Fast BK-Type Channel Mediates the $\text{Ca}^{2+}$-Activated $\text{K}^+$ Current in Crayfish Muscle

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Araque, Alfonso and Washington Buño. Fast BK-type channel mediates the $\text{Ca}^{2+}$-activated $\text{K}^+$ current in crayfish muscle. J. Neurophysiol. 82: 1655–1661, 1999. The role of the $\text{Ca}^{2+}$-activated $\text{K}^+$ current ($I_{\text{K(Ca)}}$) in crayfish opener muscle fibers is functionally important because it regulates the graded electrical activity that is characteristic of these fibers. Using the cell-attached and inside-out configurations of the patch-clamp technique, we found three different classes of channels with properties that matched those expected of the three different ionic channels mediating the depolarization-activated macroscopic currents previously described ($\text{Ca}^{2+}$, $\text{K}^+$, and $\text{Ca}^{2+}$-dependent $\text{K}^+$ currents). We investigated the properties of the ionic channels mediating the extremely fast activating and persistent $I_{\text{K(Ca)}}$. These voltage- and $\text{Ca}^{2+}$-activated channels had a mean single-channel conductance of ~70 pS and showed a very fast activation. Both the single-channel open probability and the speed of activation increased with depolarization. Both parameters also increased in inside-out patches, i.e., in high $\text{Ca}^{2+}$ concentration. Intracellular loading with the $\text{Ca}^{2+}$-chelator bis(2-aminophenoxo) ethane-N, N,N',N'-tetraacetic acid gradually reduced and eventually prevented channel openings. The channels opened at very brief delays after the pulse depolarization onset (<5 ms), and the time-dependent open probability was constant during sustained depolarization (~360 ms), matching both the extremely fast activation kinetics and the persistent nature of the macroscopic $I_{\text{K(Ca)}}$. However, the intrinsic properties of these single channels do not account for the partial apparent inactivation of the macroscopic $I_{\text{K(Ca)}}$, which probably reflects temporal $\text{Ca}^{2+}$ variations in the whole muscle fiber. We conclude that the channels mediating $I_{\text{K(Ca)}}$ in crayfish muscle are voltage- and $\text{Ca}^{2+}$-gated BK channels with relatively small conductance. The intrinsic properties of these channels allow them to act as precise $\text{Ca}^{2+}$ sensors that supply the exact feedback current needed to control the graded electrical activity and therefore the contraction of opener muscle fibers.

INTRODUCTION

$\text{Ca}^{2+}$-activated $\text{K}^+$ currents ($I_{\text{K(Ca)}}$) are of key functional importance because they regulate the excitability of neurons and muscle fibers, participating in action potential repolarization, in the regulation of the graded electrical activity, and in AP frequency adaptation (e.g., Araque and Buño 1995; Araque et al. 1998; Blatz and Magleby 1987; Crest and Gola 1993; Gola et al. 1990; Hille 1992; Madison and Nicoll 1984; Marty 1981; Yarom et al. 1985). On the basis of their single-channel conductance, calcium sensitivity, voltage dependence, and pharmacology, the channels underlying these currents have been classified in two main types, BK or SK channels with high (>75 pS) and small (<20 pS) conductance, respectively.

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channels do not inactivate (Araque and Buño 1995; Araque et al. 1998).

To experimentally test the above conclusions, we characterized the intrinsic properties of the single channels mediating the \( I_{\text{K(Ca)}} \) of crayfish opener muscle fibers. Special attention was paid to the \( \alpha \) kinetics and to the voltage and \( \text{Ca}^{2+} \) dependence of the ion channels that may explain the extremely fast kinetics of the macroscopic \( I_{\text{K(Ca)}} \). Finally, we discuss how the intrinsic properties of these BK channels contribute to the characteristics of the macroscopic \( I_{\text{K(Ca)}} \).

We have found that BK channels mediating \( I_{\text{K(Ca)}} \) in crayfish muscle show voltage and \( \text{Ca}^{2+} \) dependence, extremely fast activation kinetics, and a persistent, noninactivating steady state. They have a single-channel conductance similar to those found in molluscan cells (Hamill et al. 1981). In four cases, seal was obtained. Single-channel recordings (cf. Hamill et al. 1981) was applied through the electrode until a high-resistance (access resistance increased after touching the fiber, a gentle suction depolarized and contracted, dislodging the patch electrode.

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M E T H O D S

Preparation

Opener muscles from the propodite of the first walking leg of crayfish (Procambarus clarkii) were isolated and transferred to a 1-ml superfusion chamber placed on the stage of an inverted microscope. The propodite was glued to the glass bottom of the chamber with cyanoacrylate glue. The preparation was treated during 30–60 min. with control solution (see composition in the following text) containing 1 mg/ml of collagenase D.

Microelectrodes and recordings

Fire-polished patch electrodes (2–4 MΩ) pulled from thick-walled 1.5-mm diam borosilicate glass (A-M System, 3060) were coated with silicone elastomer (Sylgard). The pipette solution and the extracellular control solution were the same and had the following composition (in mM): 210.0 NaCl, 5.4 KCl, 13.5 CaCl₂, 2.6 MgCl₂, and 10.0 Tris buffer; pH was adjusted to 7.2 with NaOH. Higher pipette and extracellular K⁺ concentrations would tend to symmetrical K⁺ concentrations on either side of the membrane and would allow accurate estimations of \( V_m \) and of the single-channel conductance (e.g., Hamill et al. 1981). However, they could not be used because the fibers depolarized and contracted, dislodging the patch electrode.

Pipettes were connected to a Cornerstone Series PC-ONE amplifier (Dagan) and positioned with a mechanical micromanipulator under direct visualization with a dissecting microscope. When the pipette’s access resistance increased after touching the fiber, a gentle suction was applied through the electrode until a high-resistance (>1 GΩ) seal was obtained. Single-channel recordings (\( n = 28 \)) were obtained in this cell-attached configuration (Hamill et al. 1981). In four cases, the inside-out configuration was obtained by gently pulling the electrode away from the fiber after recording in the cell-attached mode. In many cases (\( n = 19 \)), the resting membrane potential (\( V_r \)) of the patched fiber also was recorded with a sharp K⁺-acetate (3 M)-filled micropipette (5–10 MΩ) using an Axoclamp 2A amplifier (Axon Instruments) in the bridge mode. These recordings provided an estimation of the mean \( V_r \), which was –70.5 ± 9.5 (SE) mV (cf. Araque and Buño 1994, 1995). Because the holding potential of the pipette was set to 0 mV, for simplicity, \( V_m \) was estimated to be –70 mV, also in those cell-attached recordings from cells in which the \( V_r \) was unknown. Even in these conditions the small dispersion of measured \( V_r \) values indicates that errors in the estimation of single-channel properties introduced by \( V_r \) to \( V_m \) differences would be small (see RESULTS). Furthermore no significant differences were found between the intrinsic properties of channels recorded from fibers in which the \( V_r \) was known and set to –70 mV and those in which the \( V_r \) was unknown and estimated to be the mean \( V_r \) (i.e., –70 mV). Membrane potential is expressed conventionally as the difference between the intracellular and extracellular side of the membrane.

In two experiments, the patched fiber was loaded with the fast \( \text{Ca}^{2+} \) chelator 1,2-bis(2-aminophenoxy) ethane-N,N,N′,N′-tetracetic acid (BAPTA) by ionophoresis after impaling the fiber with a sharp micropipette filled with 0.16 M BAPTA (see Araque and Buño 1995).

Stimulus pulse and ramp generation, data acquisition, and analysis were done with a PC 486-based computer and the pClamp software (Axon Instruments) through a LabMaster TM-100 (Scientific Solution) interface board. Currents were filtered >1 kHz and digitally sampled >2 kHz.

Uncompensated capacitative currents and Ohmnic leak currents were subtracted from the data using averaged currents obtained from voltage pulses that failed to evoke channel openings. A patch was considered to contain a single channel when openings to only a single conductance level were observed for several minutes at strong depolarization. The mean channel open probability was calculated by dividing the time spent in the open state by the total duration of the pulse. Experiments were performed at room temperature (21–23°C). Chemicals were purchased from Sigma-Aldrich (Spain). All values were expressed as means ± SE. Statistical differences were established using the Student’s \( t \)-test.

R E S U L T S

The depolarization-activated macroscopic current of the opener muscle fibers shows three different components: an L-type voltage-gated \( \text{Ca}^{2+} \) current (\( I_{\text{Ca}} \), a voltage- and \( \text{Ca}^{2+} \)-dependent \( I_{\text{K(Ca)}} \)), and a voltage-gated \( K^{+} \) current (\( I_{\text{K}} \) (Araque and Buño 1994, 1995; Araque et al. 1994; Erxleben and Rathmayer 1997). Patch-clamp recordings in the cell-attached configuration mode of the opener muscle fibers, showed single channels with properties that matched those expected of the three different ionic channels mediating the macroscopic currents that we have described previously.

Three different voltage-gated ionic channels could be observed by membrane depolarization from \( V_m = –70 \) to more than –30 mV. Two channels carried outward current, one displayed a relative low conductance <40 pS (15.1 ± 3.6 pS; six patches), and the other had a relative high conductance >40 pS (67.6 ± 15.9 pS; 7 patches) and was \( \text{Ca}^{2+} \)-dependent (see following text), suggesting that they corresponded to channels mediating the macroscopic \( I_{\text{K}} \) and \( I_{\text{K(Ca)}} \), respectively. The latter channels will be termed BK channels because the electrophysiological and pharmacological properties of \( I_{\text{K(Ca)}} \) suggest that BK-type channels mediate it (see following text) (see also Araque and Buño 1995).

The ionic channel carrying inward current was encountered less frequently and probably corresponded to that mediating the macroscopic \( I_{\text{Ca}} \). This channel showed a much lower conductance and was extremely difficult to resolve from the background noise (not shown).

The present study was focused on the characterization of the high-conductance BK-type channel, and the properties of other channels were not further analyzed.

Large-conductance channel is voltage sensitive

Depolarizing ramps (from –70 to 130 mV) applied in the cell-attached configuration evoked BK channel openings above...
FAST-ACTIVATING BK CHANNELS

A: ramp voltage command from −70 to 130 mV. B, I–2: records showing single-channel openings. C: averaged (n = 25) single-channel current evoked by ramp depolarization. D: I–V relationship of a single-channel recording fitted to a linear regression (solid line). Single-channel conductance (g = 86.1 pS) was obtained from the slope of the linear regression, and the reversal potential was estimated from the 0 current value (−62.5 mV, *). E: I–V relationship of the averaged single-channel current shown in C. Horizontal line indicates the zero current and the slanted straight line the current that corresponds to the expected I–V relationship of single-channel openings (indicated by arrows). All records are from the same channel in cell-attached recording.

In some experiments (n = 4), the large-conductance channel was recorded in the cell-attached configuration, then the patch was excised and the same channel was recorded in the inside-out mode (Fig. 3). In our cell-attached conditions, the [Ca^{2+}]i is expected to be low because the cell is at rest and Vm is at the Vc, whereas in the inside-out mode a high Ca^{2+} concentration (13.5 mM) is in contact with the intracellular phase of the membrane. Figure 3 shows a representative example where the channel open probability at 50 mV increased from 0.05 in low [Ca^{2+}]i to 0.84 in high Ca^{2+} conditions. On average, the mean single-channel open probability at 50 mV increased from 0.17 ± 0.06 to 0.58 ± 0.09 (n = 4; P < 0.01) in low and high Ca^{2+} conditions, respectively, indicating that in addition to their voltage dependence, these channels are also Ca^{2+} dependent. Further analysis is needed to elucidate the partial contribution of both variables to the behavior of the channel.

We confirm this Ca^{2+} dependence by loading the patched fibers with the Ca^{2+} chelator BAPTA (n = 2), which reduced the [Ca^{2+}]i, and prevented I_{K(Ca)} activation (see Araque and Buño 1995; Araque et al. 1998). In these conditions, channel openings evoked by pulse depolarization (from −70 to 30 mV) were reduced gradually and eventually abolished (not shown), confirming the Ca^{2+} dependence of the BK channels.

We have hypothesized that the rate of activation of the macroscopic I_{K(Ca)} was limited by the Ca^{2+} inflow rather than by membrane depolarization (Araque and Buño 1995). Figure 3 shows that at similar depolarization channels tended to activate at briefer delays in the inside-out configuration, i.e., in high Ca^{2+} conditions. The latency histograms between the
onset of the depolarizing pulse and the first channel openings in low and high Ca\(^{2+}\) conditions, respectively, show the clearly different latency distributions (Fig. 3, C and D). In low Ca\(^{2+}\) conditions, the histogram was asymmetric, having most values grouped at brief latencies and showing a tail of few long latency values (Fig. 3C). The long latency values disappeared in high Ca\(^{2+}\) conditions, and values grouped at brief latencies (Fig. 3D). The different channel opening latencies in high and low Ca\(^{2+}\) conditions are also obvious when comparing their respective cumulative probability plots (P < 0.001, Kolmogorov-Smirnov test), again demonstrating that these channels are Ca\(^{2+}\) dependent (Fig. 3E).

These results confirm previous data on the \(I_{K(Ca)}\) activation kinetics obtained by analysis of the behavior of the macroscopic current and indicate that the binding of Ca\(^{2+}\) is the rate limiting step for the opening of BK channels (cf. Araque and Búnó 1995).

**Large-conductance channel activates fast and does not inactivate**

In agreement with the extremely fast activation kinetics of the macroscopic \(I_{K(Ca)}\), the underlying large-conductance BK channels displayed fast open kinetics (Fig. 4). Successive responses evoked by \(V_m\) pulses (from −70 to 30 mV) show that channels could occasionally open in <5 ms from the onset of the pulse (Fig. 4A). Moreover, the average of successive responses (n = 500) shows that the open probability increased markedly in the first 10 ms after the \(V_m\) pulse onset (Fig. 4A, bottom). Likewise, longer pulse depolarizations revealed that the open probability increased steeply during the initial 50 ms (i.e., reaching 90% of the maximum probability in ~10 ms) and tended to stabilize thereafter. The averaged channel current evoked by long \(V_m\) pulses reached a persistent steady state as shown in Fig. 4B (bottom), where n = 300 responses where averaged from a patch containing a channel with a relatively high open probability. The uniform late (>50 ms) averaged channel current indicates an invariable open probability during the constant \(V_m\) depolarization. It is noteworthy that the open probability also remained stable during the depolarizing pulse in inside-out patch recordings (not shown).

Therefore these BK channels open with extremely fast activation kinetics and show a persistent noninactivating state that lasts as long as the depolarizing pulse, during which the channel open probability is invariable.

**DISCUSSION**

Ca\(^{2+}\)-dependent K\(^+\) channels have been described in most excitable cells (Blatz and Magleby 1987; Hille 1992; Latorre et al. 1989) and have been classified in two main groups according to their single-channel conductance, calcium sensitivity, voltage dependence, and pharmacology. SK channels have small unitary conductance (<20 pS) (Blatz and Magleby 1986, 1987; Lang and Ritchie 1987; Latorre et al. 1989), are generally voltage independent (Barret et al. 1982; Marty 1981; Moczydlowski and Latorre 1983) and are sensitive to apamin (Blatz and Magleby 1986; Latorre et al. 1989; Romey and Lazdunski 1984). BK channels have high unitary conductances (ranging from 75 to 250 pS) (e.g., Blatz and Magleby 1987; Lang and Ritchie 1987; Reinhart et al. 1989; Wang et al. 1998), are voltage dependent (Barret et al. 1982; Marty 1981; Moczydlowski and Latorre 1983), and are sensitive to TEA and CTX (Blatz and Magleby 1984; Crest et al. 1992; Hermann and Erxleben 1987; Latorre et al. 1989; Miller et al. 1985; Tauc et al. 1993; Villarroel et al. 1988).

The macroscopic \(I_{K(Ca)}\) of crayfish opener muscle fibers is fast activating, persistent, voltage and Ca\(^{2+}\) dependent, and TEA and CTX sensitive (Araque and Búnó 1995), suggesting that the current is mediated by BK type channels. Our present results demonstrate that single channels with relatively large conductance are Ca\(^{2+}\) and voltage sensitive, activate fast, and do not inactivate, matching most of the properties of the macroscopic \(I_{K(Ca)}\).

We have found that BK channels in crayfish muscle show voltage and Ca\(^{2+}\) dependence. Such voltage dependence is not
merely apparent (due to the voltage dependence of the associated Ca\(^{2+}\) currents) because the single-channel open probability increased monotonically as a function of \(V_m\) (from −50 to 50 mV; Fig. 2). If such voltage dependence was due to the voltage dependence of the Ca\(^{2+}\) inflow and the subsequent rise in \([Ca^{2+}]_i\), the channel open probability first would increase and then decrease as \(I_{Ca}\) initially rises and subsequently drops at positive \(V_m\) when the reversal potential of Ca\(^{2+}\) is approached. Because the channel open probability increased monotonically with \(V_m\), we conclude that these BK channels are also voltage dependent (see Araque and Buño 1995).

In most cells studied, BK channels have a very high single-channel conductance, ranging from 100 to 250 pS. However, channels with BK properties but with smaller conductance (40–100 pS) have been reported in molluscan neurons (Crest et al. 1992; Gola et al. 1990; Hermann and Erxleben 1987) and vertebrate smooth muscle cells (Van Renterghem and Lazdunski 1992). Our present results show that BK channels in crayfish opener muscle have a single-channel conductance of ~70 pS. Therefore in invertebrate cells, although BK channels in insect muscle have a high single-channel conductance (>100 pS) (Gorczynska et al. 1996), BK channels found in molluscs (cf. Crest et al. 1992; Gola et al. 1990) and crustaceans (our present work) show similar and relatively small single-channel conductances (<100 pS).

The current results show that the intrinsic properties of the ionic channels mediating the \(I_{K(Ca)}\) of crayfish opener muscle fibers explain most of the characteristics of the macroscopic current (Araque and Buño 1995). Indeed, the channels are voltage and Ca\(^{2+}\) sensitive and they activate fast, in harmony with the similar sensitivities and fast activation kinetics of the macroscopic \(I_{K(Ca)}\) (Araque and Buño 1995). In agreement with the behavior of the macroscopic \(I_{K(Ca)}\), which increased and activated faster with increasing membrane depolarization as a result of the augmented Ca\(^{2+}\) inflow caused by the increased activation of \(I_{Ca}\) (Araque and Buño 1995), the channels mediating \(I_{K(Ca)}\) opened faster and the open probability increased in high-Ca\(^{2+}\) conditions. Therefore these intrinsic channel properties would favor the extremely fast activation that typifies the macroscopic \(I_{K(Ca)}\).

The open probability of these channels tended to be invariant during a prolonged depolarizing pulse, in accord with the persistent property of the macroscopic \(I_{K(Ca)}\). However, although the properties of BK channels explain the persistent nature of \(I_{K(Ca)}\), they do not correspond with the complex

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**Fig. 3.** Large-conductance channel is Ca\(^{2+}\)-dependent. A: single-channel openings evoked by depolarizing pulses in cell-attached configuration (low Ca\(^{2+}\)). B: same as A, but in high-Ca\(^{2+}\) conditions, i.e., after excising the patch and recording the channel in inside-out configuration (13.5 mM Ca\(^{2+}\)). Single-channel current decreases in the inside-out configuration after the patch was excised from the membrane, probably because of the greatly diminished K\(^+\) concentration in the extracellular solution compared with that of the cytoplasm. Channel open probability increased and the channel tended to open at briefer delays from pulse onset. C and D, histograms of the latency between the onset of the depolarizing pulse and the 1st channel opening in low- and high-Ca\(^{2+}\) conditions, respectively. E: cumulative probability plot of the channel opening latencies in low- and high Ca\(^{2+}\)-conditions (thin and thick lines, respectively). Histograms and cumulative probability plots were constructed from the responses to 100 depolarizing pulses from −70 to 50 mV. Bin width: 4 ms.

**Fig. 4.** Fast activation and deactivation characterize channel openings in cell-attached recordings. A: single-channel recordings (middle traces) showing brief opening delays (*), and averaged (n = 500) single-channel current also showing fast openings (↓; bottom) evoked by depolarizing pulses (top). B: single-channel recordings from a different patch showing responses to long-duration voltage pulses (middle traces); averaged single-channel current was calculated from 300 responses (bottom).
profile of the macroscopic current in response to depolarizing pulses. Indeed, we have reported that this $I_{K(Ca)}$ showed an incomplete inactivation, declining from its maximum value to reach a persistent steady state within 10 ms, but we could not elucidate if inactivation of the macroscopic $I_{K(Ca)}$ was due to intrinsic channel properties or simply reflected temporal $[Ca^{2+}]_i$ variations (Araque and Buño 1995). Present results show that the single-channel open probability displayed a fast initial increase to a steady state without peaks in both low- and high-Ca$^{2+}$ conditions, indicating the BK-type single channels mediating this $I_{K(Ca)}$ exhibit different behaviors when activated by patch depolarization as compared with depolarization of the whole fiber. Therefore the complex profile of the macroscopic $I_{K(Ca)}$ may be due to rapid changes of the $[Ca^{2+}]_i$ (Araque and Buño 1995).

Two confronting dynamic mechanisms control $[Ca^{2+}]_i$ in opener fibers during depolarization, namely, the Ca$^{2+}$ influx through $I_{Ca}$ channels and the intracellular Ca$^{2+}$-buffering mechanisms. The interactions between these two dynamic processes may result in rapid variations of the $[Ca^{2+}]_i$, that explain the complex macroscopic $I_{K(Ca)}$ profile. Because these variations are absent in cell-attached and inside-out conditions, our present data indicate that the apparent incomplete inactivation of $I_{K(Ca)}$ corresponds to temporal $[Ca^{2+}]_i$ variations and is not due to the intrinsic properties of the channels mediating this current.

BK channel inactivation has been reported in several cell types such as vertebrate skeletal muscle (Pallotta 1985), hippocampal pyramidal neurons (Hicks and Marrion 1998), and rat adrenal chromaffin cells (Solano et al. 1995). Our data indicate that BK channels in crayfish muscle are noninactivating, matching the behavior of BK channels in most cells that exhibit a sustained activation in the presence of a constant $[Ca^{2+}]_i$, (e.g., Barret et al. 1982; Blatz and Magleby 1986; Latorre et al. 1989).

Two findings were interesting and unexpected and should be underscored because they could be of key functional importance. First, in the cell-attached mode, openings could be evoked by depolarization at very negative potentials of about −50 mV, well below the activation threshold of the L-type $I_{Ca}$ (Araque and Buño 1994, 1995; Araque et al. 1994). Second, in these conditions of low $[Ca^{2+}]_i$, openings were fast and channels could open at latencies <5 ms and reached 90% of the maximum open probability in ~10 ms. This results suggest that BK channels in crayfish muscle may be activated by very low $[Ca^{2+}]_i$. Very high Ca$^{2+}$ sensitivity of BK channels has been reported in mammalian salivary gland cells (Maruyama et al. 1983) and recently in locust muscle (Gorczynska et al. 1996), although its physiological relevance in these cells is unclear. However, such a property may be of key importance for the functional role of $I_{K(Ca)}$ in the crayfish muscle. We have proposed that $I_{K(Ca)}$ provides a rapid and continuous feedback that controls the depolarization-evoked Ca$^{2+}$ inflow, thereby regulating the depolarization and the $I_{Ca}$ activation during the graded action potentials that typify these muscle fibers (Araque and Buño 1995; Araque et al. 1998). This feedback allows a graded and persistent Ca$^{2+}$ inflow needed for the graded and sustained contraction and prevents the uncontrolled depolarization that this Ca$^{2+}$ inflow would otherwise evoke (Araque et al. 1998). Accordingly, the high Ca$^{2+}$ sensitivity of these channels, in addition to their voltage dependence, contributes to the extremely fast activation kinetics of the macroscopic $I_{K(Ca)}$ (even at low resting $[Ca^{2+}]_i$). Therefore these channels act as precise Ca$^{2+}$ sensors, providing the exact feedback current needed to control the graded electrical activity and the contraction of these muscle fibers.

In conclusion, we have demonstrated that Ca$^{2+}$ and voltage-dependent BK-type channels mediate the $I_{K(Ca)}$ in opener crayfish muscle. We show that the intrinsic properties of these channels are responsible for most of the characteristics of the macroscopic current. However, we report that owing to these intrinsic properties, the behavior of these channels is different when studied isolated (i.e., in single-channel recordings) than when studied in the whole cell, when the interaction with other channel types is significantly relevant.


