A Ba^{2+} Chelator Suppresses Long Shut Events in Fully Activated High-Conductance Ca^{2+}-Dependent K^+ Channels

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ABSTRACT High-conductance Ca^{2+}-activated K^+ channels from rat skeletal muscle were incorporated into planar lipid bilayers, and the channel kinetics were studied with a high internal Ca^{2+} concentration (Ca_i). Raising the Ca_i is known to increase the channel open probability. This effect is due to an increase in openings frequency and duration, and saturates at a Ca_i around 100 μM. Raising the Ca_i also increases the occurrence of less frequent but very long (>5 s) shut events. The mechanism underlying this slow kinetic process was studied. Raising Ca_i above 100 μM does not further increase the frequency of the long shut events. This was not consistent with the hypothesis that the long closures result from a classical channel-block mechanism induced by internal Ca^{2+}. The transmembrane voltage and the presence of K^+ ions in the external compartment both affect the slow kinetic process. A comparison of these effects with the properties of the channel block induced by Ba^{2+} ions added to the internal compartment strongly suggested that the long shut events are due to a contamination of the internal solutions by Ba^{2+}. This was confirmed by showing that a crown-ether compound that strongly chelates Ba^{2+} completely suppresses the long shut events when added to the inner compartment.

INTRODUCTION

The activation of high-conductance Ca^{2+}-dependent K^+ channels (BK channels) is a complex voltage-dependent process involving the binding of at least two Ca^{2+} ions (Magleby and Pallotta, 1983; Moczydlowski and Latorre, 1983b). In some preparations, high cytoplasmic Ca^{2+} concentrations and depolarized potentials have been reported to induce an additional inactivation-like process (Latorre et al., 1982; Barrett et al., 1982; Methfessel and Boheim, 1982; Magleby and Pallotta, 1983; Marty et al., 1984; Findlay et al., 1985; Pallotta, 1985) in which the channels enter a long-lived (hundreds of milliseconds to seconds) shut state. Vergara and Latorre (1983) presented data indicating that the divalent cations Ca^{2+} and Ba^{2+} both induced a slow block of the BK channels with long blocking events similar to the previously described long shut events, and it was proposed that a slow channel block by Ca^{2+} was the mechanism involved in BK channel inactivation (Methfessel and Boheim, 1982; Vergara and Latorre, 1983; Marty et al., 1984; Findlay et al., 1985).

Many nonpermeant cations block BK channels from the intracellular compartment. Most of them do this with very fast kinetics, as shown for Na^+ (Marty, 1983; Yellen, 1984), tetraethylammonium TEA^+ (Yellen, 1984; Benham et al., 1985), Mg^{2+}, Sr^{2+}, and Ni^{2+} (Oberhauser et al., 1988; Ferguson, 1991), which all produce an apparent reduction of the single-channel conductance at depolarized potentials. On the other hand, the slow block produced by Ba^{2+} is well documented in BK channels as well as in other K^+ channels (Armstrong and Taylor, 1980; Eaton and Brodwick, 1980; Armstrong et al., 1982). The fact that Ba^{2+} "sticks" in BK channels (Vergara and Latorre, 1983; Miller et al., 1987; Neyton and Miller, 1988) has been tentatively explained by the similarity between the Ba^{2+} ionic radius (1.35 Å) and that of K^+ (1.33 Å). It has been proposed that Ba^{2+} ions can penetrate deep inside the K^+-selective channels and interact strongly with their selectivity filter. In support of this hypothesis, Ba^{2+} was actually shown to permeate BK channels when K^+ ions are removed from the extracellular compartment (Neyton and Miller, 1988). However, this interpretation cannot be extended to Ca^{2+} ions, because they have an ionic radius (0.99 Å) close to that of Na^+ (0.95 Å) and much smaller than the ionic radii of both Ba^{2+} and K^+. Therefore, the hypothesis that the long shut events observed in high internal Ca^{2+} were due to Ca^{2+} blocking the channel raised a difficult problem, and the possibility that they were not directly induced by Ca^{2+} could be considered.

The present study reexamines the role of Ca^{2+} in the slow kinetic process observed in the recordings of fully activated BK channels (high Ca_i and depolarized potentials). No correlation between Ca_i and the frequency of occurrence of the long shut events was observed, ruling out a direct involvement of Ca^{2+} in this slow kinetic component. The close similarity of the effects of the transmembrane voltage and external K^+ ions on both the long shut events and the Ba^{2+} block kinetics suggested, however, that the long "closures" may result from the presence of contaminant Ba^{2+} ions in the internal compartment. This interpretation was tested by using a macrocyclic polyether compound, the crown-ether (+)-18-crown-6-tetra-carboxylic acid, which was shown, in the present study, to chelate Ba^{2+} very strongly (estimated K_d, 3.5×10^{-10}M at pH 7.4) and selectively. When added in the inner compartment, the

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crown-ether completely suppressed the long shut periods observed at high Ca_i.

MATERIALS AND METHODS

Biochemical

Plasma membrane vesicles were prepared from rat skeletal muscle as described previously (Moczydlowski and Latorre, 1983a) and stored in 0.4 M sucrose at −70°C. The lipids used were 1-palmitoyl, 2-oleoyl phosphatidylethanolamine (POPE) and the analogous phosphatidylcholine (POPC), obtained from Avanti Polar Lipids (Birmingham, AL) and stored as stock solutions in chloroform under N_2 at −70°C. All inorganic salts were of analysis grade and were obtained from Prolabo (Paris, France). In this study, the salt most likely to contain contaminating Ba^{2+} was KCl. The "analysis" grade salt from Prolabo (batch 7847) was preferred to the "suprapur" grade salt from Merck (Darmstadt, Germany) or the "ultrapure" grade salt from Alfa Products (Karlsruhe, Germany) because less spontaneous long "closures" were observed when this salt was used; it was thus concluded that it contained less contaminant Ba^{2+} (see Results). HEPES was obtained from Sigma (St. Louis, MO). The two polyether compounds, crown-ether (+)-18-crown-6-tetra-carboxylic acid and Kryptofix 222, were obtained from Merck and kept as 50 mM stock solutions in water (pH adjusted to 7.4 with KOH). To reduce Ba^{2+} contamination from the glassware, all solutions were prepared and stored in plastic containers.

Bilayer formation, channel incorporation, and recording conditions

BK channels were inserted into planar lipid bilayers as described previously (Neyton and Pelleschi, 1991) by fusion of plasma membrane vesicles with painted bilayers made with a mixture of POPE and POPC (ratio 7:3) solubilized at 20 mg/ml in *n*-decane. The orientation of the channel insertion was determined from the polarity of the channel voltage dependence. After channel insertion, the inner ("cytoplasmic") and external ("extracellular") compartments were washed with solutions containing (in mM) 150 KCl, 10 HEPES (pH adjusted to 7.4 with KOH) and 150 NaCl, 1 KCl, 10 HEPES (pH adjusted to 7.4 with NaOH), respectively. Ca_i was adjusted by adding the appropriate amount of a 1 M solution of $CaCl_2$ to the inner compartment. Contaminant Ca^{2+} (2–5 μM) was present in the bidistilled water used to make all solutions. Unless specified, the transmembrane voltage was maintained constantly at +30 mV (the electrophysiological voltage convention is always used here, with the external side of the channel defined as zero voltage). All experiments were performed at room temperature (20–23°C).

Recording system, data acquisition, and analysis

The electronics of the voltage-clamp system followed the design of Hanke and Miller (1983). The current signal was filtered at 500 Hz using an eight-pole Bessel filter (Frequency Devices, Haverhill, MA) and then collected at a sampling interval of 0.5 ms with a MINC 1123 computer (Digital Equipment Corp., Marlboro, MA).

The procedures used to measure the mean duration and frequency of the long shut events occurring at high Ca_i, the open probability during the bursts separating the long shut events, as well as the slow Ba^{2+} kinetics have already been described by Neyton and Pelleschi (1991). The cutoff time used to define long shut events was fixed at 0.5 s. Long "closures" and burst durations are given in the figures as mean ± SEM and were obtained from the recordings containing at least 30 events. The fits performed in some of the figures were made using SigmaPlot 4.0 software. The true dissociation constant of the crown-ether 18C6TCA for Ba^{2+} has been estimated from the apparent dissociation constant measured in the presence of KCl using a numerical approach with Mathematica software.

RESULTS

Different groups have reported the occurrence of long shut periods lasting on the order of a few seconds in the recordings of BK channels obtained in conditions expected to fully activate the channels (Latorre et al., 1982; Barrett et al., 1982; Methfessel and Boheim, 1982; Vergara and Latorre, 1983; Marty et al., 1984; Findlay et al., 1985). Fig. 1 shows examples of such long "closures." In this experiment, 5 μM $CaCl_2$ was initially added to the inner compartment. This induced a low activation of the single channel inserted in the bilayer. Over a period of 12 min, no closure longer than 2 s was observed, and the mean open probability (p_o) was 0.13. Raising Ca_i to 20 μM increased the level of activation of the channel but also induced long silent periods (six closures lasting more than 2 s were observed over 15 min). The mean

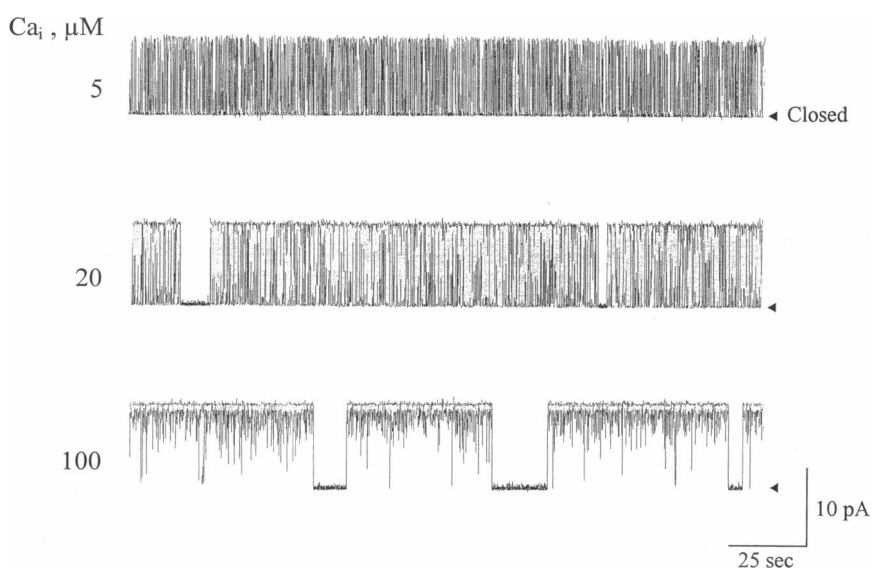

FIGURE 1 Long shut events in BK channels recorded in high internal Ca^{2+}. Single-channel recordings of the same BK channel were obtained successively in three different internal Ca^{2+} concentrations, increasing the channel open probability from low (*top*), to intermediate (*middle*) and high (*bottom*).

open probability during the bursts separating the long closures (p_{oburst}) was 0.60. The recording obtained at 100 μM Ca_i showed a similar alternation of bursts of high p_o (0.98) and long shut periods. These long shut events appeared at a higher frequency (11 closures longer than 2 s over 15 min) and were more than two orders of magnitude longer than the mean intraburst closed time (9 ± 2 s versus 2 ± 0.5 ms).

If the long shut events were due to a slow Ca^{2+} block, the probability that Ca^{2+} would terminate a burst should increase with Ca_i and thus, the duration of the bursts between the long closures should decrease with the increase in Ca_i (in the case of a bimolecular blocking mechanism, it would be inversely proportional to Ca_i). Changing Ca_i in the range 0–100 μM did increase the frequency of occurrence of the long shut events (Fig. 1). However, as shown in Fig. 2, when Ca_i was raised beyond 100 μM, the burst length decrease predicted by the slow Ca^{2+} block hypothesis was not observed. These results indicated that, despite the fact that it can affect the long closure phenomenon, internal Ca^{2+} does not induce a classical slow block in BK channels. The results did not rule out the possibility that Ca^{2+} could produce a partial inhibition of the channel that would saturate above 100 μM. Another possibility was that Ca^{2+} did affect the slow kinetic process by changing the open probability of the channel. Indeed, both the long "closure" frequency and p_o increased within the same range of Ca_i and saturated around 100 μM.

Long shut events and Ba^{2+} block have similar properties

Block of BK channels by Ba^{2+} induces long shut times of several seconds, and their frequency increases with the channel open probability (Miller et al., 1987). The similarities between these properties of Ba^{2+} block and those of the slow kinetic process observed under high Ca_i prompted a more detailed comparison of these two phenomena.

Fig. 3 illustrates the effects of the transmembrane voltage on the two phenomena for a channel recorded before and after the addition of 0.6 μM Ba^{2+} in the inner compartment. BK channels have such a high affinity for Ba^{2+} that, in the presence of 0.6 μM internal Ba^{2+}, more than 90% of the long shut events are induced by the addition of Ba^{2+}, and the long shut events present before Ba^{2+} addition make only a negligible contribution (see Fig. 6 *A* and Neyton and Miller, 1988). Fig. 3 *A* shows that, at the three voltages studied, the duration of the long "closures" and that of the Ba^{2+} blocking events are indistinguishable and thus present the same voltage dependence (an *e*-fold decrease for ≅42 mV). (The polarity of the voltage dependence may appear surprising for Ba^{2+}, acting here as an internal blocker, but we showed in a previous work (Neyton and Miller, 1988) that, under low external K^+, Ba^{2+} dissociates from its

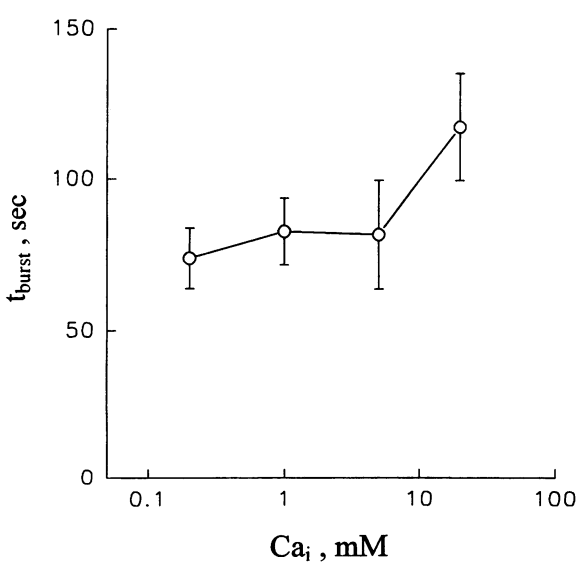

FIGURE 2 In fully activated BK channels, the duration of the bursts separating the long shut events does not decrease with the increase in the internal Ca^{2+} concentration. The mean duration of the bursts between the long closures was measured at different Ca_i varying between 0.2 and 20 mM. Note the logarithmic scale on the *x* axis.

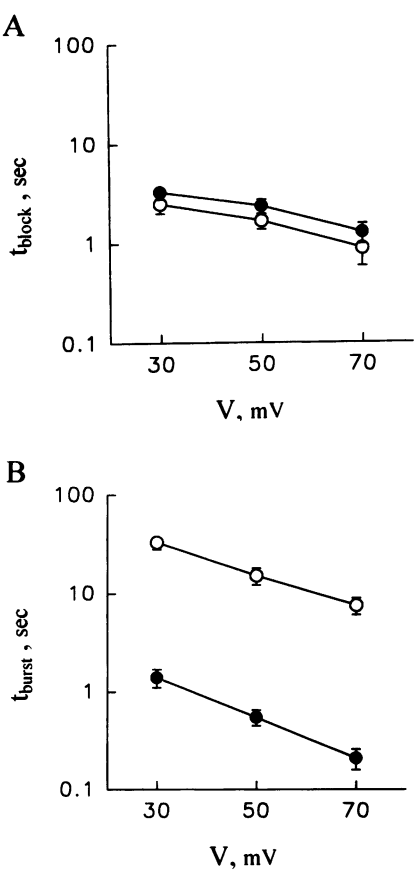

FIGURE 3 Voltage dependence of the Ba^{2+} block and of the slow kinetic process observed at high Ca_i. The kinetic measurements were done on the same single channel recorded successively before and after addition of 0.6 μM Ba^{2+} to the inner compartment (0.5 mM Ca^{2+} was always present in the internal solution). Mean durations of the Ba^{2+} blocking events (●) and of the long shut events (○) are plotted as a function of voltage in *A*. (*B*) Variation with voltage of the mean duration of the corresponding bursts.

blocking site, mainly toward the external compartment.) Fig. 3 *B* shows that the voltage dependence of the mean durations of the bursts is also very similar for the bursts separating the long closures and those separating the Ba^{2+} blocking events (*e*-fold decrease for 22 mV and 25 mV, respectively).

The effects of external K^+ ions on the two slow kinetic processes were also compared. External K^+ in the 0–10 mM range exerts a "lock-in" effect on Ba^{2+} block, which appears as an inhibition of Ba^{2+} dissociation from its blocking site with an inhibition constant (K_i) on the order of 0.2 mM when measured at +50 mV in the presence of 150 mM NaCl in the external compartment (Neyton and Miller, 1988). External K^+ ions have an additional effect on Ba^{2+} block: they inhibit the Ba^{2+} binding reaction with a K_i around 0.5 mM under the same experimental conditions (Neyton and Miller, 1988). In an experiment started in the absence of K^+ in the outer compartment, the external K^+ concentration was progressively raised, and the duration and the frequency of the long closures were measured at +50 mV. The inverse of the mean long closure duration was found to decrease with $[K^+]_{out}$ following a simple rectangular hyperbolic function, the K^+ concentration of half-inhibition being 0.15 mM (Fig. 4 *A*). The frequency of the long closures also decreased with a K_i of 0.65 mM (Fig. 4 *B*).

The results presented in this section demonstrate that the slow kinetic process observed at high Ca_i and the block induced by Ba^{2+} share strikingly similar properties. This suggested that contaminating Ba^{2+} ions present in the internal solution might be responsible for the long shut events in fully activated BK channels. From the Ba^{2+} on rate measured in experiments with added Ba^{2+}, one can calculate that a contamination of the internal solution by ≅30 nM Ba^{2+} would account for the frequency of occurrence of the long closures in the nominal absence of Ba^{2+}.

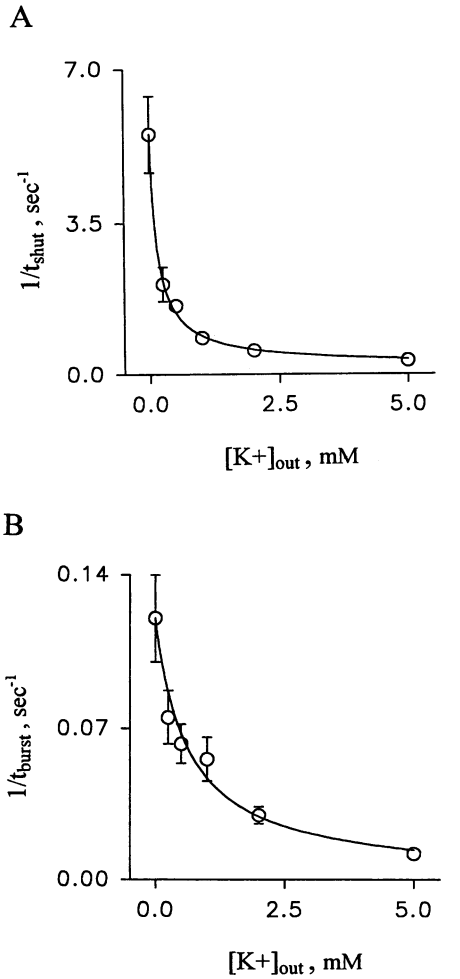

FIGURE 4 Dependence of the long "closures" process on external K^+ concentration. The inverse of the durations of the long shut events (*A*) and of the bursts separating the long closures (*B*) are plotted as a function of external K^+ concentration. Data points were fitted as in Neyton and Miller (1988), with simple hyperbolic functions (lines through the data points). In this experiment, the voltage was +50 mV and the internal compartment contained 0.5 mM Ca^{2+}.

The crown-ether (+)-18-crown-6-tetra-carboxylic acid can be used to selectively chelate Ba^{2+} ions

To more directly support this interpretation, I looked for a molecule able to chelate Ba^{2+} ions specifically. Ideally such a chelator should remove contaminant Ba^{2+} without decreasing Ca_i, thus leaving the channel activation unaffected. A macrobicyclic polyether compound, the cryptand Kryptofix 222, known to avidly chelate Ba^{2+} ($K_d = 10^{-9}$M in water) with a strong selectivity over Ca^{2+} ($K_d(Ba^{2+})/K_d(Ca^{2+}) = 2.5 \times 10^{-5}$) (Dietrich, 1985) was tested initially. Unfortunately, this molecule appeared unsuitable for the study of BK channels, because it induced, already in the low micromolar range, a pronounced flickery behavior of the single-channel current, presumably because of a direct interaction with the channel protein (data not shown). I then tried another macrocyclic polyether compound, the crown-ether (+)-18-crown-6-tetra-carboxylic acid (called hereafter 18C6TCA), the structure of which is shown in Fig. 5). This molecule was known to strongly chelate K^+ ($K_d = 3.3 \times 10^{-6}$ M in water; see Dietrich et al., 1991). Its affinity for Ba^{2+} had not been measured, but because the selectivity of this type of molecule is partly based on the size of the polyether ring that preferentially coordinates the cations of the appropriate diameter, it was also expected to chelate

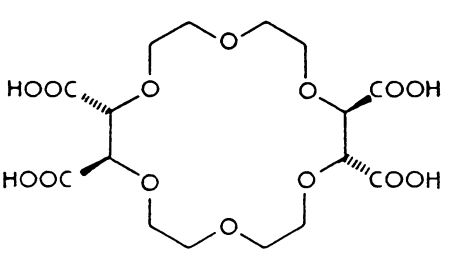

FIGURE 5 Chemical structure of the crown-ether (+)-18-crown-6-tetra-carboxylic acid.

Ba^{2+} strongly. Moreover, the four carboxylic groups on the lateral chains should increase its hydrophilicity, thus preventing chelator/protein interactions, and they might increase the selectivity of the molecule for divalent over monovalent ions for electrostatic reasons.

The Ba^{2+} chelating affinity of 18C6TCA was evaluated by measuring the effects of increasing concentrations of the molecule on the kinetics of the blockade of BK channels induced by 1 μM internal Ba^{2+}. Fig. 6 *A* shows recordings obtained in such an experiment before (*top recording*) and after (*middle*) the addition of Ba^{2+}. Ba^{2+} dramatically increased the frequency of the long shut periods, which were already present in control conditions. The addition of 200 μM 18C6TCA to the Ba^{2+}-containing solution very effectively decreased the frequency of the blocking events (Fig. 6 *A*, *bottom*), making the record very similar to that for the control. This indicated that the 18C6TCA had been able to chelate the Ba^{2+} ions added in the inner compartment. Moreover, this showed that 18C6TCA, unlike Kryptofix 222, had no detectable effects on the fast intrinsic gating of BK channels.

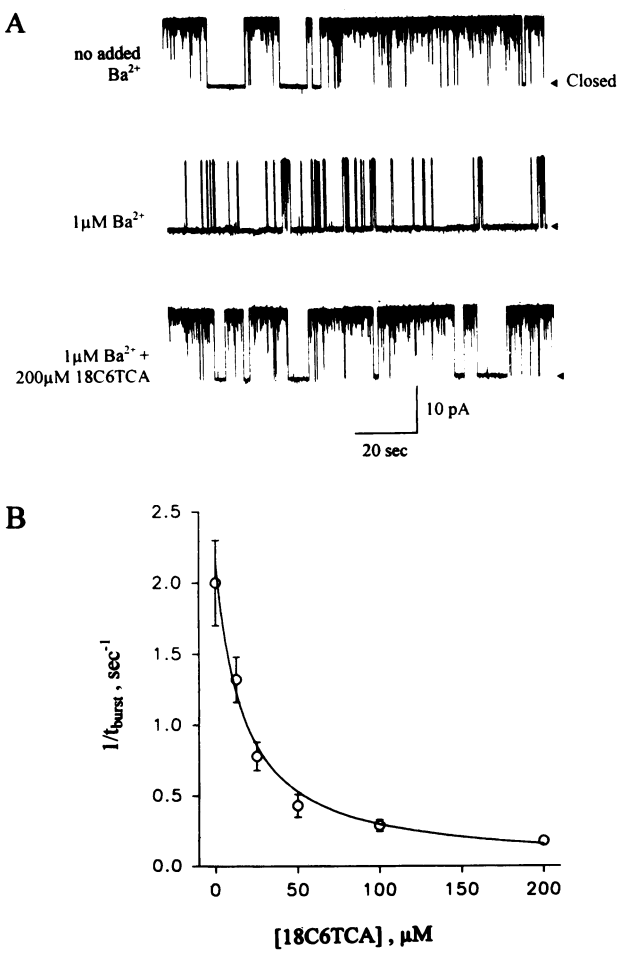

FIGURE 6 18C6TCA is a strong Ba^{2+} chelator. (*A*) Single-channel recordings of a highly activated BK channel (Ca_i = 0.5 mM) successively bathed with the control internal solution, the same solution with 1 μM Ba^{2+} added to the inner compartment, and finally, with the further addition of 200 μM 18C6TCA. (*B*) The burst duration in the presence of 1 μM Ba^{2+} was measured at different 18C6TCA concentrations. The inverse of the mean duration of the bursts separating Ba^{2+} blocking events (the Ba^{2+} blocking rate) is plotted as a function of the 18C6TCA concentration. The data points were fitted with Eq. 1.

Fig. 6 *B* shows the variation of the Ba^{2+} blocking rate (measured as the inverse of the mean burst duration) as a function of the 18C6TCA concentration. Assuming a bimolecular binding reaction between Ba^{2+} and 18C6TCA, data points were fitted with the following equation:

$$(t_{burst})^{-1} = (k_{on}/2) * \{B - K_{app} - c + [(c - B + K_{app})^2 + 4 \cdot K_{app} \cdot B]^{1/2}\}, \quad (1)$$

in which k_{on}, the Ba^{2+} blocking rate, was a free parameter in the fit; B, the total concentration of Ba^{2+} (free + chelated), was fixed at 1 μM; K_{app}, the apparent dissociation constant of 18C6TCA for Ba^{2+}, was another free parameter in the fit; and c was the added 18C6TCA concentration. Equation 1 satisfactorily describes the inhibition by 18C6TCA of the Ba^{2+} blocking rate, using for the two free parameters $k_{on} = 2.3 \times 10^6\ s^{-1}\ M^{-1}$ and K_{app} = 16 μM.

The apparent affinity of 18C6TCA for Ba^{2+} may appear disappointingly low at first view, but the titration curve was obtained in the presence, in the inner compartment, of 150 mM K^+, which also binds to the chelator (see above) and thus acts as a strong competitive antagonist against Ba^{2+} chelation. Making the appropriate correction yields an estimate of at most 3.5×10^{-10} M for the true dissociation constant of 18C6TCA for Ba^{2+} (pH 7.4). In making this correction, the presence of 0.5 mM Ca^{2+} in the internal solution was not taken into account, despite the fact that 18C6TCA also chelates Ca^{2+} (see Dietrich, 1985). Because I was unable to find a K_d value for the 18C6TCA/Ca^{2+} binding equilibrium in water, I could not enter the appropriate correction for the antagonism of Ca^{2+} on Ba^{2+} chelation.

18C6TCA suppresses the long shut events in fully activated BK channels

Fig. 7 shows the effect of 18C6TCA on fully activated BK channels in control conditions (no added Ba^{2+}). The record shown in Fig. 7 *A* (*top*) was obtained before the addition of 18C6TCA in the inner compartment; it lasted 3 min and contains 12 shut events longer than 1 s. The presence of 100 μM 18C6TCA (Fig. 7 *A*, *bottom*) almost completely prevented the occurrence of long closures.

The suppression by 18C6TCA of the slow kinetic process is dose dependent. This is illustrated in Fig. 7 *B*, where the results of a few experiments have been pooled together to quantify the relation between the frequency of long shut events and the 18C6TCA concentration. Data points have been again fitted with Eq. 1, assuming that the long closures were due to the presence of contaminant Ba^{2+} in the inner compartment. B (the amount of contaminant Ba^{2+}) was the

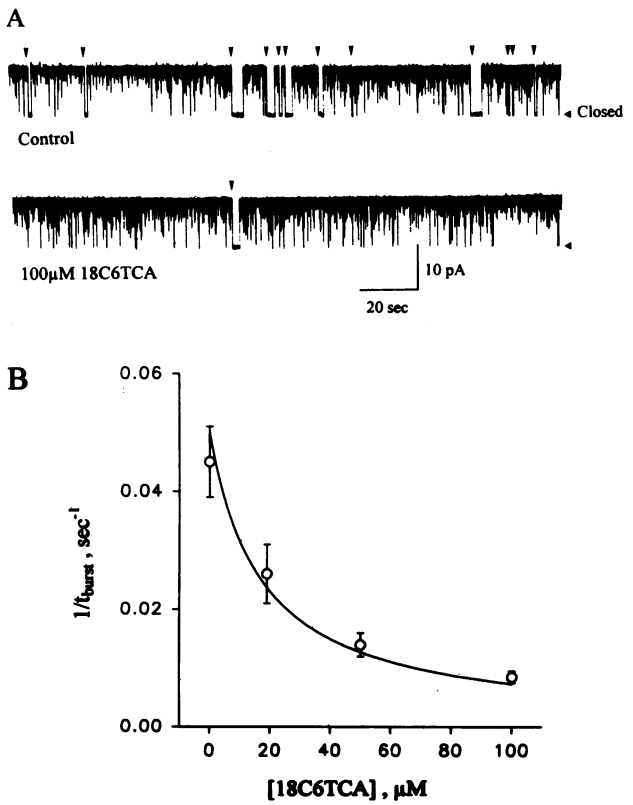

FIGURE 7 18C6TCA eliminates long shut events in fully activated BK channels. (*A*) Single-channel recordings of a BK channel in the presence of a high activating Ca_i concentration (0.5 mM) before and after addition of 100 μM 18C6TCA to the internal solution. Vertical arrowheads indicate shut events lasting more than 1 s. (*B*) Variation of the inverse of the mean duration of the bursts separating long shut events as a function of the 18C6TCA concentration. Data points were fitted with Eq. 1.

only free parameter in the fit; the values of k_{on} and K_{app} were those obtained in the previous fit (Fig. 6 *B*). In Fig. 7 *B*, the best fit was obtained with $B = 23$ nM.

DISCUSSION

In a previous study of Ba^{2+} block (Neyton and Miller, 1988), it was already suggested that the long shut events observed in fully activated BK channels before Ba^{2+} addition could be due to channel block by contaminating Ba^{2+} ions present in the internal compartment. The present study confirms this interpretation. The kinetics of the long shut state observed in no added Ba^{2+} solutions are very similar to those of Ba^{2+} block. Moreover, a crown-ether compound potently chelating Ba^{2+} suppressed the occurrence of the long shut events. It could be argued that the crown-ether acts by chelating an ion other than Ba^{2+} like Ca^{2+} or a contaminating divalent cation other than Ba^{2+}. Pb^{2+} and Cd^{2+} have been claimed to induce, like Ba^{2+}, long blocking events (Oberhauser et al., 1988). Because the frequency of the long shut events does not increase when Ca_i is raised to a range where the channel open probability is saturated (see Fig. 2), the contaminating ion cannot be Ca^{2+}. The fact that the long shut events have a mean duration indistinguishable from that of Ba^{2+} blocking events (Fig. 3 *A*) implies that the unknown ion should interact with the BK channel in a manner very similar to that of Ba^{2+} and, in particular, should have the same rate of dissociation from the channel. Furthermore, the fact that a good fit of the data points of Fig. 7 *B* was obtained using the 18C6TCA apparent dissociation constant measured for Ba^{2+} (Fig. 6 *B*) implies that any other candidate ion should possess the same affinity as Ba^{2+} for the chelator. These two requirements make it very unlikely that an ion species other than Ba^{2+} is responsible for the long shut events.

A Ba^{2+} contamination is likely to account for some previous reports on highly activated BK channels, where long shut events resembling those studied here have been shown to be responsible for an inactivation-like phenomenon (Latorre et al., 1982; Barrett et al., 1982; Methfessel and Boheim, 1982; Vergara and Latorre, 1983; Marty et al., 1984; Findlay et al., 1985; Pallotta, 1985). The presence of contaminating Ba^{2+} ions in the solutions bathing the intracellular face of the channels in these experiments (bath solution bathing bilayers and inside-out patches or pipette solution in contact with outside-out patches) could explain the inactivation: Ba^{2+} only blocks open channels, and both the channel activation process (Moczydlowski and Latorre, 1983b; Magleby and Pallotta, 1983) and the blocking reaction (Vergara and Latorre, 1983; Miller et al., 1987) are voltage dependent; therefore, during depolarizing voltage jumps that activate the channels, Ba^{2+} block is expected to be time and voltage dependent. A Ba^{2+} contamination of the internal solutions in these experiments is plausible in light of the present study, where contamination could not be avoided, despite the precautions used in preparing the internal solutions (see Materials and Methods). Contamination is even more probable in outside-out experiments, in which Ba^{2+} released by the pipette glass can constitute an important additional source of contamination (Copello et al., 1991). On the other hand, the Ba^{2+} concentration in fresh water as well as in sea water is very low (below 0.5 μM; see Martin and Whitfield, 1983). As no particular Ba^{2+}-concentrating mechanism has been described in animals, the in vivo Ba^{2+} concentration in the extracellular space is also, very probably, extremely low. Because Ba^{2+} is chelated to some extent by the intracellular Ca^{2+}-buffering systems, in vivo internal Ba^{2+} is expected to be even lower than external Ba^{2+}. This makes it unlikely that the Ba^{2+} mechanism described in this paper plays a physiological role.

The crown-ether (+)-18-crown-6-tetra-carboxylic acid molecule may be be considered by those interested in studying K^+-selective channels from two different points of view. First, because of its strong affinity for Ba^{2+} ions ($K_d \leq 3.5 \times 10^{-10}$M), the 18C6TCA is able to remove from a solution traces of Ba^{2+} (on the order of a few tens of nanomoles/liter), even in the presence of 150 mM K^+ and 0.5 mM Ca^{2+}. This property has been used here to eliminate a slow artifactual component in the kinetic behavior of BK

channels without affecting their intrinsic gating; it might be useful in studying other K^+ channels very sensitive to Ba^{2+}. A second interesting property of 18C6TCA is that it also strongly chelates K^+ ($K_d = 3.3 \times 10^{-6}$ M). This may be used in experiments designed to study K^+ channels in the presence of a very low K^+ concentrations on one side of the membrane: the flux of K^+ through the channels under study will not contaminate the low K^+ compartment in the presence of the crown-ether.

Note added in proof: While this paper was in revision, very similar results obtained on cloned BK channels from human smooth muscle have been published (Diaz et al., 1996).

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