Large-Conductance Ca\(^{2+}\)-Activated Potassium Channels in Secretory Neurons

JESÚS LARA, JUAN JOSÉ ACEVEDO, AND CARLOS G. ONETTI
Centro de Investigaciones Biomédicas, Universidad de Colima, Colima 28000, Mexico

INTRODUCTION

Calcium-activated potassium currents (K\(_{Ca}\)) have been observed in a number of neurons in invertebrates (Crest and Gola 1993; Deitmer and Eckert 1985) as well as in vertebrates (Kawai and Watanabe 1986; Lancaster et al. 1991; Pennefather et al. 1985; Smart 1987; Wang et al. 1998). Several types of potassium ion channels can mediate this K\(_{Ca}\) current: in addition to “small” conductance (SK) Ca\(^{2+}\)-activated K\(^{+}\) channels, “big” conductance (200–300 pS) Ca\(^{2+}\)-activated K\(^{+}\) (BK) channels have been described (Latorre et al. 1989). These channels couple the membrane potential to the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) in such a way that an increase in internal Ca\(^{2+}\) leads to an efflux of K\(^{+}\) ions and a subsequent hyperpolarization of the membrane. Because of this property, Ca\(^{2+}\)-activated K\(^{+}\) channels are believed to play a role in a number of different cellular processes that depend on the influx of Ca\(^{2+}\) through voltage-dependent pathways, including regulated secretion (McManus 1991; Petersen and Maruyama 1984). These channels also link internal Ca\(^{2+}\) and membrane excitability and thus are believed to play a role in regulating action potential frequency and duration in neurons. For instance, it has been proposed that BK channels underlie interburst intervals; therefore they could contribute to the control of hormone secretion in rat neurohypophysial terminals (Dopico et al. 1996).

In the X-organ sinus gland (XO-SG) system, the most important neurosecretory system of the crayfish, a periodic increase in [Ca\(^{2+}\)]\(_i\) in bursting neurons modifies membrane conductances and regulates electrophysiological activity (Martínez et al. 1991; Onetti et al. 1990). Calcium sensitivity is a very important phenomenon because the firing patterns have been associated with the ability for secretion in neurons (Stuenukel 1985). Several potassium currents contributing to modulate action potential firing patterns have been previously described in X-organ neurons: the delayed rectifier, the transient inactivating outward current (Martínez et al. 1991), and the ATP-sensitive K\(^{+}\) current (García et al. 1993; Onetti et al. 1996). Experimental evidence supports a key role of intracellular Ca\(^{2+}\) in the modulation of these currents. It has been suggested that K\(_{ATP}\) channels are involved in the regulation of spontaneous spike firing, due to their sensitivity to intracellular ATP and Ca\(^{2+}\) ions as well as to the membrane potential. In whole cell patch-clamp studies performed earlier in crayfish XO neurons, Ca\(^{2+}\)-activated potassium current was negligible because a cytoplasmic solution with EGTA was used (Onetti et al. 1990). However, recent experiments with inside-out cell-free patches suggest the presence of large-conductance Ca\(^{2+}\)-activated potassium channels (BK) in somata membranes from XO neurons. To study the relative contribution of BK and K\(_{ATP}\) channels to the macroscopic outward current and their participation in electrophysiological activity, we shall compare the properties of both types of channels in terms of their sensitivity toward intracellular Ca\(^{2+}\) and ATP as well as to the membrane potential.

METHODS

The experiments were carried out in neurons of the XO-SG system obtained from adult Procambarus clarkii crayfish of either sex. The dissection procedures and experimental conditions were described...
CITY, CA) using pClamp 6.0.2 software (Axon Instruments, Foster City, CA) and capacitive current and were fire polished. From 1.5-mm borosilicate capillaries, coated with silicone elastomer to face was bathed with solutions, the compositions of which are described in Table 1. Both cell-attached and inside-out patch pipettes were filled with 200 K solution (Table 1). Inside-out patch pipettes used in the experiments performed to test single-channel activity at quasi-physiological potassium gradient, were filled using 5 K solution. For outside-out patch current recording, the pipettes were filled with control solution, and the extracellular surface was bathed with 200 K solution (Table 1).

**Electrophysiological recordings**

The perforated patch-clamp technique (Horn and Marty 1988) was used to record membrane potential and macroscopic currents in the current- and voltage-clamp mode. Single-channel current recordings were performed in cell-attached, outside-out, and inside-out patch configurations of the patch-clamp technique (Hamill et al. 1981). All the records of membrane potential and membrane currents obtained in perforated patch-clamp experiments were made using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). To obtain single-channel currents we used a GeneClamp 500 amplifier (Axon Instruments, Foster City, CA). Pipettes with resistance ranging from 4 to 6 MΩ were used for perforated patch-clamp experiments, and from 8 to 10 MΩ for single-channel recordings. Patch pipettes were pulled from 1.5-mm borosilicate capillaries, coated with silicone elastomer (Sylgard; Dow Corning, Midland, MI) to reduce background noise and capacitive current and were fire polished.

Membrane potential and macroscopic current records were acquired with a Digidata 1200 A/D converter (Axon Instruments, Foster City, CA) using pClamp 6.0.2 software (Axon Instruments, Foster City, CA). Membrane potential records were filtered at 5 kHz ( ~ 3 dB). Macroscopic current signals were filtered at 2 kHz and sampled at 5 kHz. Single-channel currents were stored with a PCM Data Recorder 200 (A. R. Vetter, Rebersburg, PA) for subsequent off-line acquisition and analysis. These signals were filtered (1 kHz), amplified, and sampled at 10 kHz using a Digidata 1200 A/D converter (Axon Instruments, Foster City, CA). Analysis was made using a 486 microcomputer with pClamp 6.0.2 software (Axon Instruments, Foster City, CA). The method described by Davies et al. (1996) was used to obtain current-voltage (I-V) relations for single-channel currents. Briefly, currents were elicited by 80-ms voltage ramps from −80 to 80 mV; a leak trace, obtained by averaging sections that had no channel openings, was subtracted from each individual record, and an ensemble ramp was constructed by averaging sections containing a single opening. The open-state probability (Po) values were obtained by using the method described by Quayle et al. (1988). We used current records of 30 s to measure the open-state probability and to obtain single-channel conductance values. Single-channel data were filtered at 1 kHz (Bessel 4-pole) and sampled at 10 kHz. Experimental values are presented as means ± SE. A P value <0.05 was considered to be significant.

**Solutions**

For inside-out patch experiments, the intracellular membrane surface was bathed with solutions, the compositions of which are described in Table 1. The unitary currents through Ca²⁺-activated K⁺ channels (BK) were obtained in both cell-attached and excised membrane patches from X-organ neurons. The BK channel activity observed in cell-attached was similar to that obtained on excising membrane patches. The open-state probability (Po) values were ~0.8 (Vf = 0 mV) in the cell-attached configuration and ~0.9 (40 mV) in the inside-out patches at 1 μM Ca²⁺ on the internal side of the membrane.

**RESULTS**

**K⁺ selectivity, permeation, and blockade of BK channels**

Unitary currents through Ca²⁺-activated K⁺ channels (BK) were observed in both cell-attached and excised membrane patches from X-organ neurons. The BK channel activity observed in cell-attached was similar to that obtained on excising membrane patches. The open-state probability (Po) values were ~0.8 (Vf = 0 mV) in the cell-attached configuration and ~0.9 (40 mV) in the inside-out patches at 1 μM Ca²⁺ on the internal side of the membrane.

**Table 1. Composition of solutions for single-channel experiments (in mM)**

<table>
<thead>
<tr>
<th>Solution</th>
<th>KCl</th>
<th>NaCl</th>
<th>HEPES</th>
<th>EGTA</th>
<th>CaCl₂</th>
<th>Free [Ca²⁺], μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>200</td>
<td>10</td>
<td>5</td>
<td>4.31</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>0.3 Ca</td>
<td>200</td>
<td>10</td>
<td>5</td>
<td>3.33</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>3 Ca</td>
<td>200</td>
<td>10</td>
<td>5</td>
<td>4.76</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Low K</td>
<td>40</td>
<td>160</td>
<td>5</td>
<td>4.31</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>External</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 K</td>
<td>200</td>
<td>10</td>
<td>5</td>
<td>4.31</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>5 K</td>
<td>5</td>
<td>200</td>
<td>5</td>
<td>4.31</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

The free [Ca²⁺] values were calculated using the software designed by Fabiato (1988). All the internal solutions were adjusted at pH of 7.2 and the external solution at pH of 7.4 with KOH (control, 0.3 Ca, 3 Ca, and 200 K) or NaOH (low K and 5 K). * MgATP was added to this solution at 0.01, 0.03, 0.1, 0.3, 1, or 3 mM.

Earlier (Garcia et al. 1993). All records were made in neuron bodies of the X-organ (XO) at room temperature (20–22°C). In the experiments for macroscopic current recording, the XO-SG nerve tract was severed at 200–300 μm after its emergence from XO.
mV (Fig. 1B). In this condition, the $I$-$V$ relation in the linear region can be described by the Goldman-Hodgkin-Katz equation

$$I_k = P_k \frac{F V_m}{RT} [K]$$

where $P_k$ is the channel permeability to $K^+$ ions and $V_m$ is the membrane potential. $R$, $T$, and $F$ have their usual meaning. The $P_k$ value obtained by fitting Eq. 1 was $2.3 \times 10^{-13}$ cm$^3$ s$^{-1}$. When the KCl concentration on the intracellular surface of the membrane patches was reduced to 40 mM (160 mM NaCl, low K solution) the reversal potential of the unitary current was shifted to 41 ± 4 mV (Fig. 2) in all five patches studied. This value is close to the equilibrium potential for $K^+$ ions predicted by the Nernst equation (40 mV), and an inward rectification was evident. When the potassium gradient was closer to physiological conditions ([K]_o = 5 and [K]_i = 200 mM), a nonlinear $I$-$V$ relation was found, exhibiting outward rectification.

In this condition, the unitary current was outwardly directed at all membrane potentials explored (−80 to 80 mV). Single-channel ensemble ramp currents at different internal and external K$^+$ concentrations demonstrate that the BK channels are highly permeable to K$^+$ ions (Fig. 2). These results suggest these channels are selective to K$^+$ ions.

To explore the sensitivity of BK channels toward blocking agents specifically directed against particular types of K$^+$ channels, we recorded the potassium currents in outside-out and whole cell patch configurations in the presence of tetraethylammonium chloride (TEA), charybdotoxin (CTX), or Cd$^{2+}$. Also, we examined the effect of removing Ca$^{2+}$ from the extracellular medium. The single currents from an outside-out patch were reversibly blocked by adding TEA (1 mM) or CTX (100 nM) to the extracellular side of the membrane patch (Fig. 3). Moreover, the transient component of macroscopic currents through BK channels was blocked by the addition of Cd$^{2+}$, TEA, or CTX. By replacing Ca$^{2+}$ with an isosmotic amount of Mg$^{2+}$, the current observed in the whole cell mode also diminished (Fig. 6A).

**Ca$^{2+}$, MgATP, and voltage sensitivity of BK channels**

To study Ca$^{2+}$ and voltage dependence of BK channels, we obtained the steady-state open probability in symmetrical distribution of K$^+$ ions, by changing the membrane potential at different intracellular Ca$^{2+}$ concentrations ([Ca$^{2+}$]) in inside-out patches, in the absence of MgATP. When the membrane potential was held at 40 mV, increasing [Ca$^{2+}$], from 0.3 to 3 μM surface caused an enhancement of the channel activity (Fig. 4A). To describe the voltage dependence of channel activity, we fitted the experimental values of $P_o$ obtained at different membrane potentials, to the Boltzmann equation

$$P_o / P_{max} = \left[1 + e^{(V_m - V_{0.5})/k}\right]^{-1}$$

where $P_o$ is determined experimentally at a specific membrane potential ($V_m$), $V_{0.5}$ is the membrane potential at which $P_o$ is one-half of its maximum ($P_{max}$) and $k$ is the logarithmic potential sensitivity. The $e$-fold increase in $P_o$ ($k$) did not significantly change (12.6–12.8 mV) at different [Ca$^{2+}$]. However, an increase in [Ca$^{2+}$] produced a shift to more negative potentials of $P_o/V_m$ relationship. The half-activation

---

**FIG. 1.** Current-voltage relationship for the BK channels. A: single-channel currents recorded from an inside-out patch at 2 different membrane potentials (indicated at the right of recordings) in symmetrical K$^+$ ([K]_o = [K]_i = 200 mM). Closed (c) and open (o) current levels are indicated to the left of the traces. B: experimental current-voltage plots for the BK channel obtained in symmetrical K$^+$. Dotted line corresponds to experimental data fittings using the Goldman-Hodgkin-Katz equation. The intracellular membrane surface was bathed with control solution. Data are presented as means ± SE (n = 5).

**FIG. 2.** Selectivity of the BK channel. Single-channel ensemble ramp currents in the inside-out configuration obtained at different K$^+$ gradients: [K]_o = 5 and [K]_i = 200 mM (a), [K]_o = [K]_i = 200 mM (b), and [K]_o = 200 mM and [K]_i = 40 mM (c). The intracellular membrane surface was bathed with control (a and b) or low K (c) solutions. The pipettes were filled with 200 K (b and c) or 5 K (a) solutions. Ramp currents in high and low intracellular K$^+$ concentrations were obtained from the same patch (b and c).
The internal face of the membrane (pipette) was exposed to the solution that contained 1 μM Ca\(^{2+}\) (control solution). The membrane potentials \(V_{0.5}\) were \(-5\), \(-26\), and \(-44\) mV at 0.3, 1, and 3 μM of \([Ca^{2+}]_i\), respectively (Fig. 4B).

The number of \(Ca^{2+}\) binding sites on the BK channel can be estimated using the following equation (Wong et al. 1982)

\[
V_{0.5}/k = N_{Ca} \ln \frac{K_D(0)}{[Ca^{2+}]_i}
\]

where \(K_D(0)\) is the concentration of \(Ca^{2+}\) that induces 50% of maximum channel activity at 0 mV, and \(N_{Ca}\) is the number of binding sites that is analogous to the Hill coefficient. Values for \(V_{0.5}\) and \(k\) were taken from the experiments of Fig. 4B. From the fitting of those data \(N_{Ca}\) was 1.32 and \(K_D(0)\) was 0.22 μM (Fig. 4C).

Figure 5A is a typical example of the activating action on the BK channels by the addition of 0.1 and 1 mM MgATP to the internal solution at 1 μM Ca\(^{2+}\) \((V_m = 40\) mV\). To study the sensitivity of the BK channel to MgATP, we used \(NP_o\) rather than \(P_O\) due to the possibility that MgATP can affect both of these parameters (Albarwani et al. 1994). A plot of the number of functional channels multiplied by their open probability \((NP_o)\), against MgATP concentration \([\text{MgATP}]\) is shown in Fig. 5B. The relationship \(NP_o/[\text{MgATP}]\) can be described by the Hill equation

\[
NP_o = NP_{o_{max}} \frac{[\text{MgATP}]^h}{[\text{MgATP}]^h + K_D^h}
\]

where \(NP_{o_{max}}\) is the maximum number of functional channels multiplied by their open probability, \(K_D\) is the half-activating concentration of MgATP, and \(h\) is the Hill coefficient. By fitting Eq. 4 to the \(NP_o\) values obtained at various [MgATP], ranging from 0.01 to 3 mM, the dissociation constant for MgATP \((K_D)\) was 119 μM and the Hill coefficient \((h)\) was 0.6 (Fig. 5B).

These results show that the BK channels are sensitive to internal Ca\(^{2+}\) concentration, voltage dependent, and activated by intracellular MgATP.

**Contribution of BK and K\(_{\text{ATP}}\) channels to the whole current**

To study the contribution of BK and K\(_{\text{ATP}}\) channels to the whole current, we obtained macroscopic current records by using the perforated patch-clamp technique in the voltage-clamp mode. We carried out these experiments under control conditions, blocking the BK currents with TEA or CTX or suppressing the calcium current by adding CdCl\(_2\) to the external solution or by replacing Ca\(^{2+}\) with Mg\(^{2+}\). The macroscopic current, obtained in the control solution, exhibited three components: the first one was inward, and the other two were outward. One of the outward current components was transient and the other was sustained. Suppressing the inward current by adding Cd\(^{2+}\) or by removing external Ca\(^{2+}\), the transient outward current was blocked, whereas the sustained component was not modified (Fig. 6, Aa and Ab). Moreover, the addition of 1 mM TEA or 100 nM CTX to the external solution decreased the transient outward current without a significant change in either the inward current or the sustained outward current (Fig. 6, Ac and Ad). The \(I-V\) relationship of the outward current obtained in the control solution, of the current in the presence of 0.4 mM of CdCl\(_2\), and of the subtraction of the currents obtained with CdCl\(_2\) from those obtained in the control solution are shown in Fig. 6B. It is evident that the addition of TEA or CTX suppresses, at least in part, the transient outward currents in the whole cells. This suggests that the outward current blocked by TEA or CTX can be carried through the BK channels. These results, together with those obtained in excised membrane patches (Fig. 3), show that part of the transient potassium currents could depend on Ca\(^{2+}\) influx and membrane potential, whereas the sustained outward current was only voltage dependent. In excised patch membranes the BK channel activity was persistent because \([Ca^{2+}]_i\) was constant throughout the entire recording. In contrast, the BK macroscopic currents, obtained in whole cell condition, were transient because calcium currents are transient in themselves.

ATP-sensitive potassium channels (K\(_{\text{ATP}}\)) from X-organ neurons are also modulated by intracellular Ca\(^{2+}\) and membrane potential (Onetti et al. 1996). To determine whether both kinds of channels (BK and K\(_{\text{ATP}}\)) coexist in the same cell, we carried out experiments recording whole currents using blockers of BK and K\(_{\text{ATP}}\) channels. Figure 7A shows recordings obtained from a representative X-organ neuron in the presence of glybenclamide (50 μM), both with and without CdCl\(_2\) (0.4 mM) in the perfusion solution. To isolate the current component blocked by glybenclamide or by Cd\(^{2+}\), the currents obtained with glybenclamide were subtracted from those obtained in the control solution; the same procedure was applied to the currents recorded with glybenclamide and Cd\(^{2+}\) and to those obtained in the presence of glybenclamide, alone. Figure 7B shows the \(I-V\) relationship of K\(_{\text{ATP}}\) currents obtained from a neuron, in the control solution, in the presence of glybenclamide, glybenclamide plus Cd\(^{2+}\), and the subtractions mentioned above. These results show that BK and K\(_{\text{ATP}}\) channels...
are distinct entities that could coexist in the same cell of the X-organ. Furthermore, our observations suggest that K currents, carried through K$_{ATP}$ channels, may contribute to the delayed rectifier in these neurons.

**How the BK and K$_{ATP}$ channels participate in electrophysiological activity**

To determine how the BK and K$_{ATP}$ channels participate in the activity of XO neurons that discharge action potential bursts, we obtained membrane potential records with the perforated patch-clamp technique in the current-clamp mode. By adding TEA (1 mM) to the perfusion solution, an increase in the duration of action potentials (25 ± 1%, n = 7) and bursts (38 ± 9%, n = 7) was produced. Only a slight increase in the burst frequency (6 ± 2%, n = 7) was observed. The effects on the duration of action potentials and bursts, as well as on the burst frequency produced during the addition of CTX (100 nM), were similar to those produced by TEA addition; they increased 31 ± 12, 9 ± 2, and 10 ± 2% (n = 5), respectively. Figure 8A shows typical membrane potential recordings, at two different time scales, in the control solution and after the addition of CTX. On the other hand, besides a membrane depolarization, the addition of glybenclamide (50 μM) produced an increase of duration (55 ± 10%, n = 5) and frequency (110 ± 15%, n = 5) of the bursts; also, a slight increase in action potential duration (6 ± 1, n = 5) was observed. These effects can be seen in the records shown in Fig. 8 (C and D), obtained from a neuron with burst activity. The increase in the action potential and burst duration and burst frequency produced by the addition of TEA, CTX, or glybenclamide to the bath solution, was significant (P < 0.01).
In summary, the most noticeable effect on the electrophysiological activity, produced by the blockage of the BK channels (with TEA or CTX), was an increase in the duration of bursts and action potentials. Nevertheless, the most noticeable effect found to block the KATP channels (with glybenclamide), besides that of membrane depolarization, was an increase in the burst duration as well as in the burst frequency.

**DISCUSSION**

We have characterized the biophysical properties of the BK channels and their contribution to the macroscopic outward current as well as their participation in the electrophysiological activity from secretory neurons of the crayfish by using the patch-clamp technique.

The electrophysiological properties and the calcium sensitivity of the BK channels in XO neurons are similar to those from other BK channels (McManus 1991), particularly the BK channels from rat neurons (Franciolini 1988; Safronov and Vogel 1998). Single-channel kinetics in cell-attached configuration were similar to those observed when the membrane patch was excised from the cell, at 1 or 3 mM Ca\(^{2+}\) concentration; even the I-V relationships of the BK channels were identical in both configurations. This leads one to think that the
internal Ca\(^{2+}\) concentration of intact X-organ neurons could fall within this interval (1–3 \(\mu\)M).

**Permeation and blocking of the BK channels**

The unitary conductance of the BK channels in symmetrical K\(^{+}\) (~220 pS) is within the range reported (180–290 pS) for BK channels in other tissues (McManus 1991). Comparison of theoretical equilibrium potentials with reversal potentials obtained experimentally by changing the K\(^{+}\) gradient (Fig. 2), reveals that BK channels in excised inside-out patches are highly selective for K\(^{+}\) ions. Like BK channels in other tissues (Latorre et al. 1989) these channels display a high K\(^{+}\) permeability \((2.3 \times 10^{-16} \text{ cm}^2 \text{s}^{-1})\); however, under symmetrical K\(^{+}\) gradient, the BK channels in XO neurons do not have a linear conductance, as the slope falls below that of a straight line at high positive voltage (Fig. 1B). For the time being we cannot explain this effect. We speculate that this nonlinearity could be attributed to a rapid voltage-dependent block, perhaps by H\(^{+}\) ions. It could also be produced by the intrinsic permeability properties of the BK channel, or by diffusion-limited ion flow (Yellen 1984). An inward rectification is seen when 40 mM K\(^{+}\) (160 mM Na\(^{+}\)) is present in the internal solution; an outward rectification is observed when using quasi-physiological K\(^{+}\) concentrations (Fig. 2); this is consistent with the I-V relationship predicted by the Goldman-Hodgkin-Katz field equation.

As previously reported for other tissues (Anderson et al. 1988; Kehl and Wong 1996; Safronov and Vogel 1998), the current carried by BK channels can be blocked by tetraethylammonium or charybotoxin applied to the extracellular medium (Figs. 3 and 8A).

**Voltage dependence, and Ca\(^{2+}\) and MgATP sensitivity of BK channels**

Steady-state activation, as a function of membrane potential for the BK channels described here, was analyzed by fitting the \(P_{o}/V_{m}\) relationship to the Boltzmann equation at different \([\text{Ca}^2+]\) (Fig. 4B). The voltage sensitivity of the BK channels from XO neurons was similar to that reported for neurons (Safronov and Vogel 1998) and other tissues (Albarwani et al. 1994; Kehl and Wong 1996; Latorre et al. 1989; Singer and Walsh 1987). It became evident that these channels are acutely sensitive to voltage \((k = 13 \text{ mV})\); also, the half-activation point depends on the internal Ca\(^{2+}\) level \((-5, -26, \text{ and } -44 \text{ mV}, \text{ at } 0.3, 1, \text{ and } 3 \mu\text{M }[\text{Ca}], \text{ respectively})\). Increasing the Ca\(^{2+}\) concentration produced a significant shift of the activation curve to more negative membrane potentials, even when the sensitivity factor \((k)\) remained unaffected. It is possible that an increase in internal Ca\(^{2+}\) shifts the voltage activation threshold for BK channels without affecting their voltage sensitivity. However, another possibility is that the affinity of BK channels for Ca\(^{2+}\), itself, is modulated by voltage; other authors (Albarwani et al. 1994; Barrett et al. 1982; Moczydlowski and Latorre 1983) have suggested this possibility. The number of Ca\(^{2+}\) binding sites \((N_{\text{Ca}} = 1.3)\) for the BK channel from XO neurons, estimated using Eq. 3 (Fig. 4C), suggests that the activation is due to binding of only one or two Ca\(^{2+}\) ions to the channel. Similar values for \(N_{\text{Ca}}\) have been reported in rat plasma membrane (Moczydlowski and Latorre 1983), smooth muscle (Benham et al. 1986; Inoue et al. 1985), and rat hippocampus neurons (Franciolini 1988). The dissociation constant at 0 mV \([K_{D}(0) = 0.22 \mu\text{M}]\) for these channels was similar to those obtained in other tissues, such as smooth muscle (Benham et al. 1986; Carl and Sanders 1989; Inoue et al. 1985; Kume et al. 1990) and rat melanotrophs (Kehl and Wong 1996).

In XO neurons, intracellular MgATP activates the BK channels. Using a symmetrical distribution of K\(^{+}\), this activation is observed when 0.01–3 mM of internal MgATP is present (Fig. 5). Similar behavior has been found for the BK channels from the main pulmonary artery of the rat (Albarwani et al. 1994). Some authors have explained this activation as a result of phosphorylation, rendering BK channels more sensitive to Ca\(^{2+}\) ions (Albarwani et al. 1994; Robertson et al. 1992). It is possible that the MgATP activation mechanism plays a physiological role in XO neurons enhancing BK channel activity and therefore producing an increase in the hyperpolarization after a burst of action potentials. It is known that intracellular ATP concentration is strictly related to the availability of

**FIG. 7.** BK and K\(_{\text{ATP}}\) channels coexist in the same cells. A: current records obtained from an axotomized neuron using the perforated patch-clamp technique in control solution (a), after the addition of 50 \(\mu\text{M}\) glybenclamide (b), and in the presence of glybenclamide (50 \(\mu\text{M}\)) plus 0.4 mM CdCl\(_{2}\) (c). Membrane potential values are signaled near of traces. Holding potential \(-50 \text{ mV}. B:\) current-voltage relationships of outward current obtained in control solution (●), in presence of 50 \(\mu\text{M}\) glybenclamide (■), 50 \(\mu\text{M}\) glybenclamide plus 0.4 mM CdCl\(_{2}\) (▲), and the subtractions: ○ = ● – ■, ▲ = ■ – ▲. Outward currents obtained with subtractions correspond to the currents carried by BK (●) and K\(_{\text{ATP}}\) (▲) channels.
energy sources; levels of extracellular glucose can determine internal ATP concentration. In XO neurons, metabolic balance could change in such a manner as to render BK channels more sensitive to Ca\(^{2+}\) due to an increase in intracellular ATP concentration (\(K_D = 119 \, \mu M\)). However, it is not known whether the intracellular ATP concentration falls within the level at which BK channels are most sensitive to Ca\(^{2+}\).

We conclude that the BK channels in XO neurons are voltage dependent, sensitive to internal Ca\(^{2+}\), and activated by intracellular MgATP; it is possible that these factors interact to play an important role in the functional profile of these neurons.

**Physiological implication of BK and K\(_{ATP}\) channel coexistence on electrophysiological activity**

It seems reasonable to assume that in spontaneously bursting XO neurons, internal Ca\(^{2+}\) concentration will attain the optimal level necessary to activate both BK and K\(_{ATP}\) channels. Due to the influx of Ca\(^{2+}\) ions during a spike burst, through voltage-dependent Ca\(^{2+}\) channels, the BK channels can regulate both spike burst and action potential duration (Fig. 8). Similar phenomena have been described in neurons from other species (Crest and Gola 1993; Wang et al. 1998). Depending on the cellular metabolic state, the K\(_{ATP}\) channels can control not only the resting potential, but both the duration and frequency of bursts. Because BK and K\(_{ATP}\) channels are distinct entities and could coexist in the same neuron of the X-organ, spike shaping and spontaneous firing pattern regulation would depend on the balance between the level of activity of both channels.

The authors express their gratitude to Dr. Esperanza García for critical review of the manuscript.

This work has been partially supported by Grants 1913P-N and 29471-N from Consejo Nacional de Ciencia y Tecnología, Secretaría de Educación Pública and Fondo para el Mejoramiento de la Educación Superior: 98-07-01 from Subsecretaría de Educación Superior e Investigación Científica, Secretaría de Educación Pública, Mexico.

Address for reprint requests: C. G. Onetti, Centro de Investigaciones Biomédicas, Universidad de Colima, Apdo. Postal 97, Colima, Col. 28000, Mexico.

Received 5 March 1999; accepted in final form 19 May 1999.

**REFERENCES**


CREST, M., AND GOLA, M. Large conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels are involved in both spike shaping and firing regulation in Helix neurons. J. Physiol. (Lond.) 465: 265–287, 1993.

DAVIES, N. W., MCKILLEN, H. C., STANFIELD, P. R., AND STANDEN, N. B. A rate theory model for Mg\textsuperscript{2+} block of ATP-dependent potassium channels of rat skeletal muscle. J. Physiol. (Lond.) 490: 817–826, 1996.


HOUS, K., KITAMURA, K., AND KURIYAMA, H. Two Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels classified by the application of tetraethylammonium distribute to smooth muscle membranes of the rabbit portal vein. Pflügers Arch. 405: 173–179, 1985.


MOZYDLOWSKI, E. AND LATORRE, R. Gating kinetics of Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels from rat muscle incorporated into planar lipid bilayers. Evidence for two voltage-dependent Ca\textsuperscript{2+} binding reactions. J. Gen. Physiol. 82: 511–542, 1983.


