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ORIGINAL ARTICLE

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Effect of the inactivating "ball" peptide of *Shaker* B on intermediate conductance Ca^{2+}-dependent inwardly rectifying K^+ channels of HeLa cells

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Abstract The patch-clamp technique was used to study the effect of intracellularly added inactivating "ball" peptide (BP) of the *Shaker* B K^+ channel upon Ca^{2+}-dependent inwardly rectifying K^+ channels of the intermediate conductance type expressed in HeLa cells. Intracellular BP caused only moderate inhibition of outward K^+ currents when assayed at an intracellular Ca^{2+} concentration of 100 nmol/l. Increasing intracellular Ca^{2+} levels led in itself to some voltage-dependent blockade of K^+ currents, which was absent when high extracellular K^+ was used. An additional strong blockade by intracellular BP was nevertheless observed both in Na^+- and K^+-rich extracellular solutions. A non-inactivating BP analogue had no effect. At this higher intracellular Ca^{2+} concentration the inhibition of these intermediate conductance Ca^{2+}-dependent channels by BP was voltage-dependent, being absent at hyperpolarizing potentials, and could be relieved by increasing extracellular K^+. These data suggest that BP acts at an internal pore site in Ca^{2+}-dependent intermediate conductance K^+ channels of HeLa cells, and that these might possess a receptor site for the peptide similar to that of other K^+ channels such as Ca^{2+}-activated maxi-K^+ channels.

Key words Intermediate conductance Ca^{2+}-dependent K^+ channel · *Shaker* B inactivating peptide

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Introduction

K^+ channels play important roles in the maintenance of the cell membrane potential, response to agonists, cell proliferation, and regulation of cell volume in numerous excitable and non-excitable cells. We have recently studied in HeLa cells currents showing moderate inward rectification, dependence upon intracellular Ca^{2+}, voltage independence, sensitivity to charybdotoxin (but not to apamin) and complete blockade by ammonium [2, 3]. This current dominates the conductance of HeLa cells with little contribution from other currents under the conditions of our experiments. Single channels corresponding to the currents referred to above have been reported in HeLa cells and found to have intermediate conductance [8, 9]. The characteristics of this channel make it probably identical to a human intermediate conductance, Ca^{2+}-dependent K^+ channel (hIK), recently characterized in detail after cloning from placental tissue [7].

In this study we addressed the issue of whether the Ca^{2+}-dependent inwardly rectifying K^+ channels of HeLa cells can be blocked by the inactivating "ball" peptide (BP) of the *Shaker* B K^+ channel. These channels show fast inactivation which has been shown to be caused by an interaction of the first 20 N-terminal amino acids that occlude the pore once it opens [6]. Deletion of the peptide removes inactivation, which can be restored by intracellular addition of synthetic BP [11]. Synthetic BP can also block some but not all [1] channels that do not normally present fast inactivation, for example, high-conductance Ca^{2+}-activated K^+ channels [10]. This suggests that the receptor region in the channel has conserved some characteristics in common throughout evolution of these membrane proteins, and also provides a potentially valuable tool to explore the inner mouth of the channel. We report that BP is indeed capable of blocking Ca^{2+}-dependent intermediate conductance K^+ channels of HeLa cells when added to the intracellular side of the membrane. This effect is seen at depolarizing but not at negative potentials, is antagonized by increas-

ing the extracellular K^+ concentration and is favored at high intracellular Ca^{2+}.

Materials and methods

HeLa cells were grown to about 50% confluence at 37 °C in a 5%/95%: CO_2/air atmosphere in Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum, 80,000 IU/l penicillin and 50 µg/l streptomycin. For experiments, cells were seeded onto 35-mm-diameter cell culture petri dishes, which could be directly mounted on the stage of an inverted microscope. Standard whole-cell patch-clamp recordings were performed using an EPC-7 amplifier (List Medical, Germany) as previously described [2]. The pipette (intracellular) solution contained (in mmol/l): 145 KCl, 1.2 $MgCl_2$, and 10 HEPES, adjusted to pH 7.4 with Tris; the 100 µmol/l Ca^{2+} solution contained (all in mmol/l) 0.1 $CaCl_2$, while the 100 nmol/l Ca^{2+} solution had 1 EGTA plus 0.65 $CaCl_2$ or 1 BAPTA plus 0.47 $CaCl_2$. When appropriate, BP was added to the pipette solution at the concentration indicated. The Na^+-rich extracellular bath solution contained (in mmol/l): 140 NaCl, 5 KCl, 0.5 $MgCl_2$, 1.3 $CaCl_2$, 10 HEPES, adjusted to pH 7.4 with Tris. K^+-rich solution was identical except for equimolar replacement of NaCl by KCl. The pipettes had a resistance of 3–5 MΩ. Voltage and current signals from the amplifier were recorded on a digital audio tape recorder (DTR-1204, Biologic, France) and processed using a Digidata 1200 (Axon Instruments, USA) AD/DA interface. Data acquisition and analysis programs used were those written by John Dempster (University of Strathclyde, Glasgow, Scotland). An Ag-AgCl wire was used as the reference electrode. All experiments were performed at room temperature (22–25 °C). Reagents used were of analytical grade and were purchased from Sigma (St. Louis, Mo., USA) and Merck (Santiago, Chile). BP and its inactive analogue were synthesized and purified as described previously [4]. The values are given as mean±SEM. Statistical significance was evaluated by Student's *t* test.

Results and discussion

Whole-cell currents were recorded in HeLa cells, obtaining typical moderately inwardly rectifying currents that have been described before as being carried by K^+ [2]. When 100 nmol/l intracellular Ca^{2+} was used, inclusion of 1–100 µmol/l BP in the pipette solution did not affect current values or rectification properties (n=31 experiments with BP and n=11 controls, not shown). When 200 µmol/l BP was used there was some effect with extracellular Na^+-rich solution (see above). Under these conditions inward current was not significantly affected: conductance values measured at –120 mV were 215±32 (n=7) pS/pF in controls and 223±35 (n=9) pS/pF with BP. Outward current was, nevertheless, slightly diminished at large positive potentials. The rectification ratio, defined as the ratio of the current measured at 120 mV to that at –120 mV (I_{120mV}/I_{-120mV}), decreased significantly (P=0.0001) from 1.6±0.1 (n=7) in controls to 1.2±0.1 (n=9) with BP. This small effect was abolished upon switching to a extracellular K^+-rich solution. In this situation, conductance values at –120 mV were 577±95 (n=7) pS/pF in controls and 639±129 (n=8) pS/pF with BP. The respective I_{120mV}/I_{-120mV} rectification ratios were 0.65±0.03 and 0.59±0.05 (not significantly different).

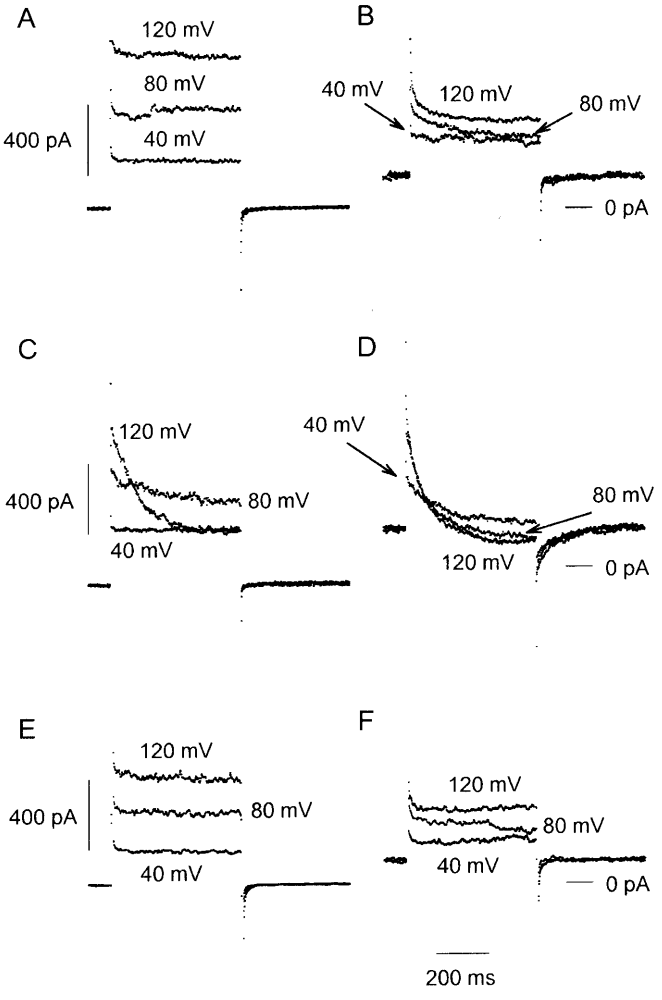

Fig. 1A–F Effect of BP on Ca^{2+}-dependent intermediate conductance K^+ channels of HeLa cells. Currents were measured with 100 µmol/l intracellular Ca^{2+} in K^+- (**A**, **C**, **E**) or Na^+-rich (**B**, **D**, **F**) extracellular solutions. Currents illustrated were elicited by square voltage pulses from a holding potential of 0 mV to the indicated voltage levels. **A, B** Control cell. **C**,**D** Cell dialyzed with 100 µmol/l BP. **E**,**F** Cell dialyzed with 100 µmol ShB-L7E mutant peptide

A recent report characterized the inactivation of Ca^{2+}-dependent maxi-K^+ channels by an associated intracellular peptide particle as dependent upon high intracellular Ca^{2+} [5]. We therefore tested whether this is the case for BP interaction with Ca^{2+}-dependent intermediate conductance K^+ channels of HeLa cells. Figure 1A and B shows K^+ currents recorded with intracellular Ca^{2+} of 100 µmol/l. The currents recorded in K^+-rich medium (Fig. 1A) gave the expected time-independent outward and inward (not illustrated) currents. When low extracellular K^+ was used, the currents showed a time-dependent decay at large (>40 mV) positive potentials. There was no current decay at negative potentials (not illustrated). We attribute this effect, which has also been observed with cloned hIK [7], to Ca^{2+} pore blockade, as it was not observed at lower intracellular Ca^{2+} concentrations [2] and was absent at low positive potentials or with high extracellular K^+.

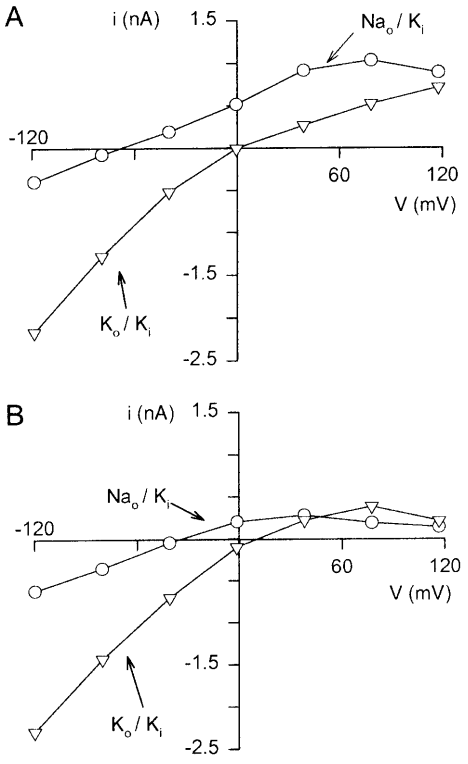

Fig. 2 Current-voltage relationship for Ca^{2+}-dependent inwardly rectifying K^+ channels of HeLa cells. Data correspond to the full voltage range for experiments shown in Fig. 1A–D. **A** Control cell. **B** Cell dialyzed with 100 µmol/l BP. Na_o/K_i Na^+-rich extracellular solution; K_o/K_i K^+-rich extracellular solution

When 100 µmol/l intracellular BP was assayed at 100 µmol/l Ca^{2+}, the results shown in Fig. 1C and D were obtained. Now a time- and voltage-dependent blockade was seen in high extracellular K^+ and a much larger decay in current took place at all positive potentials tested in Na^+-rich extracellular solution. At negative potentials there was no marked effect of BP; conductance values measured in Na^+-rich extracellular solution at –120 mV were 180±50 (n=4) pS/pF in controls and 282±72 (n=4) pS/pF with BP. This difference was not statistically significant. Outward current at positive potentials was diminished by BP when measured in Na^+-rich extracellular solution. This can be gauged from comparing the rectification ratio I_{120mV}/I_{-120mV} values. These decreased significantly (P=0.03) from 1.09±0.18 (n=4) in controls to 0.68±0.07 (n=4) with BP. This effect was also present in high extracellular K^+ solution, with conductance values at –120 mV of 708±161 (n=4) pS/pF in controls and 755±155 (n=5) pS/pF with BP (not significantly different). The respective I_{120mV}/I_{-120mV} rectification ratios were 0.34±0.02 and 0.16±0.02 (P=0.0004). A synthetic non-inactivating BP (ShB-L7E) used at 100 µmol/l produced no effect. An example of outward currents measured in the presence of this non-inactivating BP is shown in Fig. 1E and F. No decay in current was observed at depolarized potentials at high extracellular K^+ and only a slight effect was seen in Na^+-rich medium (Fig. 2).

In this report we demonstrate that intracellular BP is capable of blocking Ca^{2+}-dependent intermediate conductance K^+ channels of HeLa cells. The effect of the peptide was marked only at high intracellular Ca^{2+} concentrations but weak at physiological Ca^{2+} levels. The blockade completely disappeared in high extracellular K^+ at low intracellular Ca^{2+} but was only attenuated at high intracellular Ca^{2+}. BP blockade was present at depolarizing potentials but was negligible at negative voltages, in contrast to the apparent intrinsic voltage independence of BP effect upon *Shaker* K^+ channels [6]. Our results, however, are similar to those reported before for maxi-K^+ channels as regards voltage dependence and reversal by K^+ [1, 10]. Interestingly, a requirement for micromolar Ca^{2+} concentrations for the blockade of an intracellular peptidic inactivating particle has been reported in maxi-K^+ channels from hippocampal CA1 neurons [5]. This might reflect, at least in part, a dependence of opening of the channels upon Ca^{2+} since only open channels can be blocked in a voltage-dependent manner.

Our observations suggest that BP is capable of interacting with an intracellular site of Ca^{2+}-dependent intermediate conductance K^+ channel pore to block it. Such a blockade is Ca^{2+}- and potential-dependent and can be displaced by a permeant cation from the extracellular side. We conclude that Ca^{2+}-dependent inwardly rectifying K^+ channels of HeLa cells possess a pore receptor site for BP that might have some homology with the peptide receptor in other K^+ channels.

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