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Potassium channels in aortic microsomes: conductance, selectivity, barium-induced blockage and subconductance states

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The activity of potassium channels of canine aortic sarcoplasmic reticulum was measured using the planar lipid bilayer-fusion technique. The channels have a conductance of 208 pS (400/100 mM K^+ in cis/trans solutions) and potassium-to-sodium permeability ratio of 7.7. Ba^{2+} ions produced two main effects: one is the interruption of channel currents for tens to hundreds of milliseconds in a voltage-dependent manner, and the other is the appearance of a second conductance level with amplitude about 60% of the main level.

Introduction

Ion fluxes through the membrane of sarcoplasmic reticulum (SR) are of great importance in the initiation and control of cell contraction. The central role is played by calcium release from SR which triggers the contractile reaction. Monovalent ions also cross the SR membrane [1] to shunt the membrane potential near zero voltage and thereby to allow Ca^{2+} ions to be released at a high rate. Channels mediating these fluxes in skeletal [2–5] and cardiac [6–9] muscles have been identified and characterized using planar lipid bilayer-fusion technique [10].

In this paper we describe some functional properties of single potassium channels from SR of aortic smooth muscle.

Materials and Methods

Aortic microsomes were isolated according to the procedure described by Watras and Benevolensky [11]. Biochemical assays did not detect any sarcolemmal pump in this preparation, only SR Ca-ATPase was found (for details, see Ref. 11). Thus, the contamination of this fraction by sarcolemmal membranes was insignificant. Pelleted membranes were frozen and stored in liquid nitrogen. Bilayers were formed by the Mueller-Rudin method from a mixture of lipids dissolved in decane. Lipids were phosphatidylethanolamine (25 mg/ml), phosphatidylcholine (12 mg/ml), and cholesterol (1 mg/ml). The phospholipids were purchased from Far East State University (Vladivostok, U.S.S.R.) and their purity was tested by TLC using a chlorform/methanol/water system. Cholesterol was from Calbiochem.

The cis side of the membrane was defined as the side to which SR vesicles were added. Unless otherwise indicated, the cis chamber contained (in mM): 400 KCl, 10 $CaCl_2$, 40 Hepes-Tris (pH 7.4); and the trans chamber contained: 100 KCl, 10 $CaCl_2$, 40 Hepes-Tris (pH 7.4). Aortic microsomes (final protein concentration 50 μg/ml) were added into the cis solution which was continuously stirred and as soon as K channel currents appeared the microsomes were removed by perfusion of the cis chamber.

The trans chamber was grounded and the cis chamber was clamped at the desired potential level. The current signal was stored on FM tape and was filtered before processing at 200 Hz using a low pass six pole Bessel filter. The probability for each channel to be open was estimated from amplitude histograms using current records longer than 25 s. The number of channels in the bilayer was assumed to be equal to the maximum number of channels open in the same time.

Experiments were performed at 21–23°C.

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Results and Discussion

Fig. 1 shows representative current records and the associated unitary current-voltage relation. Unitary currents reversed direction at −31 mV, which is reasonably close to expected equilibrium potential (−35 mV) for potassium ions under experimental conditions used

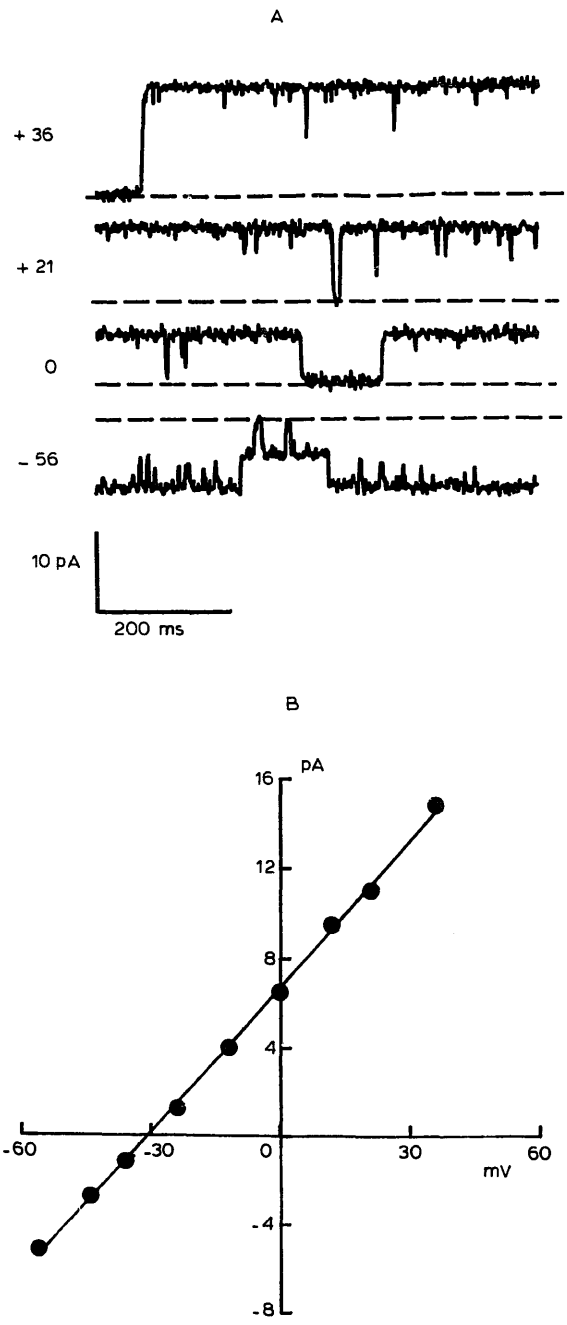


Fig. 1. Currents through K channels of aortic SR microsomes (A) and unitary associated current-voltage relation (B). Potassium concentrations are 400 and 100 mM in cis and trans chambers, respectively. The membrane potential is indicated on the left side of the current traces in mV. Cis to trans currents (at 0, +21, +36 mV) and trans to cis (−56 mV) currents are shown as upward and downward deflections from the basal level (dashed line), respectively. The unitary conductance estimated from current-voltage relation is equal 220 pS.

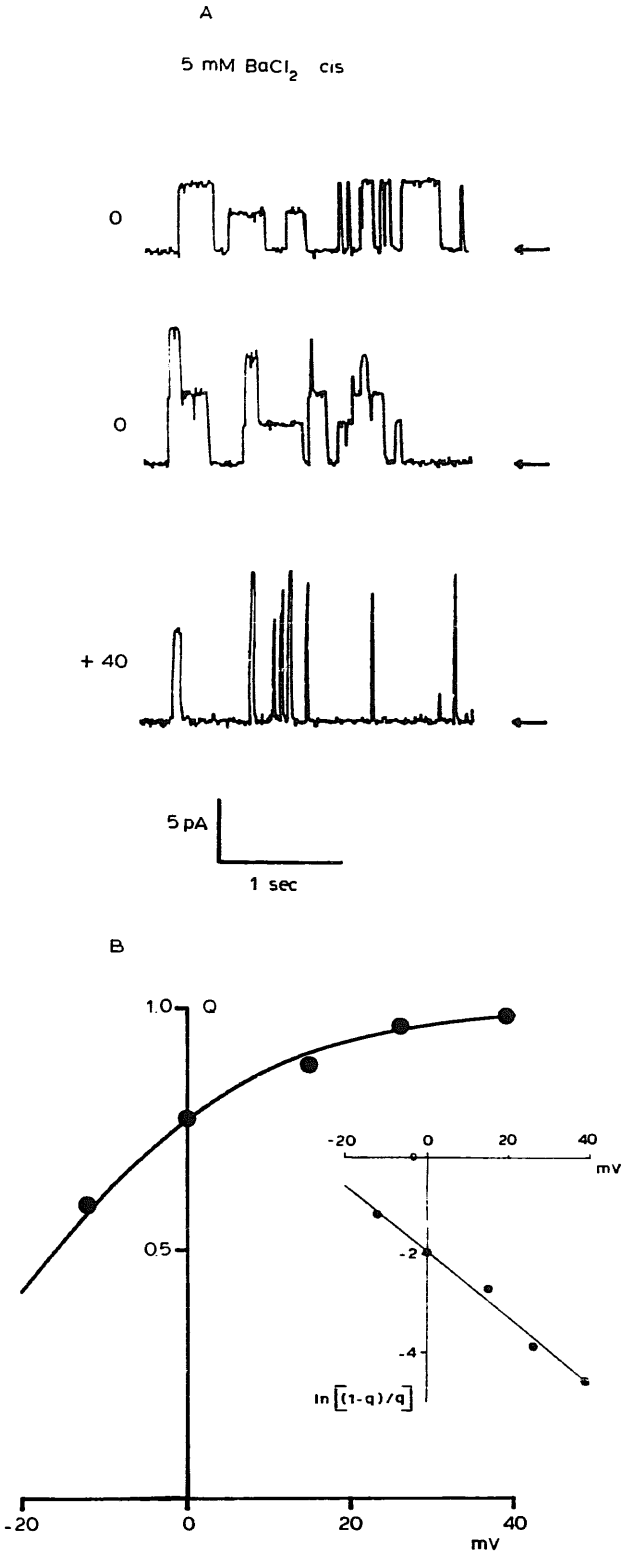
(400 and 100 mM K^+ in cis and trans solutions, respectively). The average value of the reversal potential (E_r) from four similar experiments was −31.3 mV. Currents through the incorporated channels remained unchanged after the substitution of Cl^- by aspartate$^-$. Thus, they pass cations but not anions. These channels are fairly selective for K^+ over Na^+. In four experiments the cis chamber was filled with 400 mM NaCl instead of KCl (the trans chamber contained the same concentration of KCl, 100 mM) and the mean E_r was +21 mV. A potasisum to sodium permeability ratio (P_K/P_{Na}) for these channels of 7.7 was calculated using the difference in E_r from both series of measurements (equal to 52.3 mV) and the Goldman-Hodgkin-Katz equation. K channels of aortic SR discriminate better between monovalent cations that those from skeletal [2] or cardiac [7] SR for which P_K/P_{Na} was estimated to be 1.8 and 2.0, respectively.

The unitary K channel conductance varied among experiments between 195 and 220 pS and averaged 208 pS (four experiments) with 400 and 100 mM K^+ in cis and trans solutions, respectively. The conductance of K channels of skeletal and cardiac SR has been reported to be close to 200 pS [5,7]; thus, K channels from the SR of three major muscle types have virtually the same conductance.

The channels were open for the most of the time either at positive or negative potentials. Although at negative potentials closings were somewhat more frequent, the open probability did not show an evident potential-dependence over the potential range −56 mV to 45 mV. It is possible that these channels are potential-dependent so that their open probability decreases as potential becomes more negative than −50 mV. Unfortunately, we could not examine this point quantitatively because of membrane nonstability at potentials more negative than −50 mV. K channels from skeletal SR have been shown to be mostly open at potentials more positive than +100 mV and predominantly closed at zero and negative potentials [5,9]. It is difficult to determine whether the discrepancy between smooth muscle and other muscle types is due to a difference in an intrinsic property of the channel protein or to some minor difference in the lipid environment.

Many potassium permeable channels have been shown to be blocked by Ba^{2+} ions (for references, see Ref. 12). This is the case for K channels of aortic SR as well. Fig. 2A and Fig. 3, B1 show K channel currents after the addition of 5 mM $BaCl_2$ to the cis solution. It can be seen that Ba^{2+} induced the appearance of interruptions in channel currents tens to hundreds of milliseconds in duration. Whereas without Ba^{2+} the channels were closed less than 1% of the observation period, with Ba^{2+} present the percentage of silent intervals was more than 50% (compare amplitude histograms A2 and B2 in the Fig. 3). We noticed that the Ba^{2+} block was

potential-dependent; as the potential became more positive, the percent of blocked time increased (on Fig. 2A traces at 0 and 40 mV are shown for illustration). In order to characterize the voltage-dependence of the Ba^{2+}-block quantitatively, we calculated the percentage of silent intervals, Q, at the different potentials used.



Numbers obtained from one experiment are plotted as a function of potential on Fig. 2B (similar results were obtained in three other experiments). In these estimations we did not distinguish between fully and partially open channels (see below).

To explain the observed voltage-dependence of the channel block, a simple model developed in the classical work of Woodhull [13] can be used. Ba^{2+} ion seems to block the channel by entering into the ion pathway and the electric field promotes Ba^{2+} entry. We assumed that there is a binding site for Ba^{2+} in the pore of the channel (for example, it can be a selective filter of the channel) and the electrical distance between the cis side of the membrane and this binding site is designated as d (a reasonable value for d should be between 0 and 1). The affinity of this binding site for Ba^{2+} ions ($k_d(V)$) can be defined as a function of the transmembrane voltage (V) using the equation:

$$k_d(V) = k_d(0) \exp(-zFdV/RT) \quad (1)$$

where $k_d(0)$ is the affinity of this site for Ba^{2+} at 0 mV, $z = 2$ (charge of Ba^{2+} ion), and F, R, T are Faraday constant, universal gas constant and absolute temperature, respectively.

Then, it was assumed that binding of Ba^{2+} to this site leads to the complete block of the channel. If binding to this site also is assumed to be described by Michaelis-Menten kinetics and the probability of the channel to be blocked is q, then:

$$q(V) = [Ba^{2+}_{cis}]/([Ba^{2+}_{cis}] + k_d(V)) \quad (2)$$

where $[Ba^{2+}_{cis}]$ is equal 5 mM in our experimental conditions.

In the experiment shown in Fig. 2 two K channels were active in the membrane. Assuming both channels are independent and the probability of each of them to

Fig. 2. Voltage-dependent slow block of the K channels by 5 mM $BaCl_2$ from the cis side. Potassium concentrations are 400 mM and 100 mM in cis and trans chambers, respectively. (A) The current traces are shown at 0 and 40 mV transmembrane potential (the potential is indicated in mV at the left side of each record). Channel openings are shown as upward deflections from the basal level (indicated by arrow at the right side of each record). (B) Application of the model of Ba^{2+}-block to the experimental data. The points show the proportion of silent intervals Q versus transmembrane potential in mV. The solid line is the expected dependence $Q(V)$ according to the model used. The equation to generate the line, $Q(V) = [1 + 0.142 \cdot \exp(-0.068 \cdot V)]^{-2}$, was written using estimations of the distance the Ba^{2+}-binding site is down the electrical gradient and the affinity at a transmembrane voltage of 0 mV ($k_d(0)$) from the linearization (see inset). (Inset) The linear transformation of the probability for a K channel to be blocked as a function of voltage. The slope of this regression line allows us to estimate the location of the binding site in the electrical field within the membrane as 0.86 from the cis side (see details in the text).

be blocked is q, then the percent of silent intervals Q will be:

$$Q = q^2 \quad (3)$$

We have assumed that the reason for silent intervals after addition of $BaCl_2$ was the Ba^{2+}-induced block in all cases, because without Ba^{2+} present channels were open with very high probability at all potentials tested.

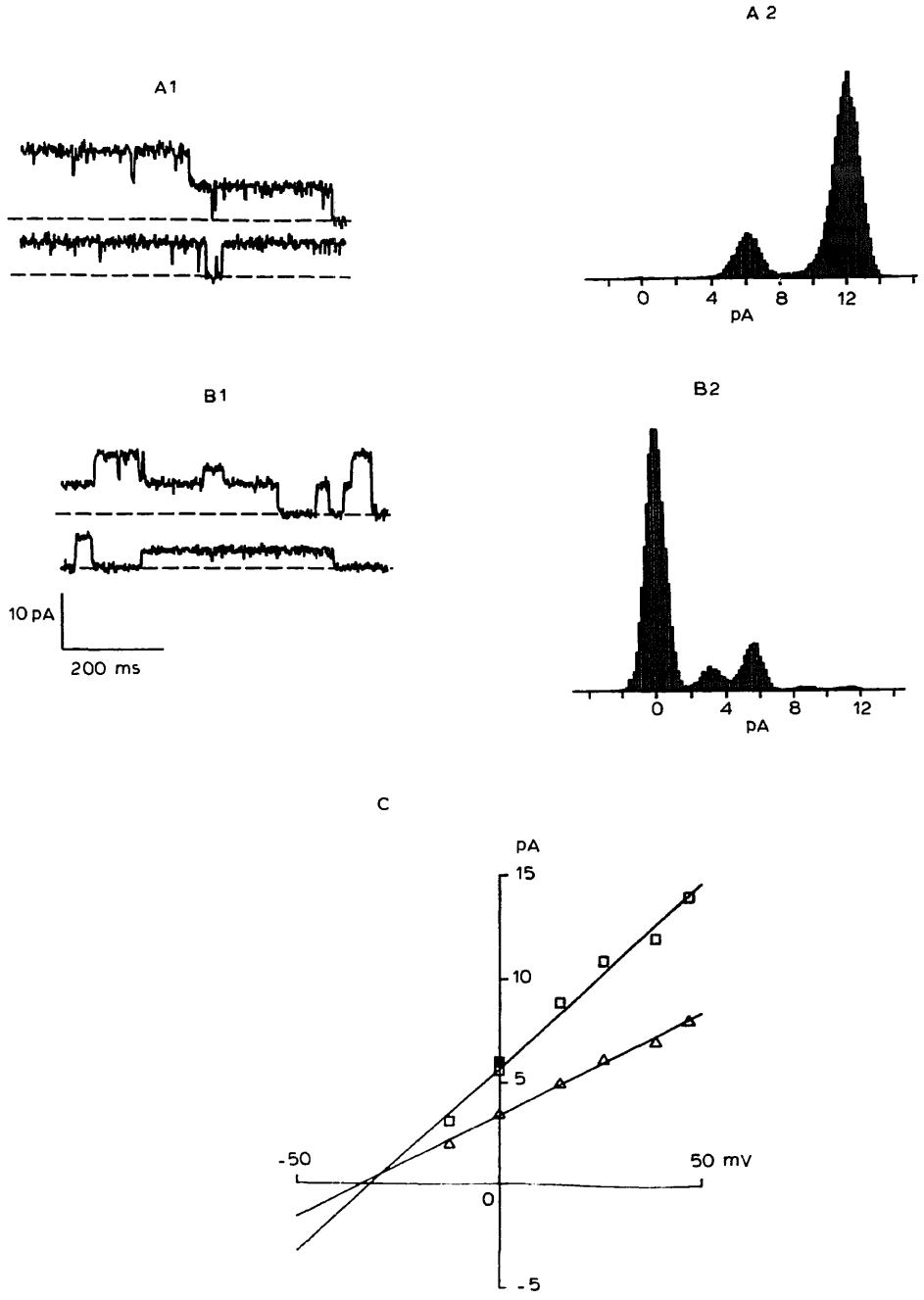


Fig. 3. Effects of Ba^{2+} ions on K channels of aortic SR. 400/100 mM KCl in cis/trans solutions. (A) Current records at 0 mV (A1) and amplitude histogram for a continuous records lasting 25-s interval (A2) under control condition. Note that the peak corresponding to the closed state of the channels (0 pA) is less than 1% of the number of sampled points. Bin width is 0.16 pA. The histogram could be fit by three gaussian curves with means equal to 0.0, 6.0, and 12.0 pA for none, one, and two simultaneously open channels. (B) Current records at 0 mV (B1) and amplitude histogram for a continuous records lasting 25-s interval (B2) after the addition of 5 mM $BaCl_2$ to the cis solution. Note that besides full-size openings there are openings with a lower current amplitude and that the channels are predominantly in nonconducting state (compare histograms A2 and B2). The B2 histogram could be fit by five gaussian curve with means equal to 0.0 pA (all channels blocked or closed), 3.4 pA (one channel in subconductance state), 5.6 pA (one channel in fully open state), 9.0 pA (one channel in fully open state and second channel in subconductance state), 11.2 pA (both channels fully open). (C) Unitary current-voltage relations for fully (open squares) and partially (open triangles) open K channels after addition of 5 mM $BaCl_2$ to the cis solution. The corresponding values for the single-channel conductance are 180 pS and 100 pS. The filled square shows the unitary current level through the same channels at 0 mV under control conditions (before addition of $BaCl_2$).

Using Eqns. 1 and 2 one can predict that the function $\ln[(1-q)/q]$ will be a linear function of the voltage with the slope $-zFd/RT$. The inset in Fig. 2B shows that this is the case. From the parameters of this regression we estimate d to be 0.86 and $k_d(0)$ to be 0.71 mM. The curve on Fig. 2B was drawn using these values and Eqns. 1–3. Unfortunately, we were not able to provide quantitative analysis at potentials less than −12 mV because it was too close to the reversal potential and currents were small. But, the fit of the model to the experimental points (see Fig. 2B) in the range between −12 mV and 40 mV is reasonable.

In two experiments 5 mM $BaCl_2$ was added to the trans solution. At zero potential the block of the channel activity was approximately the same as with Ba^{2+} at the cis side at the same voltage. Variations in the membrane potential from −15 mV to 42 mV did not affect significantly the block from the trans side. This result is consistent with our model of Ba^{2+} block. For Ba^{2+} on the trans side the electrical distance to the binding site will be only 0.14 and the potential dependence of the block will be weak.

It should be mentioned that we consider the model described above as only one of the possible explanations for this phenomenon and additional experiments must be done in order to confirm this hypothesis.

In addition to the slow block, Ba^{2+} ions produced one more alteration in channel behaviour. Whereas under control conditions (Fig. 3, A1 and A2) only two current (conductance) levels were observed: closed (nonconducting) and fully open ones (O_f level), in the presence of Ba^{2+} on either side of the membrane one more intermediate current level (O_i level) became evident with an amplitude of about 60% of the fully open level (O_f) (see Fig. 3, B1 and B2). The channels spent approximately 30% of their total open time at this subconductance level (Fig. 3, B2).

The fully open conductance level in the presence of Ba^{2+} was slightly lower than under control conditions: in different experiments (the same 400/100 mM KCl cis/trans concentrations with 5 mM $BaCl_2$ on the either side of the membrane) the conductance varied from 173 to 193 pS with a mean of 180 pS. In Fig. 3C the unitary current level through the K channel at 0 mV in control conditions is shown by a filled square for comparison with the current through the same channel after addition of 5 mM $BaCl_2$ to the cis side (the fully open state is shown by open squares, the substate is shown by open triangles). This decrease seemed to be a result of fast blockage of the channel by Ba^{2+} ions. A qualitatively similar decrease of the fully open conductance level was observed when the Ca^{2+} concentration was increased: with 50 mM $CaCl_2$ in the trans solution the channel conductance was decreased 2-fold (data not shown) as compared with control conditions (10 mM $CaCl_2$). It should be noted that Ca^{2+}, unlike Ba^{2+}, produced neither long lasting interruptions of channel currents nor additional conductance sublevels. Similar sublevels (56% of full open conductance) were displayed by K channel from cardiac SR [7,8] without any experimental interventions. Several factors (temperature, some drugs) have been reported to increase the occurrence of conductance sublevels in many channel types (for references, see Refs. 14 and 15). The data presented here provide an example of an efficient way to generate conductance sublevels.

To explain conductance sublevels several models have been proposed. The most widely cited 'subunit' model [16–18] views the whole channel as composed of two or more conducting subunits, which are predominantly gated in a coupled way. Occasional deviations from the primary mode of operation result in the appearance of conductance sublevels. Slow blockers such as Ba^{2+} ions are potent probes to test the subunit model, because they are expected to occlude channel subunits asynchronously allowing one to measure current through each subunit separately. Using this model one might interpret Ba^{2+}-induced sublevels in the K channel as indicating that the channel is composed of two subunits with nonequal conductances of O_i and $(O_f - O_i)$. There are, however, some peculiarities in channel behaviour which are hardly consistent with the subunit model. According to the model, Ba^{2+} ions would leave both pores asynchronously and, consequently, openings from the nonconducting level to the fully open level should occur extremely rarely. However, experiments show that full-size conductance steps (from zero to O_f) account for at least 50% of all openings. A second problem is that channel openings to the level equal to the difference between the full open level and the intermediate level $(O_f - O_i)$ never were observed in the experiments as predicted by the 'subunit' model. In addition, one more sublevel was observed very rarely with a conductance of about 130 pS, which is not easily consistent with the two pore model. Thus, the subunit model does not appear to provide a good description of the SR K channel.

The proposed mechanism of slow Ba^{2+}-blockade seems to involve binding of Ba^{2+} inside the conductance pathway of the potassium channels. When Ba^{2+} is bound no current passes through the channel. The binding of Ba^{2+} is voltage-dependent when $BaCl_2$ is added to the cis side suggesting that the localization of the binding site is 86% into the electrical field within the membrane from the cis side. The binding of Ba^{2+} appears to lead to changes in the channel conformation, because after dissociation of Ba^{2+} and unblock of the channel, a second level of conductance, equal to 60% of the main level, appears. The unblocked channel in this new state can jump between partially and fully open levels of conductance or stay in one of them until it is blocked by next Ba^{2+} ion.

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After submission of this manuscript Zn^{2+}-induced subconductance states for cardiac sodium channels were reported by Ravindran et al. [19].

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