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# A $K^+$-Selective, Three-State Channel from Fragmented Sarcoplasmic Reticulum of Frog Leg Muscle

Pedro P. Labarca* and Christopher Miller**

Graduate Program in Biophysics and Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254

**Summary.** Sarcoplasmic reticulum (SR) vesicles from frog leg muscle were fused with a planar phospholipid bilayer by a method described previously for rabbit SR. As a result of the fusion, $K^+$-selective conduction channels are inserted into the bilayer. Unlike the two-state rabbit channel, the frog channel displays three states: a nonconducting ("closed") state and two conducting states "$\alpha$" and "$\beta$". In 0.1 M $K^+$ the single-channel conductances are 50 and 150 pS for $\alpha$ and $\beta$, respectively. The probabilities of appearance of the three states are voltage-dependent, and transitions between the closed and $\beta$ states proceed through the $\alpha$ state. Both open states follow a quantitatively identical selectivity sequence in channel conductance: $K^+ > NH_4^+ > Rb^+ > Na^+ > Li^+ > Cs^+$. Both open states are blocked by $Cs^+$ asymmetrically in a voltage-dependent manner. The zero-voltage dissociation constant for blocking is the same for both open states, but the voltage-dependences of the $Cs^+$ block for the two states differ in a way suggesting that the $Cs^+$ blocking site is located more deeply inside the membrane in the $\beta$ than in the $\alpha$ state.

**Key words.** Sarcoplasmic reticulum, K-channel, planar bilayer, ion selectivity, Cs-block, excitation-contraction coupling.

Muscle contraction in vertebrate skeletal muscle is triggered by a rapid and massive release of calcium from the sarcoplasmic reticulum (SR) into the sarcoplasm [3, 9]. It is necessary that the release of calcium be accompanied by the movement of other ions across the SR membrane in order to compensate for the charge imbalance produced [18, 19].

In the last few years a number of studies have produced evidence indicating that the fragmented SR of skeletal muscle is selectively permeable to small monovalent cations. The experimental approaches used in these studies include flux experiments [14], light scattering techniques [10], and fusion of SR vesicles with planar bilayer membranes [15]. The latter approach has allowed a detailed study of a channel that is selective for small monovalent cations, especially $K^+$, derived from the fragmented SR of rabbit skeletal muscle [8, 11, 15, 16]. While the studies of permeability of fragmented SR have been carried out using SR from rabbit skeletal muscle, much of the electrophysiological information concerning muscle contraction, including that on the mechanism of calcium release, has been derived from frog leg muscle. The electrophysiological work has led to models for calcium release requiring a "leak" permeability in the SR membrane [13, 19].

We therefore consider it important to study the permeability properties of fragmented SR from the same source used in electrophysiological studies: frog leg fast-twitch muscle. In this paper we describe a $K^+$-selective channel from frog SR incorporated into planar phospholipid bilayers. The novel feature of this channel is its presentation of two distinct conducting states, differing in electrical conductance but identical in ionic selectivity.

## Materials and Methods

### *Biochemical*

Northern bullfrogs *(Rana catesbiana)* were obtained from West Jersey Biological Supply. Fragmented SR from frog leg muscle was prepared by the method of Batra [1], modified as follows: Frog leg white muscle (100–300 g), dissected away from fascia and visibly red muscle, was chopped in a Cuisinart food processor for 5 sec and then homogenized in a Waring blendor in 2 vol of 0.15 M KCl, 5 mM MOPS-KOH, pH 6.8, for 15 sec.[1] One additional volume of buffer was added and the homogenization repeated. The homogenate was spun at 8,000 × *g* for 12 min. The

* *Present address:* Department of Physics, University of California at San Diego, La Jolla, Calif. 92093.

** *To whom correspondence should be directed.*

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supernatant was passed through cheesecloth and was neutralized with KOH. The pellet was rehomogenized in 1 vol of 0.15 M KCl buffer and spun at $10,000 \times g$ for 15 min. The supernatant was filtered through cheesecloth and adjusted to pH 7. The combined supernatants were centrifuged at $90,000 \times g$ for 40 min. The pellets were resuspended in 0.6 M KCl, 5 mM MOPS-KOH, pH 7.0, and centrifuged at $90,000 \times g$ for 40 min. The pellets were resuspended in 0.4 M sucrose, 5 mM MOPS-KOH, pH 7.0, and spun at $8,000 \times g$ for 10 min. The supernatant of this centrifugation was spun at $90,000 \times g$ for 40 min; the pellets were resuspended in a small volume of the sucrose buffer. The SR vesicles were stored in small aliquots at $-70$ °C.

Calcium loading and oxalate-dependent calcium uptake were found to be 100–200 nmol/mg-min and 1–1.5 μmol/mg-min, respectively. Rates of $Mg^{2+}$-dependent ATPase activities were typically 0.2–0.3 μmol $P_i$/mg min. $Ca^{2+}$-dependent ATPase activities ranged 0.3–0.4 μmol $P_i$/mg min in the absence of the calcium ionophore A 23157 and 1–1.3 μmol $P_i$/mg min in the presence of the ionophore. Cytochrome oxidase specific activities were usually in the order of 0.01 nmol/mg-min. (All activities were reported at 25°.)

Mixed soybean phospholipid (asolectin) was purchased from Sigma and washed free of calcium and proteolipid as described [11]. Phosphatidylethanolamine (PE) was prepared as described previously [11].

Planar bilayers were cast from decane solutions according to Mueller and Rudin [17] as described [15]. The aqueous phase of the bilayer system consisted of salt at the appropriate concentration, 5 mM HEPES-0.1 mM EDTA, neutralized to pH 7.0, with Tris base.

### *Fusion of SR Vesicles with Planar Bilayers*

Fusion of SR vesicles with the planar phospholipid bilayer was brought about as described [15]. SR vesicles (2–10 μg/ml) were added to the *cis* side of the bilayer; voltage is defined as zero on the opposite, *trans*, side of the bilayer. Single-channel reversal potentials under asymmetric ionic conditions were measured as described previously [8].

## Results

### *Interaction of SR Vesicles with Planar Bilayers*

The interaction of SR vesicles with a negatively charged membrane in the presence of calcium (~1 mM) induces a step-like increase in the conductance of the membrane (Fig. 1*A*). The occurrence of this phenomenon is dependent on the presence of negatively charged phospholipid in the bilayer and calcium on the same side (the *cis* side) into which vesicles are added. The conductance of the membrane could be stabilized at any time after the addition of vesicles by adding into the *cis* chamber excess EDTA. Large increases in membrane conductance (3–4 orders of magnitude) could be obtained if the SR vesicles were made to interact with

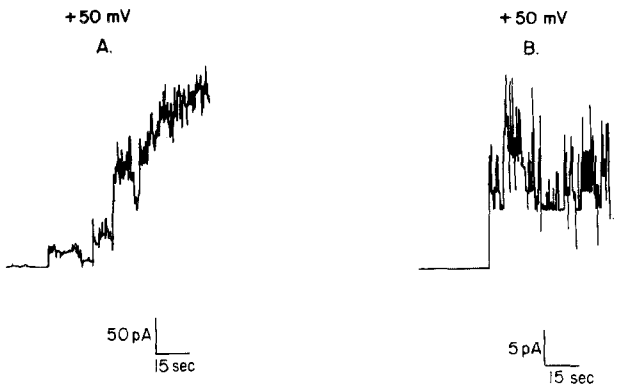


**Fig. 1.** (*A*): Increase in the conductance of artificial membranes in the presence of SR vesicles from frog leg muscle. The conductance of the membrane was monitored at +50 mV. Recording was begun after the addition of calcium (1 mM, final concentration) and vesicles (10 μg/ml, final protein concentration) into the *cis* chamber. About 30 sec after addition of calcium and vesicles the conductance of the membrane started to increase in steps as indicated by arrows. The planar bilayer consisted of 90% PE-10% AL. The aqueous phase contained 100 mM $K^+$ glucuronate, 5 mM Hepes-Tris-0.1 mM EDTA, pH 7.0. The temperature was 20 °C. (*B*): Single fusion event. A single step-like increase in the conductance of the planar bilayer is shown at higher gain

the planar bilayer in the presence of an osmotic gradient such that the *cis* side was ~200 mosM hyperosmotic with respect to the *trans* side. This condition has been shown by Cohen, Zimmerberg and Finkelstein [4] to induce the fusion of phospholipid vesicles with planar bilayers.

The elemental event responsible for the conductance increase described in Fig. 1*A* is shown in Fig. 1*B*. It consists of a sudden increase in both the mean conductance of the bilayer and in the conductance noise about the mean value. A closer look at this noise reveals that the conductance fluctuates among well-defined levels. In Fig. 2 we observe two conductane levels of 50 and 150 pS above the zero-level conductance. This behavior could be the result of the incorporation of two types of channels into the bilayer with conductances 50 and 100 pS or of a single channel with two conducting states. The latter explanation is strongly suggested by the experiment shown in Fig. 2. In this membrane only one channel is fluctuating above the zero-level conductance most of the time. As seen, transitions from the zero to the 50 pS level are observed; transitions from the 50 to the 150 pS level are also present. However, transitions from the zero to the 100 pS level *never* occur, and this has been our experience in general for this system. Therefore we propose that these fluctuations are due to the existence of a channel which can exist in *three* electrically distinguishable states: a nonconducting or "closed" state (*C*), a

[1] *Abbreviations used:* MOPS, morpholinopropane sulfonic acid; Hepes, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; EDTA, (ethylenedinitrilo)-tetraacetic acid; Tris, Tris (hydroxymethyl)aminomethane; AL, soybean phospholipid.

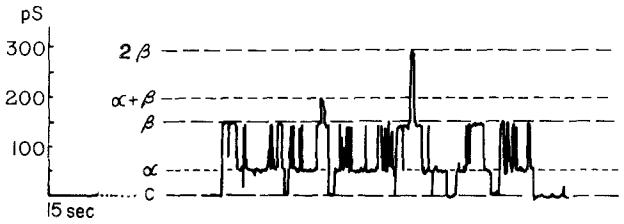


**Fig. 2.** Single channel fluctuations. Planar bilayers made of PE-AL (8/2) were formed in 100 mM $K^+$ glucuronate buffer. Calcium (0.1 mM, final concentration) and vesicles (2 μg/ml final protein concentration) were added into the *cis* chamber. After a single fusion event took place, EDTA was added into the *cis* chamber up to a concentration of 0.2 mM. Fluctuations were recorded at +50 mV. In this case only one channel was fluctuating most of the time. The line labeled "$C$" indicates the zero-level conductance; line labeled "$\alpha$" was drawn at 50 pS, "$\beta$" at 150 pS, "$\alpha+\beta$" at 200 pS and "$2\beta$" at 300 pS

small-open state, $\alpha$, with a conductance of 50 pS in 0.1 M $K^+$, and a large-open state, $\beta$, of 150 pS conductance in 0.1 M $K^+$.

An alternative explanation for these three levels is that monomeric 50-pS channels could dimerize and interact such that in the dimeric state each monomer acts as a channel with a 75-pS conductance. This explanation is unlikely, however, since the mean conductance of the membrane increases linearly, not quadratically, with the number of fusion events (data not shown). Thus, as in the case of the SR $K^+$ channel from rabbit muscle [15], this channel appears to act as a monomer. Only rarely do we obtain records such as Fig. 2, taken from a membrane in which only one channel is fluctuating most of the time. Much more often, channels are inserted in "packages" of several channels, and so the conductance levels appearing are quite difficult to interpret unambiguously.

### *Channel Gating Properties*

*Transition model.* Since the channel displays three electrically distinguishable states, one closed ($C$) and two open ($\alpha$ and $\beta$), we would like to know whether the mechanism of transitions among these states is linear (*I*) or triangular (*II*).

$$C \rightleftarrows \alpha \rightleftarrows \beta \qquad (I)$$

$$C \rightleftarrows \alpha \quad (\alpha \rightleftarrows \beta) \qquad (II)$$

We can decide between these two possibilities by examining records of a large number of conductance transitions, in a membrane containing only one channel. We define the single-step transition probability, $P_{ij}$, from the state $i$ to the state $j$ as:

**Table 1.** Single-step transition probabilities

| $i \rightarrow j$ | $P_{ij}$ | Probability of missing an intermediate state |
|---|---|---|
| $C \rightarrow \alpha$ | 0.36 | 0.10 |
| $\alpha \rightarrow C$ | 0.30 | |
| $\alpha \rightarrow \beta$ | 0.14 | 0.05 |
| $\beta \rightarrow \alpha$ | 0.10 | |
| $C \rightarrow \beta$ | 0.06 | 0.08 |
| $\beta \rightarrow C$ | 0.05 | |

Single-channel fluctuations were measured at −20 mV in PE-AL membranes. The aqueous phase contained 100 mM KCl buffer. Calcium (0.1 mM) and SR vesicles (2 μg/ml) were added into the *cis* chamber. After the occurrence of a single fusion the EDTA concentration was raised in the *cis* chamber to 0.15 mM and recording of channel fluctuations was started. A large number of fluctuations (~200) were recorded on chart paper and the $P_{ij}$ obtained as explained in the text. The probability of missing an intermediate state was obtained as follows. Dwell times for $C$, $\alpha$, and $\beta$ states were measured by hand; these values were used to build frequency *vs.* time histograms. From the exponential distribution of dwell times for each state, $\tau_i$, the time constant of the exponential was was obtained. $P_i^*$, the probability of the state $i$ having a lifetime less than or equal to time $t$ is given by:

$$P_i^* = (1 - \exp(-t/\tau_i)).$$

In the present case $t$ corresponds to the time response of the recorder used (0.1 sec)

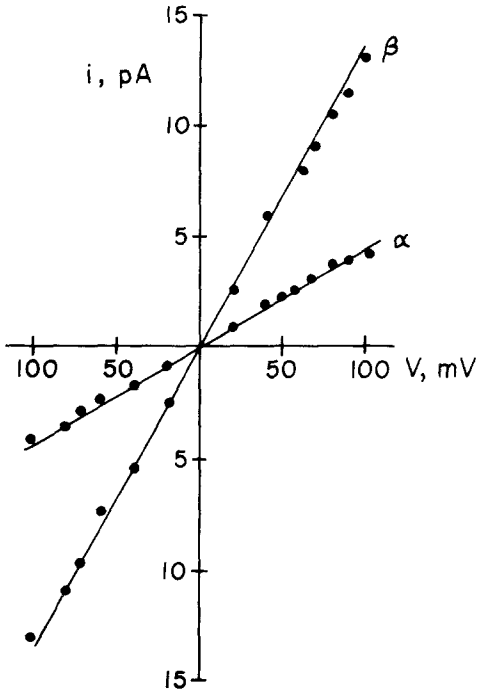


**Fig. 3.** Current *vs.* voltage relation for $\alpha$ and $\beta$ states. Single channel fluctuations were studied at different voltages under conditions of Fig. 2

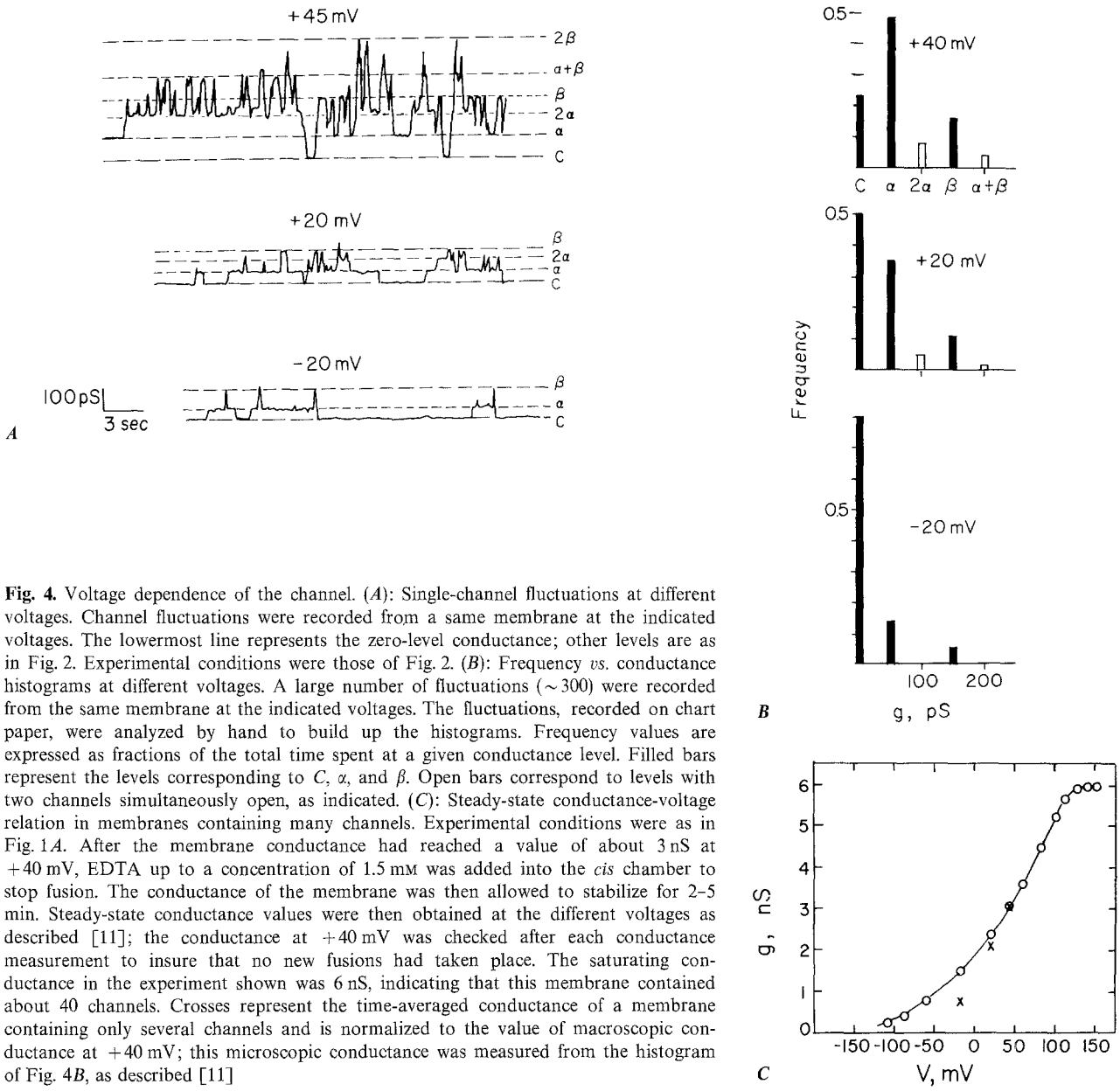


**Fig. 4.** Voltage dependence of the channel. (*A*): Single-channel fluctuations at different voltages. Channel fluctuations were recorded from a same membrane at the indicated voltages. The lowermost line represents the zero-level conductance; other levels are as in Fig. 2. Experimental conditions were those of Fig. 2. (*B*): Frequency *vs.* conductance histograms at different voltages. A large number of fluctuations (~300) were recorded from the same membrane at the indicated voltages. The fluctuations, recorded on chart paper, were analyzed by hand to build up the histograms. Frequency values are expressed as fractions of the total time spent at a given conductance level. Filled bars represent the levels corresponding to *C*, α, and β. Open bars correspond to levels with two channels simultaneously open, as indicated. (*C*): Steady-state conductance-voltage relation in membranes containing many channels. Experimental conditions were as in Fig. 1*A*. After the membrane conductance had reached a value of about 3 nS at +40 mV, EDTA up to a concentration of 1.5 mM was added into the *cis* chamber to stop fusion. The conductance of the membrane was then allowed to stabilize for 2–5 min. Steady-state conductance values were then obtained at the different voltages as described [11]; the conductance at +40 mV was checked after each conductance measurement to insure that no new fusions had taken place. The saturating conductance in the experiment shown was 6 nS, indicating that this membrane contained about 40 channels. Crosses represent the time-averaged conductance of a membrane containing only several channels and is normalized to the value of macroscopic conductance at +40 mV; this microscopic conductance was measured from the histogram of Fig. 4*B*, as described [11]

$P_{ij}$=(number of $i \rightarrow j$ transitions)/
(total transitions observed).

Table 1 shows the result of such an experiment at −20 mV. We see that transitions between α and *C* and α and β are most probable. Occasionally transitions between *C* and β are observed; however, the probabilities of these transitions fall within the range expected for failures to observe a short-lived, α-state, given the time response of the recorder and the exponential distribution of α-state dwell times (*see* legend to Table 1). The results shown in Table 1 are consistent with a linear mechanism in which transitions between *C* and β must proceed through α. Similar results (not shown) were obtained at +20 mV.

*Voltage dependence.* The conductance of both open states of the channel was found to be constant over the range ±100 mV (Fig. 3). The probability of forming an open channel, however, depends on the applied voltage as revealed by observations made in membranes containing several channels (Fig. 4*A*).

Notice that in these membranes, a conductance level at 100 pS is observed, in contrast to the absence of such a level in a one-channel membrane (Fig. 2). It is reasonable to suggest that this represents a "2α" level, i.e., with two channels simultaneously

occupying an $\alpha$-state. Conductance histograms obtained from the same membrane, at different voltages, demonstrate that at negative voltages the closed and $\alpha$ states are favored. At positive voltages the $\alpha$ and $\beta$ states predominate (Fig. 4*B*).

This effect of voltage is reflected in the macroscopic steady-state conductance *vs.* voltage relation ($g-V$ curve) obtained in membranes containing many channels. Here we measure the macroscopic conductance after the membrane has relaxed to its steady-state conductance following the application of the desired voltage (typically 0.5–5.0 sec, depending on lipid composition, voltage, and $K^+$ concentration). One such $g-V$ curve is shown in Fig. 4*C*. The voltage dependence of the macroscopic conductance agrees qualitatively with our observations in membranes containing only a few channels: the macroscopic conductance increases as voltage is made increasingly positive. The macroscopic $g-V$ curve, however, cannot be precisely superimposed upon the microscopic, time-averaged conductance-voltage curve (crosses in Fig. 4*C*). This difference is perhaps due to the "leak" conductance present in the macroscopic curve, which is effectively subtracted out of the single-channel fluctuations. Note that at highly positive voltage, the macroscopic conductance approaches a maximum level, as if all channels are driven into their conducting states, as is the case for the channel from rabbit SR [11].

### *Channel Conduction Properties*

*A. Ionic selectivity.* The conductance of both open states of the channel measured in the presence of small monovalent cations, at 1 M concentration, are shown in Table 2. Both open states are more selective for $K^+$. Furthermore, the ionic selectivities of the two states are quantitatively identical (Table 2). The selectivity sequence $K^+ > NH_4^+ > Rb^+ > Na^+ > Li^+$ for both open states is identical to that of the $K^+$-selective channel found in the SR of rabbit skeletal muscle [8]. The permeability ratio ($P_{K^+}/P_{Na^+}$) is the same for both open states, as illustrated in Fig. 5*A*, averaging $2.1 \pm 0.03$ for the $\alpha$-state and $2.1 \pm 0.05$ for the $\beta$-state (SEM for 3 determinations); note that this ratio is less than the $K^+/Na^+$ conductance ratio in Table 2, as is also the case for the rabbit channel [8]. Chloride ions do not permeate through either open state of the channel, as demonstrated in Fig. 5*B*.

*B. Blocking of the $\alpha$ and $\beta$ states by $Cs^+$.* We have found that $Cs^+$ blocks both open states of the channel in a voltage-dependent manner. This effect of $Cs^+$ can be accounted for in terms of the single-site block model described for the rabbit channel [7].

**Table 2.** Selectivity sequence of $\alpha$ and $\beta$ states for monovalent cations

| Cation | $\gamma_\alpha$ (pS) | $\gamma_\beta$ (pS) | $\gamma_{K^+}/\gamma_{X^+}$ | | $\gamma$ rabbit (pS) |
|---|---|---|---|---|---|
| | | | $\alpha$ | $\beta$ | |
| $K^+$ | 97 ±3 | 240 ±7 | – | – | 214 ±3 |
| $NH_4^+$ | 57 ±2 | 140 ±4 | 1.7 | 1.7 | 157 ±2 |
| $Rb^+$ | 48 ±2 | 115 ±4 | 2.0 | 2.1 | 125 ±2 |
| $Na^+$ | 23 ±2 | 61 ±2 | 4.3 | 4.0 | 72 ±1 |
| $Li^+$ | 7.6 ±0.2 | 17 ±0.4 | 13 | 14 | 7.8 ±0.3 |

Single-channel conductance measured in PE membranes using 1 M solutions of the chloride salt indicated. Each value is the average of at least five determinations obtained in different membranes ±SE. Data for the rabbit SR channel are taken from Coronado, Rosenberg and Miller [7], and were obtained under similar conditions.

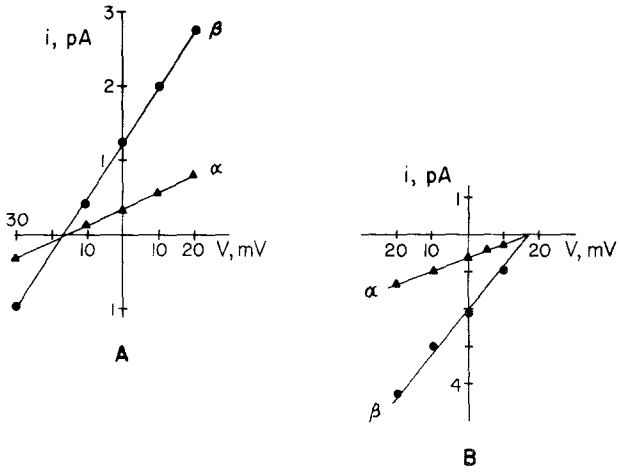


**Fig. 5.** (*A*): Determination of ($P_{K^+}/P_{Na^+}$) ratio. Planar bilayers made of PE were formed in 200 mM NaCl, 5 mM Hepes-Tris-0.1 mM EDTA; other experimental conditions were as in Fig. 2. After a single fusion occurred, the *cis* chamber was perfused with 200 mM KCl buffer. The current *vs.* voltage curve was then measured for both open states in order to determine the zero-current voltage (*Vo*). *Vo* relates to the permeability ratio according to [7]:

$$P_{K^+}/P_{Na^+} = (a_{Na^+}/a_{K^+}) \exp(-FV_o/RT)$$

where $a_{Na^+}$ and $a_{K^+}$ represent activities of $Na^+$ and $K^+$. In the experiment shown $V_o$ was found to be $-17 \pm 0.6$ mV for both open states, which correspond to ($P_{K^+}/P_{Na^+}$) equal to 2.0 for $\alpha$ and $\beta$. (*B*): $K^+$ over $Cl^-$ selectivity. PE membranes were formed in 200 mM KCl buffer; other conditions were as in Fig. 2. After a single fusion event took place, the *cis* chamber was perfused with 100 mM KCl buffer. The current *vs.* voltage curve was measured for both open states. In the experiment shown the zero-current voltage was 17.5 mV for both open states, which equals the Nernst potential for $K^+$ under the experimental conditions. In three similar experiments the zero-current voltage averaged 17.3 mV for the $\alpha$ state and 17.5 mV for the $\beta$ state, showing that the channel is ideally selective for $K^+$ over $Cl^-$

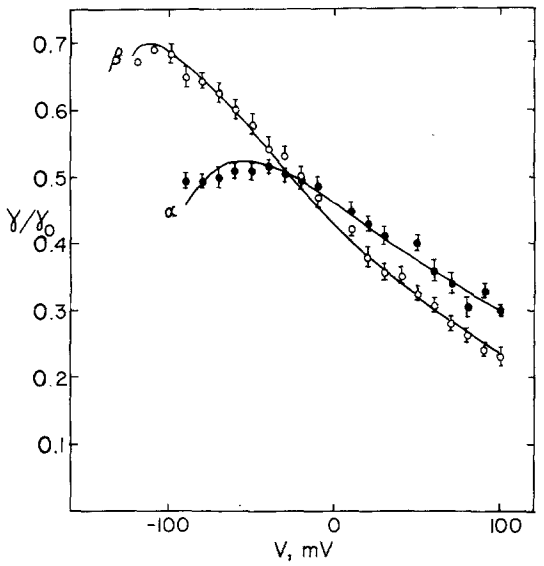


**Fig. 6.** $Cs^+$ blocking of the channel. PE-AL membranes were formed in 80 mM KCl buffer; other experimental conditions were as in Fig. 2. After a single fusion event EDTA was added into the *cis* chamber; the $Cs^+$ concentration of the *cis* and *trans* chambers was then adjusted to 40 mM by adding small aliquots of a concentrated solution of CsCl. The conductance of the $\alpha$ and $\beta$ states was then measured at different voltages. The conductance of both open states was also determined in a buffer containing 80 mM KCl and 40 mM TrisCl to obtain values for $\gamma_0$. Solid curves are drawn according to Eq. (1), with parameters given in Table 3

According to such a model $Cs^+$ binds to a site within the open channel, thus preventing movement of $K^+$. Since the site lies inside the channel, the binding equilibrium will be affected by the applied voltage. Moreover, the binding site is not, in general, equally accessible from the *cis* and from the *trans* side of the membrane. Therefore we can define $K(0)_{cis}$ and $K(0)_{trans}$ as the zero-voltage apparent dissociation constants for *cis* and *trans* blocking. A formal expression for the voltage-dependent $Cs^+$ blocking of both open states can be written as [6, 7]:

$$\frac{\gamma}{\gamma_0}=\left[1+\frac{[Cs^+]_{cis}}{K(0)_{cis}}\exp\left(\delta\frac{FV}{RT}\right)+\frac{[Cs^+]_{trans}}{K(0)_{trans}}\exp\left((\delta-1)\frac{FV}{RT}\right)\right]^{-1} \quad (1)$$

where $\gamma$ is the conductance of either open state in the presence of $Cs^+$, $\gamma_0$ is the conductance of either open state in the absence of $Cs^+$ and $\delta$ is the fraction of the total voltage drop that occurs in the site of binding. Fig. 6 shows plots of $\gamma/\gamma_0$ *vs.* voltage for both open states of the channel. The solid lines have been drawn according to Eq. (1), with blocking parameters $K(0)_{cis}$, $K(0)_{trans}$, and $\delta$ given in Table 3. The results obtained indicate that a transition from the $\alpha$ to the $\beta$ state does not affect the *cis* binding reaction, since $K(0)_{cis}$ is the same for both open states, about 35 mM. $K(0)_{trans}$, however, is 8 times smaller for the $\alpha$(1 M) than for the $\beta$ state (8 M), indicating that the binding site becomes less accessible to *trans* $Cs^+$ as a result of a transition from $\alpha$ to $\beta$. Finally, $\delta$, the fraction of the total voltage drop experienced at the binding site, is larger for the $\beta$ (0.27) than for the $\alpha$(0.18) state. This difference, which is manifested in a 50% stronger voltage dependence of $Cs^+$ block for $\beta$ than for $\alpha$, suggests that a transition from $\alpha$ to $\beta$ moves the binding site deeper into the membrane or, equivalently, moves the field configuration around the binding site.

**Table 3.** $Cs^+$ blocking parameters

| Channel state | $K(0)_{cis}$ (mM) | $K(0)_{trans}$ (mM) | $\delta$ |
|---|---|---|---|
| $\alpha$ | 36±2 | 1000± 200 | 0.18±0.01 |
| $\beta$ | 35±2 | 8000±1200 | 0.27±0.01 |
| Rabbit channel | 43±3 | 1400± 100 | 0.35±0.02 |

$Cs^+$ blockade of the frog SR channel was measured as in Fig. 6. The table shows the best fit (by eye) parameters for the solid curves in the figure, drawn according to Eq. (1). Errors on these parameters represent the extreme values generated by the subjective fit of the curves to the data. For determining these parameters, $K(0)_{cis}$ and $\delta$ were first found by using data at positive voltages. These values were then held fixed to find the best value for $K(0)_{trans}$. Parameters for the rabbit SR channel are included for comparison, and were taken from references [6] and [7].

## Discussion

It is our intention to describe several of the basic properties of a $K^+$-selective channel from SR of frog skeletal muscle and to compare these with a similar channel from rabbit muscle – a channel which has been studied quantitatively in detail [11, 15]. We conclude that, overall, the two channels display qualitatively similar behavior in planar phospholipid bilayers. The experimental conditions under which the incorporation of these channels occurs are similar to those required to obtain the fusion of phospholipid vesicles with planar bilayers: presence of negatively charged phospholipid, $Ca^{2+}$, and osmotic gradients [4]. Moreover, the SR-induced conductance increase consists of discrete events, each one of which incorporates several channels into the bilayer simultaneously. It is most reasonable to think that each incorporation event represents the fusion of a single SR vesicle with the planar bilayer [15].

The $K^+$ channel from frog differs from that of rabbit in that it displays multiple open states. Our experience indicates that this feature represents a true property of this channel since it is observed

under different conditions of pH, ionic strength, and in membranes of different lipid compositions. The most straightforward explanation for the observed conductance levels is that each channel can exist in at least three states: closed, $\alpha$, and $\beta$. Multistate channels have been previously observed both in model channels, like hemocyanin and alamethicin channels [12], and in channels from biological membranes such as the voltage-dependent anion channel present in the mitochondrial outer membrane [5].

Histograms like those shown in Fig. 4*B* suggest that both equilibria ($C \rightleftarrows \alpha$) and ($\alpha \rightleftarrows \beta$) are affected by the applied voltage. We have not yet determined how the voltage controls the probability of the channel existing in either state. Therefore no attempt was made to fit the conductance *vs.* voltage relation obtained in membranes containing many $K^+$ channels with any theoretical model. Further work will be required to develop and test such a model.

The selectivity sequence of both open states of the channel was found to be the same; the two open configurations are more selective to potassium. Moreover, the intercationic selectivities of the two open states are quantitatively identical, for both conductance and permeability. This result suggests quite strongly that the effect of the channel protein's conformational change from the $\alpha$ to the $\beta$ state is *not* upon the intimate ion-protein interactions within the channel. Rather, the conformational transition might cause a difference in *nonspecific* electrostatic energies (such as those due to image forces) to be superimposed upon an invariant protein structure inside the channel's conduction pathway.

Our data on $Cs^+$ blockade, however, indicate that the transition between $\alpha$ and $\beta$ states does involve some kind of structural change of the conduction pathway. First, the transition seems to affect the location of the $Cs^+$ binding site in the imposed electric field. Second, the conformational change affects the accessibility of $Cs^+$ from the *trans* side of the membrane to the binding site, as reflected by the different $K(0)_{trans}$ for $\alpha$ and $\beta$ states. The equality of $K(0)_{cis}$ for both open states suggests that the same site is involved. The fact that the fraction of the applied voltage seen at the blocking site is larger for the $\beta$ than for the $\alpha$ state tells us that a transition from the smaller to the larger conductance state moves the binding site deeper within the channel.

The physiological role of this channel remains obscure. Previous work on SR from rabbit muscle has led to the conclusion that this membrane contains specific permeability pathways for small monovalent ions [8, 10, 11, 14, 15]. It is noteworthy that SR from frog leg muscle, the electrophysiological preparation of choice for studies on excitation-contraction coupling, has now been shown to display a $K^+$-channel qualitatively similar to that described in rabbit SR. It is a worthwhile though speculative exercise to estimate, on the basis of the channel behavior in planar bilayers, the specific conductance of the SR membrane, and to compare this to that estimated by other methods. On the basis of the mean conductance per fusion event, we can assume that an "average" SR vesicle of 1000 Å radius contains three channels, each with an "average" conductance of 100 pS in 0.1 M $K^+$. Then, assuming that at zero voltage about 25% of the channels are open (Fig. 4*C*), we would calculate a specific conductance of the SR membrane of $6 \times 10^{-2}$ S/cm$^2$ at zero voltage; a similar value is calculated for rabbit SR from the appropriate data [8, 11]. Of course, this calculation is subject to the assumption that the channel behaves in SR as in the artificial system. There have been two other estimates of SR conductances. Vergara, Bezanilla, and Salzberg [19] estimated a conductance of $7 \times 10^{-5}$ S/cm$^2$ in frog semitendonosus muscle fibers; McKinley and Meissner [13], using rabbit SR vesicles, estimated a $K^+$ permeability of at least $10^{-6}$ cm/sec, which would be equivalent to a zero-voltage conductance in the order of at least $10^{-3}$ S/cm$^2$. This small survey leads to the unhappy conclusion that there are great discrepancies in the values for SR membrane conductance. Since all of the measurements are indirect, we are faced with a host of underlying assumptions which may be incorrect. At this time, we have no way of identifying the source of the discrepancy, and further work on all fronts is clearly necessary. It seems reasonable, however, to accept tentatively the proposal that the SR $K^+$ channel provides a mechanism for allowing $K^+$ movement to compensate for the charge imbalance produced by $Ca^{++}$ fluxes across the SR membrane during the contraction-relaxation cycle.

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