Ca-dependent regulation of Na^+-selective channels via actin cytoskeleton modification in leukemia cells

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Received 28 April 1997; revised version received 30 May 1997

Abstract With the use of the patch-clamp technique, physiological mechanisms of Na^+ channel regulation involving submembranous actin rearrangements were examined in human myeloid leukemia K562 cells. We found that the actin-severing protein gelsolin applied to cytoplasmic surface of membrane fragments at a high level of $[Ca^{2+}]_i$ (1 μM) increased drastically the activity of Na-selective channels of 12 pS unitary conductance. In the experiments on intact cells, the elevation of $[Ca^{2+}]_i$ using the ionophore 4Br-A23187 also resulted in Na^+ channel activation. Addition of actin to the cytoplasmic surface of membrane patches reduced this activity to background level, likely due to actin polymerization. Our data imply that Ca-dependent modulations of the actin cytoskeleton may represent one of the general mechanisms of channel regulation and cell signalling.

Key words: Na^+ channel; Cytoskeleton; Actin; Gelsolin; Ca regulation; Leukemia cell

1. Introduction

The actin-based cytoskeleton is involved in the control of ion channel activity in the plasma membrane of different cell types [1–4]. The rearrangements of cytoskeleton were shown to be Ca-dependent [5]. Dynamic assembly and disassembly of the F-actin network is affected by a variety of actin-binding proteins [6], particularly the Ca-sensitive actin-severing protein gelsolin [7,8]. Studying the regulation of non-voltage-gated amiloride-insensitive Na-selective channels in leukemia cells, we have recently shown that cytochalasin D-induced F-actin disruption [9] resulted in a significant increase in P_o values [4]. Here we elucidated Na^+ channels modified by exogenous gelsolin in excised membrane fragments. Furthermore, a combination of cell-attached and inside-out mode experiments was employed to study the effect of Ca^{2+} elevation in intact cells. The main outcome of the present work is that ion channel activation induced by a rise of $[Ca^{2+}]_i$ in native cells could be mediated by rearrangements of submembranous actin cytoskeleton.

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Abbreviations: P_o, probability for single channel being open; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; EGTA, ethylene glycol-bis(β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetic acid; HEDTA, *N*-hydroxyethylethylenediaminetriacetic acid

2. Materials and methods

Experiments were performed on human myeloid leukemia K562 cells (Cell Culture Collection, Institute of Cytology, Russia) at 22–24°C. The cells were grown as described earlier [4]. Single-channel currents were recorded using cell-attached and inside-out configurations essentially as described in [10]. Channel activity was estimated as P_o using the following equation: $P_o = I/iN$, where I is the mean current determined from the amplitude histograms, i is the unitary current amplitude and N is the number of functional channels in the patch. Averaged data are given as the mean ± S.E.M. (number of experiments).

Recording pipettes were filled with normal external solution containing (in mM) 145 NaCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES/TrisOH (pH 7.3). The bath solution for cell-attached measurements contained (in mM) 145 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES/TrisOH (pH 7.3). The cytosol-like solution for inside-out experiments contained (in mM) 10 KCl, 63 K_2SO_4 (or 140 KCl), 10 HEPES-KOH (pH 7.3), 1 $MgCl_2$, 2 EGTA (or HEDTA) and different $[Ca^{2+}]_i$ varied in the range from 0.01 to 1 μM. Pig stomach gelsolin was isolated as described [11], precipitated with ammonium sulfate and stored at −70°C. G-actin isolated from rabbit skeletal muscle [12] was stored in a low ionic strength solution (2 mM Tris-HCl, pH 7.5, 0.2 mM $CaCl_2$, 0.5 mM ATP) within a week.

3. Results and discussion

Single-current recordings revealed a low background activity of Na^+ channels measured as rare inwardly directed unitary currents in excised patches on leukemia cells (Fig. 1a). Variations in $[Ca^{2+}]_i$ in the bath cytosol-like solution did not affect open probability (P_o) of Na^+ channels in membrane fragments (Fig. 1b). Fig. 1d shows that addition of exogenous gelsolin to the bath solution containing 1 μM $[Ca^{2+}]_i$ caused a drastic increase in sodium channel activity in inside-out patches. Single-channel conductance measured prior to and after gelsolin-induced activation was about 12 pS (Fig. 3), that is similar to the value reported previously for non-voltage-gated Na-permeable channels in leukemic cells [4]. Addition of gelsolin at 0.01 μM $[Ca^{2+}]_i$ had no effect on the Na^+ channels (Fig. 1c). Thus, the gelsolin-induced channel activation is Ca-dependent suggesting that this effect may be due to the specific severing by gelsolin of actin filaments associated with the membrane fragment. Upon application of gelsolin, P_o values reached maximum without any measurable delay. The increased level of channel activity remained after washing out of gelsolin and replacing the bath with Ca^{2+}-free solution. In contrast, addition of G-actin to the bath solution reduced the channel activity to the background level (Fig. 1e). This seems to be due to the polymerization of actin induced by a physiological concentration of salts in the bath solution. These results suggest that disassembly of the submembranous actin cytoskeleton could evoke Na^+ channel activation in leu-

PII S0014-5793(97)00754-0

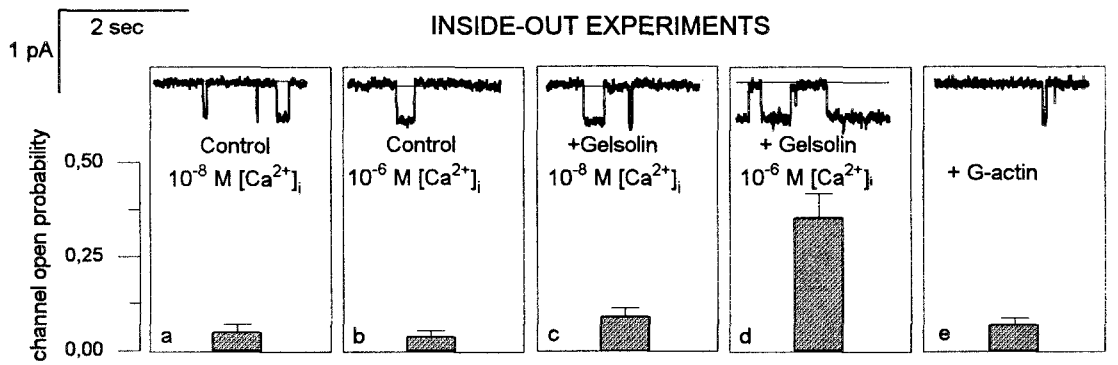

Fig. 1. The effect of exogenous gelsolin and actin on Na^+ channel activity in excised membrane patches on K562 cells. Inside-out current records (traces above, holding potential −30 mV, filter 50 Hz) and corresponding mean values of P_o (plots below) show the activity of Na^+-selective channels in controls at different levels of $[Ca^{2+}]_i$ ($n = 14$), after the application of 25 μg/ml gelsolin ($n = 14$) and after the subsequent addition of 300 μg/ml G-actin ($n = 4$) to the cytosol-like solution.

kemic cells while actin assembly causes inactivation of these channels.

Taking into account that in macrophages and platelets gelsolin is shown to localize near the plasma membrane being bound to actin filaments [13,14], we can assume that variations in $[Ca^{2+}]_i$ in native cells may affect channel activity through a mechanism involving modifications of actin filaments by the cytoplasmic gelsolin. To test this hypothesis under physiological conditions, we investigated the effect of the Ca^{2+} ionophore 4Br-A23187 [15] on ionic currents in plasma membrane using the cell-attached mode of the patch-clamp technique. Indeed, the elevation of cytosolic $[Ca^{2+}]_i$ by the ionophore induced the typical activity of Na^+ channels in cell-attached patches on leukemic K562 cells (Fig. 2). Fig. 3 shows that current–voltage relations were the same in controls, after gelsolin-induced channel activation in excised fragments and after activation caused by $[Ca^{2+}]_i$ elevation in native cells; unitary conductance was 12 pS. Upon ionophore application, inward Na^+ currents appeared with a delay of 60 ± 22 s ($n = 12$) suggesting that some intermediate process is involved in Na^+ channel activation. Channel activity of high P_o remained after patch excision and was not affected by lowering $[Ca^{2+}]_i$ in the bath solution (Fig. 2). Similarly to the experiments with the exogenous gelsolin (Fig. 1), addition of G-actin to the patches excised from the ionophore-treated cells reduced channel activity to the original values (Fig. 2) with a very fast reaction kinetics. These data allow us to assume that actin polymerization at the cytoplasmic surface of the membrane fragment may abolish the Na^+ channel activity induced previously by elevation of $[Ca^{2+}]_i$ in intact cells. Disassembly of membrane-associated actin filaments caused by cytoplasmic actin-modulating Ca-sensitive protein, such as endogenous gelsolin, is the most probable intracellular process mediating the Na^+ channel activation in response to the Ca^{2+} increase in K562 cells.

Gelsolin is known to bind phosphatidylinositol lipids [5]. Therefore, a direct effect of gelsolin on membrane lipid bilayer cannot be excluded. However, the involvement of actin filaments in channel function and the structural link of cytoskeleton with channel subunits was established for several types of ion channels [1–4,16]. Specifically, the Na^+ channels were shown to be activated upon treatment by cytochalasin D and DNase I [1,4]. Together with these data, our results suggest that actin cytoskeleton may play a regulatory role in the channel activity. It is plausible that in vivo variations in $[Ca^{2+}]_i$ may affect ion channel properties in the plasma membrane through F-actin rearrangements. Our results imply that submembranous cytoskeleton modifications controlled by actin-binding proteins – in particular, membrane-associated gelsolin – may provide an important mechanism of ion channel regulation. Cytoplasmic gelsolin is a target for intracellular (cytosolic or membrane-associated) effectors like Ca^{2+},

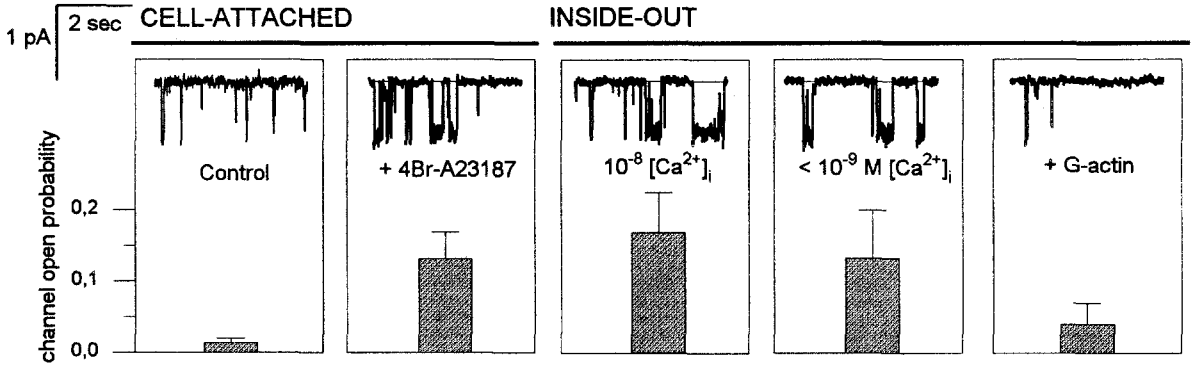

Fig. 2. Elevation of $[Ca^{2+}]_i$ in intact cells results in Na^+ channel activation through a mechanism involving modifications of microfilaments. Cell-attached and inside-out records (traces above, membrane potential −50 mV, filter 100 Hz) and corresponding mean P_o (plots below) show the effect of extracellular application of 10 μM 4Br-A23187 on Na^+ channel activity in intact cells ($n = 12$) and then, after patch excision, the effect of lowering $[Ca^{2+}]_i$ ($n = 5$) and of actin addition ($n = 3$).

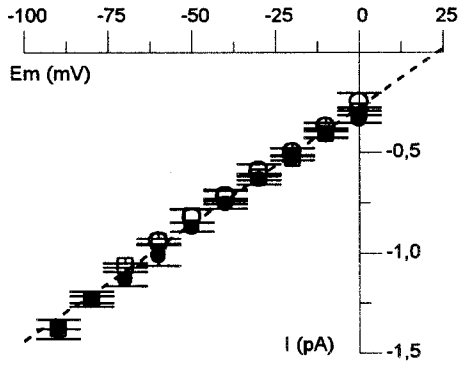

Fig. 3. Mean current–voltage relation curves of Na^+ channels obtained in controls (filled circles, $n=5$), after activation by gelsolin (squares, $n=14$) and by ionophore 4Br-A23187 (hollow circles, $n=12$).

phosphoinositides or GTP-binding proteins which are important elements of the intracellular signal transduction pathways [5,17] and are involved in the modulation of gelsolin–actin interactions [7,18]. The effects of these messengers and signal proteins on ion channels could be mediated through gelsolin-induced F-actin rearrangements.

Acknowledgements: This work was supported by the Russian Basic Research Foundation, Grants 95-04-11420a, 96-15-97653 (to A.V.M., E.A.V., S.Y.K. and Y.A.N.) and by the Deutsche Forschungsgemeinschaft, SFB223 (to H.H.).

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