum

Since no morphological studies have been done to identify the membrane blebs prepared with the experimental procedures, a physiological approach has to be used to determine the possible origin of the membrane blebs: mainly, a comparison of the channels from this preparation to the channels of outer membranes determined in other studies.

McKinley and Meissner (1978) discovered the K^+ channel in the sarcoplasmic reticulum using flux studies of SR vesicles and proposed that this channel allows charge to flow to neutralize charge moved during Ca^{2+} release. The properties of the channel have been studied extensively in a model membrane system, the planar lipid bilayer (Miller, 1978; Labarca & Miller, 1981). The K^+ channel is blocked by Cs^+, decamethonium and hexamethonium in a voltage-dependent manner (Coronado & Miller, 1980; Tang, Wang & Eisenberg, 1989; Stein, Nelson & Palade, 1989). Our experiments (n = 10, *data not shown*) showed that the channel was blocked by 5 mM hexamethonium and 1 mM decamethonium from the cytoplasmic side, and the block reduced single channel conductance. Decamethonium block was asymmetrical; it exerted a stronger effect from the cytoplasmic side, which is consistent with the results from other groups.

As shown in the Results section, the Ca^{2+} conducting channel in the membrane blebs of skinned muscle fibers has at least three conductances: 70 pS, 50 pS and 37 pS. It is weakly selective for Ca^{2+} versus K^+ with the permeability ratio P_{Ca}/P_K = 3.4. It is not sensitive to 10 μM nitrendipine (n = 4, *results not shown*). These characteristics of the Ca^{2+} conducting channel are obviously different from those of surface membrane Ca^{2+} channels. Surface membrane Ca^{2+} channels (Tsien, 1983) have been divided (Bean, 1989) into three groups. All have relatively small conductance, typically under 25 pS. They are quite selective for divalent ions versus monovalent ions with permeability ratios of divalent over monovalent usually greater than 1000 (Lee & Tsien, 1984). Their activation and inactivation depend steeply on membrane potential. Each of these properties is quite different from those we record.

It seems likely then that the membrane blebs of our preparation do not come from surface membrane, and most likely come from the sarcoplasmic reticulum, which is not surprising given that during the preparation of membrane blebs, outer membranes could be clearly seen to roll up after single muscle fibers were torn in two.

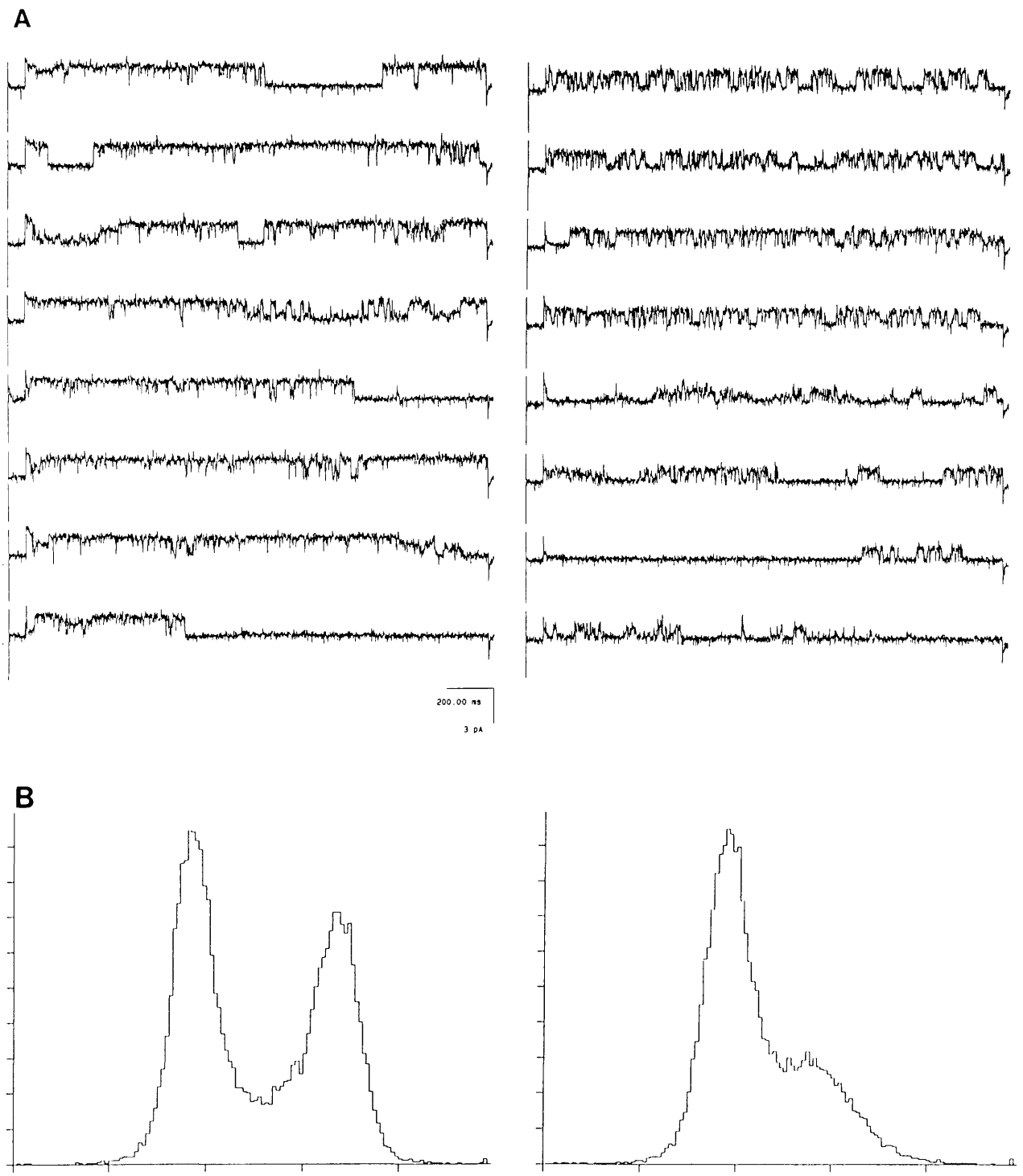

Fig. 8. Effects of 1 μM cyclopiazonic acid (CPA) on the Ca^{2+} channel. (*A*) Currents were recorded in symmetrical 52.5 mM Ca^{2+} recording solution with perfusion pipette. Left eight traces were recorded without CPA and right eight traces were recorded after the perfusion of 1 μM CPA into the pipette. Applied potential was +30 mV. Flickering events and reduction of amplitude can be seen clearly. Data were filtered at 1 kHz. (*B*) Amplitude histograms of the channel at +30 mV membrane potential before (left) and after (right) the addition of CPA from the same experiment. The open probability was reduced from $P_o = 0.47$ before to $P_o = 0.31$ after the application of 1 μM CPA. The channel current amplitude was reduced from 1.48 pA to 0.68 pA. (*C*) Channel open lifetime histogram shows the CPA greatly reduced the channel open time. The histogram was fitted with two exponentials and both time constants were reduced by CPA (right), especially the τ_2. The τ_1 was changed from 4.75 ms to 1.70 ms and the τ_2 was changed from 74.4 ms to 3.11 ms by CPA. Please note that the time scale is different in the two histograms.

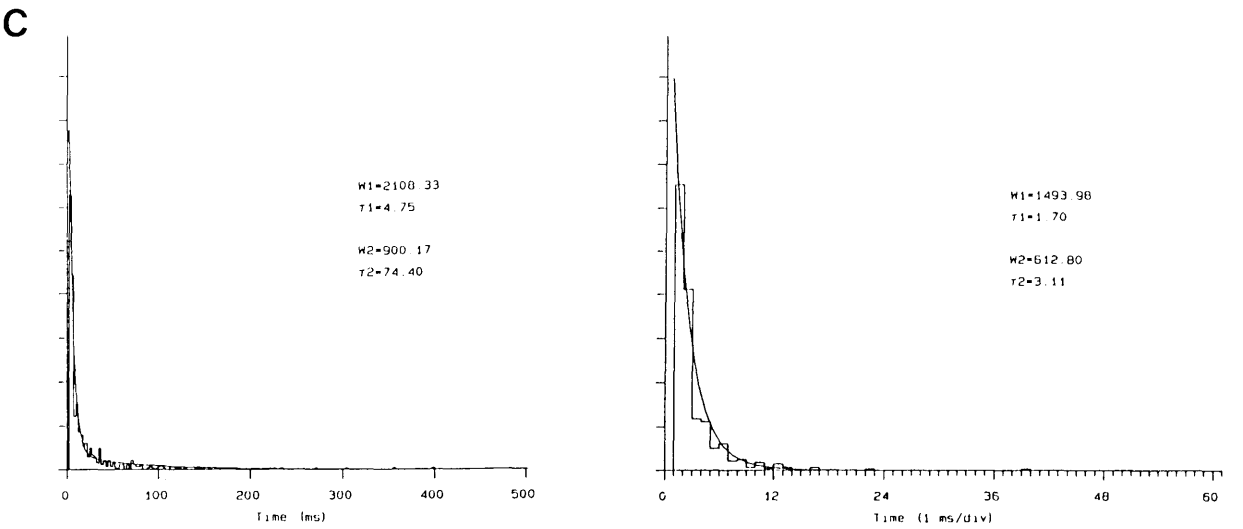

Fig. 8. (*Continued*).

Vivaudou and colleagues (1991) reported an ATP sensitive K^+ channel from the membrane blebs of frog skeletal muscle prepared in a similar way. This K^+ selective channel had a conductance of 53 pS in 140 mM K^+ solution and was blocked by micromolar ATP. The ATP inhibition could be reduced by millimolar Mg^{2+} and ADP. These membrane blebs might form from surface membrane (sarcolemma): ATP-sensitive K^+ channels are known to exist on surface membrane of pancreatic β-cells and mouse skeletal muscle cells (Quast & Cook, 1989; Ashcroft & Ashcroft, 1990; Woll, Lonnendonker & Neumcke, 1989).

Vivaudou's procedure for preparing the membrane blebs is somewhat different from ours. In their method, single fibers were dissected and skinned in very low Ca^{2+} concentration ($pCa^{2+} > 8$); the membrane blebs can be produced directly after the skinning process *without* undergoing Ca^{2+}-induced contracture (Vivaudou et al., 1989, 1991; Vivaudou & Villaz, 1991). Indeed, the membrane blebs obtained from this procedure might come from different membranes than those produced by our caffeine or Ca^{2+}-induced contractures. The K^+ channel found in our preparation has different properties from that studied by Vivaudou, e.g., it is *not* sensitive to millimolar ATP (added to the bath: $n = 6$, *results not shown*), supporting the idea that the membrane blebs prepared by these two different methods have different origins, reinforcing our conclusion that our K^+ channel is not the ATP sensitive K^+ channel of the T-tubule. Reinforcement is hardly necessary, however, because it would be a miracle if the T-membrane could form vesicles or donate channels so easily and regularly to our sacroballs: it is important to remember that K^+ channels were observed in 36 out of 72 patches (in blebs prepared with caffeine).

Inhibitory Effect of Ca^{2+} on the K^+ Channel

As mentioned earlier, membrane blebs of skinned muscle fibers can be prepared by two methods: caffeine-induced or Ca^{2+}-induced contracture. If the sarcoplasmic reticulum membrane blebs were prepared by high Ca^{2+}, the K^+ selective channel was almost never seen. However, the K^+ channel could be identified in about 50% of patches if the membrane blebs were prepared by caffeine-induced contracture. Since caffeine has no known effects on the K^+ channel, the difference in frequency of observing the K^+ channel in these two procedures may be due to the difference in Ca^{2+} concentration.

Miller (1978) studied the action of Ca^{2+} ions on the K^+ channel of the SR, using channels in planar bilayers reconstituted from vesicles. He reported that the K^+ conductance of the K^+ channel was reduced by 60% when 3.5 mM Ca^{2+} was added on the cytoplasmic side. Ca^{2+} had no effect from the lumenal side. Liu & Strauss (1991) also reported Ca^{2+} blocked cardiac SR K^+ channel. In our experiments, K^+ channels disappeared in 5 to 10 min after the perfusion of 2 mM Ca^{2+} into the pipette (five out of eight experiments, *result not shown*). In other words, Ca^{2+} changed the open state of the channel into a permanently closed state. No flickering events were observed. If the K^+ channel was blocked directly by Ca^{2+}, the blocking effect should reverse after Ca^{2+} ions are removed, but we did not see such

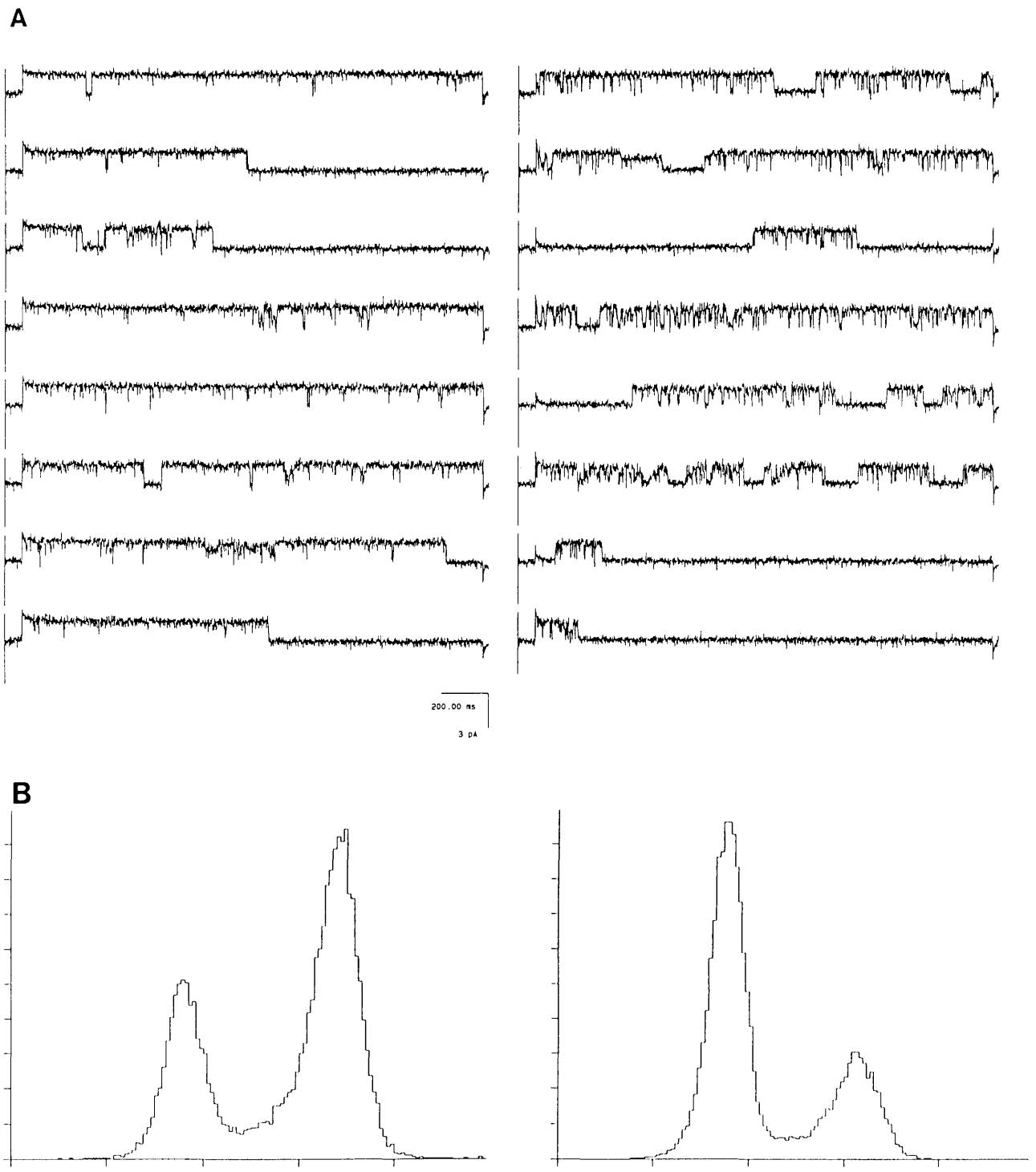

Fig. 9. Effects of 1 mM AlF_4^- on the Ca^{2+} conducting channel. (*A*) Channel was recorded in symmetrical 52.5 mM Ca^{2+} with the membrane potential of +30 mV, and the AlF_4^- was added on the cytoplasmic side through the perfusion pipette. The left eight traces were recorded without AlF_4^- and the right eight traces were recorded with AlF_4^-. The flickering events could be detected readily, but without obvious reduction of current amplitude. Data were filtered at 1 kHz. (*B*) Amplitude histogram of the same experiment at +30 mV applied potential to show the effect of 1 μM AlF_4^-. The open probability was reduced from P_o = 0.67 before the addition of AlF_4^-, to P_o = 0.29 after the addition of AlF_4^-. The current amplitude was changed from 1.63 pA to 1.4 pA, which is not as dramatic as in other drugs. (*C*) Channel open lifetime histogram from the same experiment at +30 mV to show the effect of AlF_4^-. Both time constants were reduced by the application of 1 mM AlF_4^- from τ_1 = 5.11 ms and τ_2 = 92.6 ms to τ_1 = 1.76 ms and τ_2 = 13.4 ms. Please note that the time scale is different in the two histograms.

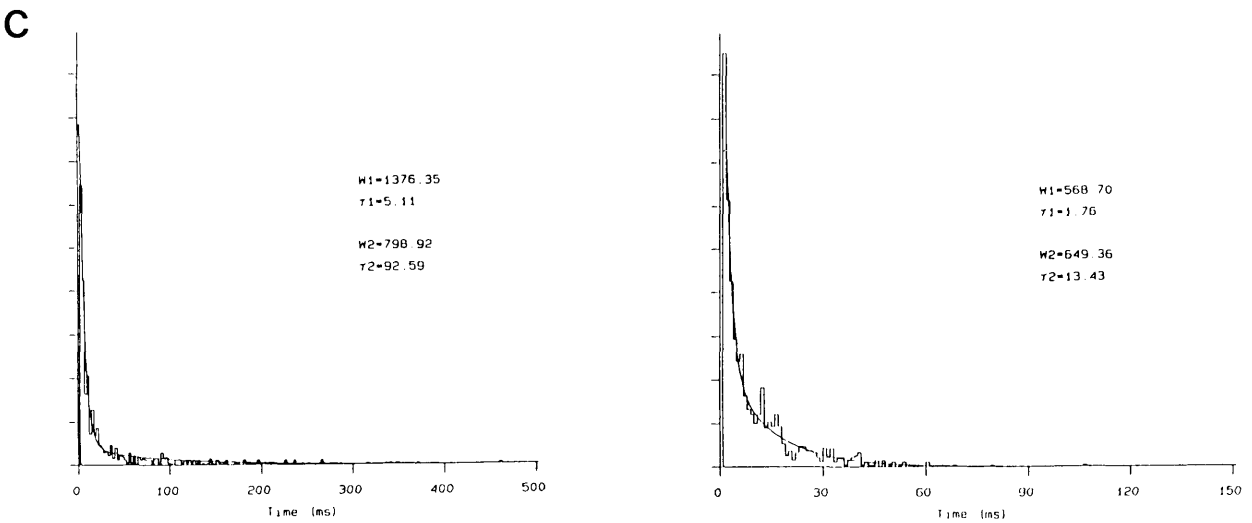

Fig. 9. (*Continued*).

reversal: the K^+ channel never reappeared, even long after Ca^{2+} ions were removed.

Ca^{2+} might change the state of the K^+ channel by phosphorylation. A Ca^{2+} dependent protein kinase system, also requiring calmodulin, that phosphorylates membrane proteins has been identified in sarcoplasmic reticulum preparations (Campbell & MacLennan, 1982; Tuana & MacLennan, 1988). It is possible that exposing the fiber to high Ca^{2+} in our experiments activated protein kinases, phosphorylating serine and threonine residues of the K^+ channel, which then caused a conformational change and inactivated the channel.

The Ca^{2+} Conducting Channel is not the Ryanodine Receptor

Single Ca^{2+} release channels of sarcoplasmic reticulum have mostly been studied in planar bilayers (Smith et al., 1985, Smith, Coronado & Meissner, 1986; Lai et al., 1988; Smith et al., 1988) exploiting the drug ryanodine, a neutral alkaloid that binds tightly to the channel (Fleisher et al., Sutko, Ito & Kenyon, 1985; Campbell et al., 1987; Lai et al., 1988). The channel has a conductance of 110 pS in 50 mM Ca^{2+} solution, or 360 pS in 250 mM K^+ solution, and is selective for divalent ions with permeability ratio $P_{B_a}/P_K = 4.3$ and $P_{C_a}/P_{C_s} = 11.4$. The open probability of the channel is increased by micromolar Ca^{2+} on the cytoplasmic side, but the lifetimes of the openings are not changed substantially. Millimolar ATP also activates the channel; however, a combination of Ca^{2+} and ATP is required for maximal activation. Millimolar Ca^{2+} on the cytoplasmic side blocks the channel. Less than 1 mM Mg^{2+} on the cytoplasmic side produces an unresolvable flickering and may inhibit the channel completely. Nanomolar ryanodine puts the channel into a long-lasting, permanently open subconductance state. Ruthenium red (1 μM), a functional specific blocker of the native Ca^{2+} release channel, blocks the channel, switching it from any of the open states to the closed state (Smith et al., 1986, 1988).

The properties of the Ca^{2+} channel reported here are very different. The channel is insensitive to 10 μM ryanodine and 5 μM ruthenium red. Millimolar concentrations of ATP, caffeine, Mg^{2+} and Ca^{2+} have no obvious effect on this channel (*results not shown*). It seems that the Ca^{2+} conducting channel studied here is not the Ca^{2+} release channel (ryanodine receptor) of sarcoplasmic reticulum.

The ease with which we observed this Ca^{2+} channel also suggests that it is not the ryanodine receptor. The probability of our seeing the Ca^{2+} channel was well above 70%. The chances of seeing the (ryanodine sensitive) Ca^{2+} release channel would be expected to be very low since the junctional membrane containing this channel is only 4.5% of the whole sarcoplasmic reticulum membrane (Mobley & Eisenberg, 1975; Franzini-Armstrong & Nunzi, 1983; Chu et al., 1986; Fleischer & Inui, 1989), unless the channels diffused away from the junctional area and distributed evenly into the SR or into our blebs.

Ca^{2+} conducting channels have also been studied in sarcoballs, prepared with a method similar to ours (Stein & Palade, 1988; Kwok & Best, 1990). The Ca^{2+} conducting channel observed was identified as the Ca^{2+} release channel (ryanodine receptor)

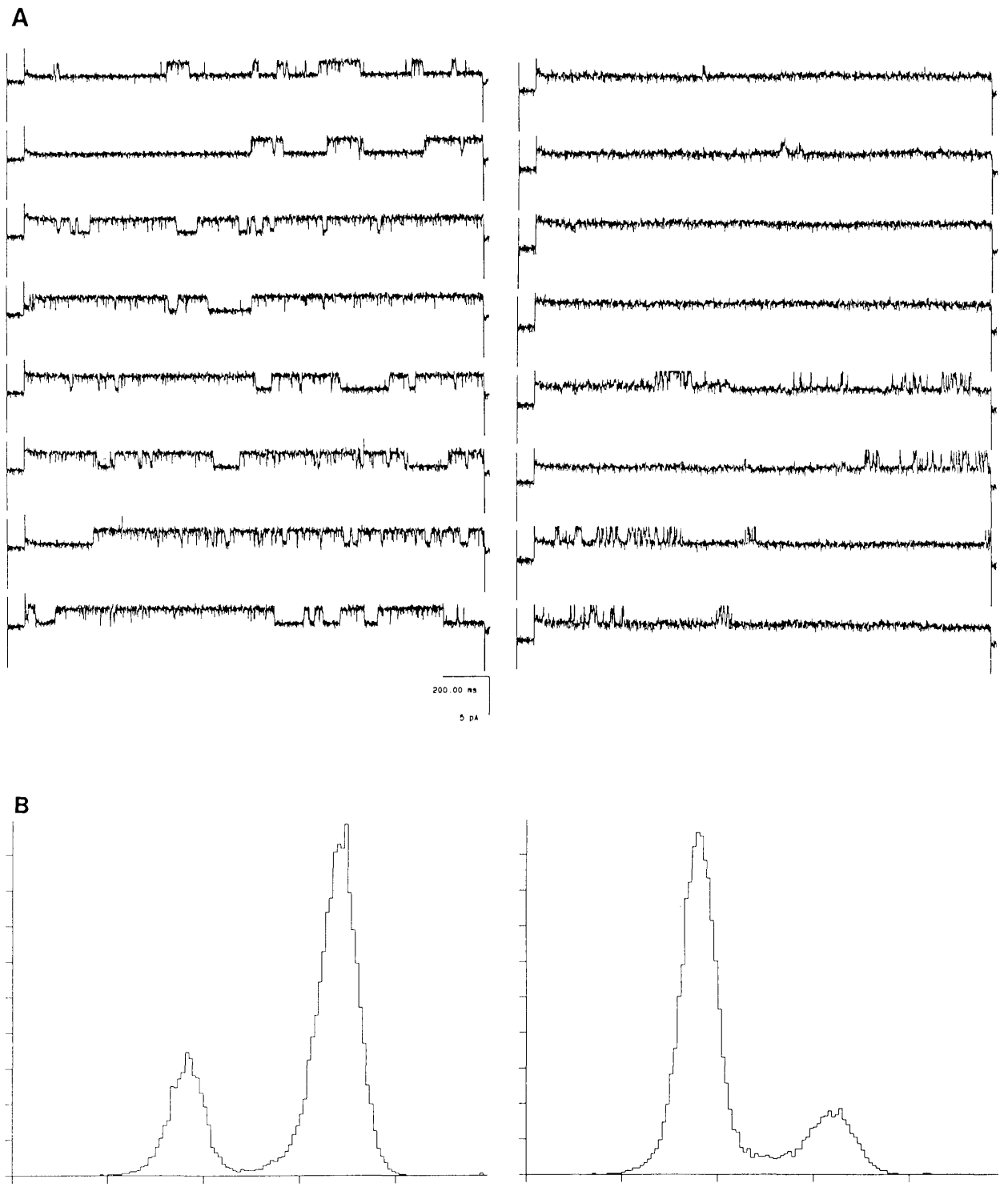

Fig. 10. Effects of 1 mM vanadate on the Ca^{2+} conducting channel. (*A*) Current traces were recorded in symmetrical 100 mM K^+ and 10 mM hexamethonium solution with the membrane potential of +40 mV. K^+ solution was used because vanadate reacts with Ca^{2+} solution, and 10 mM hexamethonium was added in the pipette to block the K^+ channel. The left eight traces wre recorded without and the right eight traces were recorded with 1 mM vanadate. The reduction in channel open probability and the open lifetime by the addition of vanadate could be seen here. The current amplitude was not altered obviously. Vanadate was applied on the cytoplasmic side through the perfusion pipette. Data were filtered at 1 kHz. (*B*) The amplitude histogram of the same channel at +40 mV membrane potential further confirm the observation of the previous figure. The open probability before perfusion was reduced from P_o = 0.67 (left) to P_o = 0.1 (right) after the addition of vanadate. The current amplitude was changed from 1.93 pA to 1.74 pA.

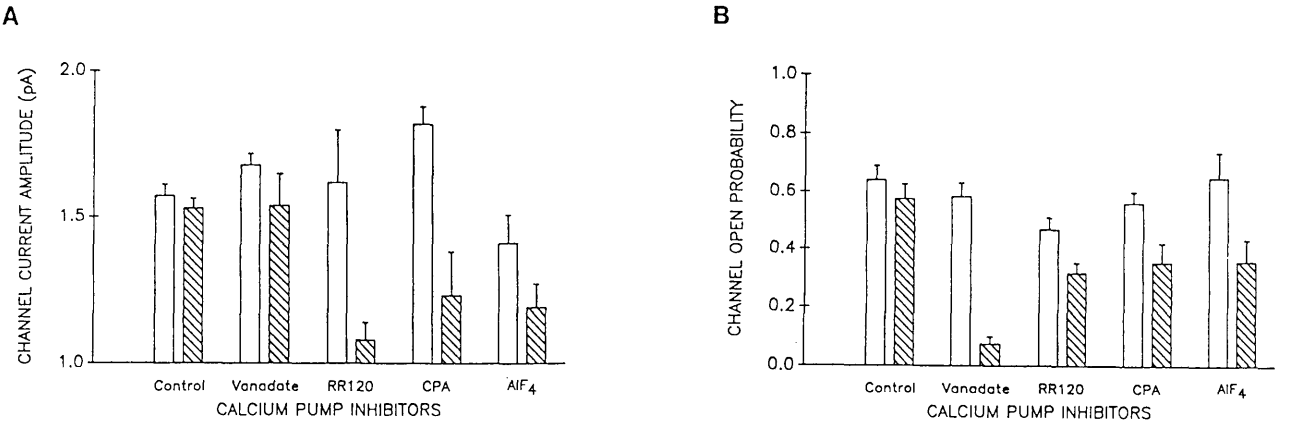

Fig. 11. Summary of effects of Ca^{2+} pump inhibitors on the Ca^{2+} conducting channel. The open bars represent the control groups (before pipette perfusion) and the hashed bars represent the groups with drug applied (after pipette perfusion) ($n = 5$). (*A*) Effect of drugs on channel current amplitude. (*B*) Effect of drugs on channel open probability.

because it responded to ruthenium red and caffeine (Stein & Palade, 1988) and to ryanodine (Kwok & Best, 1990). The channel was reported to have multiple conductance states. Stein and Palade reported two predominant conductance levels: 80–100 pS and 120–160 pS, while Kwok and Best identified four different conductance levels: 31 pS, 49 pS, 81 pS and 128 pS, with the 49 pS and 81 pS being the dominant states. We found three conductance levels: 37 pS, 50 pS and 70 pS and the predominant level in our experiments was 50 pS. We never saw a state with 120 pS conductance. The differences in our results may be due to different experimental conditions, or to other laboratories reporting a conductance that in fact came from two different channels (in different states) that happened to be open at the same time, e.g., one channel open in a 70 pS state, the other in a 50 pS state.

Stein and Palade tested the effect of ruthenium red on the Ca^{2+} channel by looking for channels with pipettes filled with 1 μM ruthenium red. In eight experiments they found no channels. Variability in experimental results can cause difficulties with this kind of experiment; indeed, such variability motivated our development of a pipette perfusion system (Tang et al., 1990) allowing the study of drug action on a specific channel molecule. In our experiments, 5 μM ruthenium red was perfused after a Ca^{2+} channel had been identified. It had no effect. Experiments were also done with pipettes filled with ruthenium red. Ca^{2+} channels were seen with properties no different from "normal." The difference in results between laboratories may be due to differences in conditions, but is probably due to variability in preparations. In our lab, the probability of seeing the Ca^{2+} channel fluctuated. It was not always high: in "bad days" or weeks, Ca^{2+} channels could not be detected in many experiments for no known reason.

Kwok and Best (1990) report (in an abstract) a ryanodine effect on the Ca^{2+} channel. Results from two experiments showed that the open probability of the channel was increased from 0.11 to 0.67 by 1 μM ryanodine and from 0.26 to 0.99 by 10 μM ryanodine added to the bath. This effect of ryanodine was not observed in our experiments. The open probability of the channel varies from channel to channel, in our experience, and, sometimes, varies from time to time even in the same channel, perhaps leading to the result cited. Other explanations are differences in experimental conditions, or the possibility that the channels Stein and Kwok studied were ryanodine sensitive Ca^{2+} release channels that had diffused into their blebs.

The Ca^{2+} Conducting Channel is Probably a Form of the Ca^{2+}-ATPase

As shown in the Results section, pump inhibitors affect the Ca^{2+} conducting channel in our experiments (Fig. 11). These results suggest that the Ca^{2+} channel is a form of the Ca^{2+} pump.

The Ca^{2+} pump of fast-twitch skeletal muscle is the predominant membrane protein in sarcoplasmic reticulum. The 110-kd Ca^{2+}-ATPase constitutes 90% of the total protein. The density of the Ca^{2+}-ATPase on sarcoplasmic reticulum is estimated to be ~20,000/μm^2 (Martonosi, 1984). This means that a patch of membrane under the tip of patch clamp pipette contains at least 20,000 Ca^{2+} pump molecules, if the bleb is made of normal SR membrane, assuming the membrane area is 1 μm^2. The channel

we see might then reflect a rare state of the pump, a state found with probability 1/20,000. One would expect the Ca^{2+} pump to behave like a channel if it opened, *unoccluded,* a small fraction (0.005%) of the time. Perhaps the affinity of Ca^{2+} binding sites (Clarke et al., 1989) forming (or governing) the occlusion is reduced under our experimental conditions, producing a Ca^{2+} pump that has lost its coupling of catalytic, occluding, and channel function, making it a Ca^{2+} conducting channel (Inesi & Kirtley, 1990) instead of an occluded uphill transporter.

We are unaware of other work reporting single channel measurements on a normal pump protein. Meis and Inesi (1992) measured efflux from SR vesicles and conclude that "a passive channel activated by phenothiazine is an integral part of the ATPase"; Narahashi and colleagues (Kim & Wu, 1992; Kim et al., 1990, 1991) found that the depolarizing toxin palytoxin converts the Na,K-ATPase of red blood cell membranes into an ion channel of 10 pS conductance carrying inward Na^+ current.

Although the Ca^{2+} pump behaving as a Ca^{2+} conducting channel is a pleasing, plausible, and thus, our favorite, interpretation of this data, other interpretations are possible. The Ca^{2+} conducting channel could be a Ca^{2+} release channel (ryanodine receptor) that diffused away from the T-SR junctional area and bound to a Ca^{2+} pump molecule, losing its native sensitivity to ryanodine (etc.) and acquiring (allosterically) the drug sensitivity of the pump.

Significance of Ca^{2+}-ATPase Forming a Ca^{2+} Channel

The pump acting as a channel may help explain the appreciable passive Ca^{2+} permeability of SR thought to come from the Ca^{2+}-ATPase molecule. (Incorporation of the Ca^{2+}-ATPase into bilayer membranes increases passive Ca^{2+} permeability: Jilka, Martonosi & Tillack, 1975; Jilka & Martonosi, 1977). At least four mechanisms for this passive permeability have been proposed: (1) reversal of the Ca^{2+}-ATPase; (2) partial reactions of the ATPase; (3) the ATPase acting as a carrier; and (4) the ATPase acting as a channel (Jilka et al., 1975; Jilka & Martonosi, 1977; Vanderkooi et al., 1977). Our results suggest that the passive Ca^{2+} permeability arises from a rare conformation of the Ca^{2+}-ATPase containing an open (un-occluded) channel spanning the SR membrane. This conformation may have nothing to do with normal active transport (except as a passive parasitic shunt path) or it might be a state of the Ca^{2+}-ATPase also used in transport of a Ca^{2+} ion against a free energy gradient.

We thank Drs. F. Cohen, A. Fox, R. Levis and E. Rios for much useful help and criticism and Dr. G. Inesi for sending us his paper while in press.

We thank the National Science Foundation for steadfast support.

References

Ashcroft, S.J.H., Ashcroft, F.M. 1990. Properties and functions of ATP sensitive K channels. *Cell. Signal.* **2:**197–214

Bean, B.P. 1989. Classes of calcium channels in vertebrate cells. *Annu. Rev. Physiol.* **51:**367–384

Campbell, K.P., Knudson, C.M., Imagawa, T., Leung, A.T., Sutko, J.L., Kahl, S.D., Raab, C.R., Madson, L. 1987. Identification and characterization of the high affinity [^{3}H] ryanodine receptor of the junctional sarcoplasmic reticulum Ca^{2+} release channel. *J. Biol. Chem.* **262:**6460–6463

Campbell, K.P., MacLennan, D.H. 1982. A calmodulin-dependent protein kinase system from skeletal muscle sarcoplasmic reticulum. *J. Biol. Chem.* **257:**1238–1246

Chu, A., Volpe, P., Costello, B., Fleischer, S. 1986. Functional characterization of junctional terminal cisternae from mammalian fast skeletal muscle sarcoplasmic reticulum. *Biochemistry* **25:**8315–8324

Clarke, D.M., Loo, T.W., Inesi, G., MacLennan, D.H. 1989. Location of high affinity Ca binding sites within the predicted transmembrane domain of the sarcoplasmic reticulum Ca-ATPase. *Nature* **339:**476–478

Coll, R.J., Murphy, A.J. 1987. Inhibition of the sarcoplasmic reticulum Ca-ATPase by reactive red 120. *Biochim. Biophys. Acta* **904:**227–238

Coronado, R., Miller, C. 1980. Decamethonium and hexamethonium block K^+ channels of sarcoplasmic reticulum. *Nature* **288:**495–497

Dupont, Y., Bennett, N. 1982. Vanadate inhibition of the Ca dependent conformational change of the sarcoplasmic reticulum Ca-ATPase. *FEBS Lett.* **139:**237–240

Fabiato, A. 1988. Computer programs for calculating total from specified free or free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. *Methods Enzymol.* **157:**378–417

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

Fleischer, S., Ogunbunmi, E.M., Dixon, M.C., Fleer, E.A.M. 1985. Localization of Ca^{2+} release channels with ryanodine in junctional terminal cisternae of sarcoplasmic reticulum of fast skeletal muscle. *Proc. Natl. Acad. Sci. USA* **82:**7256–7259

Franzini-Armstrong, C., Nunzi, G. 1983. Functional feet and particles in the triads of a fast-twitch muscle fibre. *J. Muscle Res. Cell Motil.* **4:**233–252

Goeger, D.E., Riley, R.T., Dorner, J.W., Cole, R.J. 1988. Cyclopiazonic acid inhibition of the Ca-transport ATPase in rat skeletal muscle sarcoplasmic reticulum vesicles. *Biochem. Pharmacol.* **37:**978–981

Hals, G.D., Palade, P.T. 1990. Different sites control voltage dependence and conductance of sarcoball anion channel. *Biophys. J.* **57:**1037–1047

Hals, G.D., Stein, P.G., Palade, P.T. 1989. Single channel characteristics of a high conductance anion channel in "sarcoball". *J. Gen. Physiol.* **93:**385–410

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch clamp techniques for high-resolution

current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Inesi, G., Kirtley, M.E. 1990. Coupling of catalytic and channel function in the Ca^{2+} transport ATPase. *J. Membrane Biol.* **116:**1–8

Inesi, G., Kurzmack, M., Coan, C., Lewis, D. 1980. Cooperative calcium binding and ATPase activation in sarcoplasmic reticulum vesicles. *J. Biol. Chem.* **255:**3025–3031

Jilka, R.L., Martonosi, A. 1977. The effect of calcium ion transport ATPase upon the passive calcium ion permeability of phospholipid vesicles. *Biochim. Biophys. Acta* **466:**57–67

Jilka, R.L., Martonosi, A., Tillack, T.W. 1975. Effect of the purified Mg^{+} Ca-activated ATPase of sarcoplasmic reticulum upon the passive Ca permeability and ultrastructure of phospholipid vesicles. *J. Biol. Chem.* **250:**7511–7524

Kim, S.Y., Wu, C.H. 1992. Ouabain-sensitive single channel currents induced by palytoxin. *Biophys. J.* **61:***A*512

Kim, S.Y., Wu, C.H., Narahashi, T., Beress, L. 1990. Modification of Na, K-ATPase by palytoxin. *Biophys. J.* **57:**352*a*

Kim, S.Y., Wu, C.H., Narahashi, T., Beress, L. 1991. Palytoxin forms ion channels through Na, K-ATPase. *Biophys. J.* **59:**460*a*

Kwok, W.M., Best, P.M. 1990. Ryanodine sensitivity and multiple conductance states of the Ca release channel from native SR membrane. *Biophys. J.* **57:**168*a*

Labarca, P.P., Miller, C. 1981. A K^{+} selective, three-state channel from fragmented sarcoplasmic reticulum of frog leg muscle. *J. Membrane Biol.* **61:**31–38

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

Lee, K.S., Tsien, R.W. 1984. High selectivity of calcium channels in single dialysed heart cells of the guinea-pig. *J. Physiol.* **354:**253–272

Liu, Q.Y., Strauss, H.C. 1991. Blockade of cardiac sarcoplasmic reticulum K^{+} channel by Ca^{2+}: two-binding-site model of blockage. *Biophys. J.* **60:**198–203

Martonosi, A. 1984. Mechanisms of Ca release from sarcoplasmic reticulum of skeletal muscle. *Physiol. Rev.* **64:**1240–1320

Meis, L. de and Inesi, G. 1992. Functional evidence of a transmembrane channel within the Ca^{2+} transport ATPase of sarcoplasmic reticulum. *FEBS Lett.* **299:**33–35

McKinley, D., Meissner, G. 1978. Evidence for a K^{+}, Na^{+} permeable channel in sarcoplasmic reticulum. *J. Membrane Biol.* **44:**159–187

Miller, C. 1978. Voltage-gated cation conductance channel from fragmented sarcoplasmic reticulum: steady-state electrical properties. *J. Membrane Biol.* **40:**1–23

Mobley, B.A., Eisenberg, B.R. 1975. Sizes of components in frog skeletal muscle measured by methods of stereology. *J. Gen. Physiol.* **66:**31–45

Norred, W.P., Morrissey, R.E., Riley, R.T., Cole, R.J., Dorner, J.W. 1985. Distribution, excretion and skeletal muscle effects of the mycotoxin [^{14}C]cyclopiazonic acid in rats. *Food Chem. Toxicol.* **23:**1069–1076

Quast, U., Cook, N.S. 1989. Moving together: K channel openers and ATP-sensitive K channels. *Trends Pharmacol. Sci.* **10:**431–435

Rios, E., Pizarro, G. 1991. Voltage sensor of excitation-contraction coupling in skeletal muscle. *Physiol. Rev.* **71:**849–908

Rousseau, E., Smith, J.S., Meissner, G. 1987. Ryanodine modifies conductance and gating behavior of single Ca^{2+} release channel. *Am. J. Physiol.* **253:**C364–368

Seidler, N.W., Jona, I., Vegh, M., Martonosi, A. 1989. Cyclopiazonic acid is a specific inhibitor of the Ca-ATPase of sarcoplasmic reticulum. *J. Biol. Chem.* **264:**17816–17823

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

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

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

Stein, P.G., Nelson, T.E., Palade, P.T. 1989. Mammalian sarcoplasmic reticulum K channels recorded in skinned fibers. *Biophys. J.* **55:**480*a*

Stein, P.G., Palade, P.T. 1988. Sarcoballs: direct access to sarcoplasmic reticulum Ca^{2+} channels in skinned frog muscle fibers. *Biophys. J.* **54:**357–363

Sutko, J.L., Ito, K., Kenyon, J.L. 1985. Ryanodine: a modifier of sarcoplasmic reticulum calcium release in striated muscle. *Fed. Proc.* **44:**2984–2988

Tang, J.M., Wang, J., Eisenberg, R.S. 1989. K^{+} selective channel from sarcoplasmic reticulum of split lobster muscle fibers. *J. Gen. Physiol.* **94:**261–278

Tang, J.M., Wang, J., Quandt, F.N., Eisenberg, R.S. 1990. Perfusing pipettes. *Pfluegers Arch.* **416:**347–350

Troullier, A., Girardet, J.L., Dupont, Y. 1991. AlF_4^- as an analog of P_i on the SR Ca-ATPase. *Biophys. J.* **59:**252a

Tsien, R.W. 1983. Calcium channels in excitable cell membranes. *Annu. Rev. Physiol.* **45:**341–358

Tuana, B.S., MacLennan, D.H. 1988. Isolation of the calmodulin-dependent protein kinase system from rabbit skeletal muscle sarcoplasmic reticulum. *FEBS Lett.* **235:**219–223

Vanderkooi, J.M., Ierokomas, A., Nakamura, H., Martonosi, A. 1977. Fluorescence energy transfer between Ca transfer ATPase molecules in artificial membranes. *Biochemistry* **16:**1262–1267

Vivaudou, M.B., Arnoult, C., Villaz, M. 1989. Patch-clamp recordings of K channels from membrane blebs (sarcoballs) from skinned frog skeletal muscle fibers. *Biophys. J.* **55:**483*a*

Vivaudou, M.B., Arnoult, C., Villaz, M. 1991. Skeletal muscle ATP-sensitive K^{+} channels recorded from sarcolemmal blebs of split fibers: ATP inhibition is reduced by magnesium and ADP. *J. Membrane Biol.* **122:**165–175

Vivaudou, M.B., Villaz, M. 1991. Mg and ADP reduce the ATP sensitivity of skeletal muscle ATP-sensitive K channels recorded from sarcolemmal blebs of split fibers. *Biophys. J.* **59:**75*a*

Woll, K.H., Lonnendonker, U., Neumcke, B. 1989. ATP sensitive potassium channels in adult mouse skeletal muscle: different modes of blockage by internal cations, ATP and tolbutamide. *Pfluegers Arch.* **414:**622–628

Xu, L., Jones, R.V., Meissner, G. 1989. Activation of the skeletal muscle Ca^{2+} release channel by the triazine dyes cibacron blue F3A-G and reactive red 120. *Arch. Biochem. Biophys.* **274:**609–616

Received 6 February 1992; revised 20 May 1992