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Amiloride-insensitive sodium channels are directly regulated by actin cytoskeleton dynamics in human lymphoma cells

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ABSTRACT

Sodium influx mediated by ion channels of plasma membrane underlies fundamental physiological processes in cells of blood origin. However, little is known about the single channel activity and regulatory mechanisms of sodium-specific channels in native cells. In the present work, we used different modes of patch clamp technique to examine ion channels involved in Na-transporting pathway in U937 human lymphoma cells. The activity of native non-voltage-gated sodium (NVGS) channels with unitary conductance of 10 pS was revealed in cell-attached, inside-out and whole-cell configurations. NVGS channel activity is directly controlled by submembranous actin cytoskeleton. Specifically, an activation of sodium channels in U937 cells in response to microfilament disassembly was demonstrated on single-channel and integral current level. Inside-out experiments showed that filament assembly on cytoplasmic membrane surface caused fast inactivation of the channels. Biophysical characteristics of NVGS channels in U937 cells were similar to that of epithelial sodium channels (ENaCs). However, we found that amiloride, a known inhibitor of DEG/ENaC, did not block NVGS channels in U937 cells. Whole-cell current measurements revealed no amiloride-sensitive component of membrane current. Our data show that cortical actin structures represent the main factor that controls the activity of amiloride-insensitive ENaC-like channels in human lymphoma cells.

1. Introduction

Sodium transport through ion channels in plasma membrane plays an important role in water-salt homeostasis, cell volume regulation and modulations of membrane potential in living cells. Specific changes in membrane permeability for monovalent ions underlie fundamental physiological processes in cells of blood origin and may be coupled with a variety of clinical pathologies [1]. In recent years, leukemia-lymphoma cell lines are extensively used as appropriate cellular models to study the role of ion-transporting systems in hematologic malignancies. Particularly, systemic analysis of monovalent ion fluxes and water balance in U937 lymphoma cells postulated specific modulations of integral channel permeability for Na^+ implicated in apoptotic water-salt cellular regulations [2]. An important question arises about the functional expression and regulation of sodium-specific channels underlying physiological reactions in human lymphoma cells.

To date, various physiological aspects and molecular basis of sodium transport in apical membranes of renal epithelia are well-established [3,4]. Particularly, epithelial Na^+ channels (ENaCs) represents the rate-limiting step for Na^+ reabsorption in the distal nephron, lungs, and colon [5–7]. In other non-excitable cells, including cells of blood origin, the membrane pathways providing selective sodium influx are much less understood. Electrophysiological studies revealed the activity of NVSG channels in myeloid blood cells, particularly in primary macrophages [8–10]. ENaC transcripts have been previously detected in human leukemic cell lines including proerythroblastic TF1, erythroblastic leukemia HEL and myeloblastic U937 [11,12]. Taken together, these results allowed us to speculate that ENaC channels may be involved in

Abbreviations: CytD, cytochalasin D; DEG/ENaC, degenerin/epithelial Na^+ channel; $NMDG^+$, *N*-methyl-D-glucamine$^+$; NVGS channel, non-voltage-gated sodium channel; P_o, channel open probability.

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sodium-transporting pathway in monocytic blood cells and their transformed counterparts. In the same time, the single channel behavior and regulation of sodium channels in human leukemia-lymphoma cells remains largely unknown. In frame of functional approach, promising data on native channel fingerprint may be obtained with the use of patch clamp technique.

One of the most important questions is to reveal physiological pathways that modulate non-voltage-gated sodium channel activity in the plasma membrane [7]. In epithelia, an involvement of cytoskeleton elements and some actin-binding proteins in the control of ENaC functions have been repeatedly documented [13–15]. However, molecular mechanisms that couple channel activity with submembranous microfilaments are still not understood. Previously, we have employed human erythroleukemia K562 cell line to examine an involvement of membrane-cytoskeleton coupling in channel regulation and cellular mechanotransduction [16,17]. Convincing evidence indicated an essential role of actin dynamics in the activation of sodium-selective channels in K562 cells which display properties of multipotent blood cell precursor [10,18,19]. The present study was designed to examine functional expression and actin-dependent gating of Na-specific channels in U937 lymphoma cells [20,21]. Our data show that sodium influx through amiloride-insensitive sodium channels in plasma membrane of U937 cells is directly controlled by the dynamics of submembranous actin structures.

2. Materials and methods

2.1. Cell culture

The human histiocytic lymphoma cell line U937 (Russian Cell Culture Collection, Institute of Cytology, St.Petersburg, Russia) were grown in RPMI-1640 medium containing 10% fetal bovine serum (Biolot, Russia) and 40 μg/ml gentamycin at 37 °C and 5% CO_2. For patch clamp measurements the cells were seeded on coverslips, coated with poly-DL-lysine (Sigma, USA).

2.2. Electrophysiology

Cell-attached, inside-out and whole-cell modes of the patch clamp technique [22] were employed to examine functional properties of native channels in plasma membrane. The patch clamp setup was based on HEKA EPC-8 (HEKA, Germany) amplifier and Zeiss Axiovert 40 CFL inverted microscope (Carl Zeiss Micro-Imaging, Germany). Pipettes were pulled from borosilicate glass capillaries (BF-150-110-10) at a P-97 puller (Sutter Instrument, USA). Resistance of the pipettes filled with standard solution was in range of 3–5 MOhm in whole-cell mode and 5–12 MOhm in other configurations. Ion currents through single channels were recorded at various levels of holding membrane potential. Whole-cell ramp currents were elicited by 1000-ms voltage pulses (from +20 to −80 mV) from a holding potential of +20 mV. For leakage current substraction in whole-cell records we use equimolar substitution of Na^+ with impermeable $NMDG^+$ cations in external solution. The membrane voltage was determined as the potential of the intracellular membrane side minus the potential of the extracellular one.

Channel open probability (P_o) was determined using 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. Data are presented as mean ± SEM (n – number of experiments) and were compared using standard paired Student's t-test, $p < 0.005$ was considered significant.

2.3. Solutions

The standard external solution contained (mM): 145 NaCl, 2 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES/TrisOH. Pipettes were filled with the external solution in cell-attached and inside-out experiments. Potassium bath solution containing (mM) 140 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES/KOH was used in cell-attached recordings to nullify the resting membrane potential. Cytosol-like solution (contacting with the intracellular membrane side) contained (mM) 140 K-Aspartate, 5 NaCl, 2 EGTA/KOH, 1 $MgCl_2$, 20 HEPES/TrisOH and the corresponding amount of $CaCl_2$ (0.176 mM) to establish free calcium concentration at the level of 0.01 mkM (pCa 8). pH of all solutions was set at 7.3. The purified G-actin isolated from the rabbit skeletal muscle [22] was stored in the low ion strength solution (2 mM Tris–HCl, 0.1 mM $CaCl_2$, 0.2 mM ATP, 0.02% NaN_3, pH 7.5) and used for 5 days. An aliquot of G-actin stock solution was added to the bath to the final concentration of 300 μg/ml.

3. Results

3.1. Activity of sodium-selective channels in intact cells and excised membrane fragments

To define single channel properties in intact cells we performed cell-attached patch clamp experiments with sodium (145 mM Na^+-containing solution) as the main cation in the patch pipette. In 6 of 23 of stable patches we observed inwardly directed currents representing the activity of the non-voltage-gated sodium (NVGS) channels in human lymphoma U937 cells. Fig. 1 demonstrates an example of background activity of NVGS channels at various holding membrane potentials. Mean current–voltage relationship corresponds to the unitary conductance of 10.4 ± 0.7 pS and indicates preferential permeability for sodium. Further, sodium channels with identical characteristics were recorded in inside-out experiments on U937 cells (10.6 ± 1.3 pS, n = 5). Sodium-selective channels of similar conductance (10–12 pS) were described earlier in rat macrophages and K562 leukemia cells [8–10].

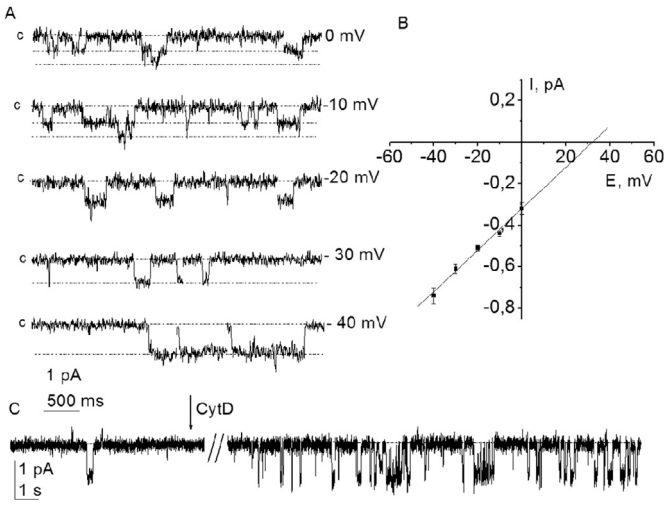

Fig. 1. The activity of non-voltage-gated sodium (NVGS) channels in plasma membrane of U937 lymphoma cells. (A) Cell-attached current records at different membrane potentials (indicated near the traces), c – closed state. (B) Mean current–voltage relationship of sodium channels calculated from 6 experiments. Unitary conductance is 10.4 ± 0.7 pS, reversal potential – +34.5 ± 4.9 mV. (C) An increase of channel activity in response to actin disrupter CytD (10 μg/ml) added to the bath solution; currents recorded at −40 mV.

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