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Functional coupling of ion channels in cellular mechanotransduction

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ABSTRACT

The major players in the processes of cellular mechanotransduction are considered to be mechanosensitive (MS) or mechano-gated ion channels. Non-selective Ca^{2+}-permeable channels, whose activity is directly controlled by membrane stretch (stretch-activated channels, SACs) are ubiquitously present in mammalian cells of different origin. Ca^{2+} entry mediated by SACs presumably has a significant impact on various Ca^{2+}-dependent intracellular and membrane processes. It was proposed that SACs could play a crucial role in the different cellular reactions and pathologies, including oncotransformation, increased metastatic activity and invasion of malignant cells. In the present work, coupling of ion channels in transformed fibroblasts in course of stretch activation was explored with the use of patch-clamp technique. The combination of cell-attached and inside-out single-current experiments showed that Ca^{2+} influx via SACs triggered the activity of Ca^{2+}-sensitive K^+ channels indicating functional compartmentalization of different channel types in plasma membrane. Importantly, the analysis of single channel behavior demonstrated that K^+ currents could be activated by the rise of intracellular calcium but displayed no direct mechanosensitivity. Taken together, our data imply that local changes in Ca^{2+} concentration due to SAC activity may provide a functional link between various Ca^{2+}-dependent molecules in the processes of cellular mechanotransduction.

1. Introduction

Mechanical forces play a crucial role in the life of cells, tissues and whole organisms. Changes in the mechanical properties of the extracellular microenvironment affect a variety of important cellular processes including proliferation, growth, differentiation, gene expression, migration and signal transduction [1,2]. The ability of cells and organisms to sense mechanical stimuli is thought to be one of the oldest signaling systems in the living world. The interest is growing towards studying of cellular mechanotransduction since numerous pathological processes are tightly linked with the changes in cell mechanics [3,4].

The major players in the processes of cellular mechanotransduction are considered to be mechanosensitive (MS) or mechano-gated ion channels, whose activation and inactivation are controlled by mechanical status of the cell. In the last decades MS channels were found in the plasma membranes of different organisms, from bacteria to mammals [5,6]. The expression and functioning of mechanically gated membrane channel proteins is likely a universal property of the living cell. In eukaryotes, these channels are known to be implicated in generation of receptor potential in specialized mechanosensors. In other excitable and various non-excitable cells, physiological contribution of MS channels in cell signaling is much less understood. Ionic mechanisms mediating mechanotransduction and regulating various aspects of cell movement are generally assumed to be linked with Ca^{2+} influx via MS channels [7,8].

Non-selective Ca^{2+}-permeable channels, whose activity is directly controlled by plasma membrane stretch (stretch-activated channels, SACs) are the most widespread type of MS channels in eukaryotic cells. It was proposed that SACs could play an important role in the different cellular processes and pathologies, including oncotransformation, increased metastatic activity and invasion of malignant cells [9]. Ca^{2+} entry mediated by SACs presumably has a significant impact on different Ca^{2+}-dependent intracellular and membrane processes, particularly, on stress-sensitive reactions in fibroblasts [8,10]. However, direct evidence for the participation of SACs in Ca^{2+}-dependent cellular responses is substantially lacking. Here, we show for the first time that Ca^{2+} influx via SACs trigger the activity of Ca^{2+}-sensitive K^+ channels indicating functional compartmentalization of different channel types in plasma membrane. The data imply that local changes in Ca^{2+} concentration

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due to SAC activity may provide a functional link between various Ca^{2+}-dependent molecules in the processes of cellular mechanotransduction.

2. Materials and methods

2.1. Cells

We used cultured mouse fibroblasts 3T3B-SV40 (Russian Cell Culture Collection, St. Petersburg, Russia) as a model to study membrane mechanisms of mechanotransduction in transformed cells. The cells were grown on coverslips to 70–80% confluency in DMEM medium containing 10% fetal bovine serum and antibiotics in 5% CO_2 at 37 °C.

2.2. Electrophysiology

Single channel currents were recorded using "cell-attached" and "inside-out" mode of patch clamp method essentially as described earlier [11,12]. Experiments were performed with HEKA EPC-8 operational amplifier at room temperature (21–23 °C). Pipettes were pulled from borosilicate glass capillaries (BF-150-110-10, Sutter Instruments) to a resistance 7–10 MOhm when filled with normal external solution containing (in mM): 145 NaCl, 2 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES/TrisOH. To nullify resting membrane potential in cell-attached recordings, we used K^+-containing bath solution (in mM): 145 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES/TrisOH. pH of all solutions was set at 7.3. "Cytosol-like" solution for inside-out experiments contained (in mM): 140 K-Aspartate, 5 NaCl, 2 EGTA/KOH, 1 $MgCl_2$, 20 HEPES/TrisOH and appropriate quantity of $CaCl_2$ to establish the level of free ionized Ca^{2+} ($[Ca^{2+}]_i$) at 0.01 μM (pCa = 8) or 0.1 μM (pCa = 7). To analyze stretch-induced channel activation, we used standard method of mechanical stimulation as application of "negative" pressure to a patch pipette. Data are presented as mean ± S.E.M. (n – number of experiments).

3. Results and discussion

Cell-attached patch-clamp experiments were performed to characterize stretch-induced activation of native channels in plasma membrane of transformed fibroblasts (Fig. 1). Typical stretch-activated currents in response to "negative" pressure (suction) were recorded in 75% of stable cell-attached patches (n = 30). The sufficient level of stimulus needed to stimulate the activity of SACs was about 45–55 mmHg. Unitary currents were recorded in the range from −60 to +40 mV; reversion was close to zero or slightly shifted to positive potentials. I–V relationship displayed no rectification and corresponded to single-channel conductance of 24.4 ± 1.4 pS. Stretch-induced channel activity with similar characteristics could be recorded in excised patches; the substitution of anions in cytosol-like solution confirmed cationic nature of mechanically-gated currents. The data obtained provide the first single current description of typical native SACs in transformed fibroblasts of mammals under quasi-physiological ionic conditions (with 2 mM Ca^{2+} in pipette). SAC currents (conductance of 58 pS at 140 mM KCl without Ca^{2+}) in cultured human skin fibroblasts were recorded earlier [13]. Mechano-gated currents in rat atrial fibroblasts were governed by cell deformation but their gating was reported to be clearly distinct from SACs [14]. The presence of SACs with unitary conductance about 55 pS in Ca^{2+}-free pipette solution has been previously demonstrated in migrating fish fibroblast-like epithelial keratocytes which respond to mechanical forces generating cytoplasmic Ca^{2+} transients [7].

Single-channel patch-clamp recordings allowed us to study membrane mechanisms of ion channel coupling in the processes of mechanotransduction. Specifically, we observed stretch-induced SAC activity followed by subsequent activation of outward K^+ currents. This effect was found in 35% of stable patches with SAC activity. K^+ channels were characterized by typical unitary conductance of 8.6 ± 0.6 pS and extrapolated reversal potential about −80 mV (Fig. 2A). Importantly, K^+ currents displayed no direct mechanosensitivity and could not be evoked by mechanical stimulus independently of SACs. In a few patches (2 out of 30) we observed background activity of K^+ channels with same unitary conductance (Fig. 2B) that was also not sensitive to stretch. The analysis of single-channel behavior in all patches with coupled activation showed that outward K^+ currents always followed inward SAC currents indicating functional link between SAC-mediated cation entry and subsequent K^+ channel activity.

We proposed that local Ca^{2+} entry via SACs is the putative mechanism of functional coupling between two channel types in the plasma membrane. SAC openings are likely to provide a pathway for miserable calcium influx into the cell thus affecting calcium-dependent molecules, particularly co-localized Ca^{2+}-activated K^+ channels (K_{Ca}). K_{Ca} are well-studied and were found in many cells and tissues of different origin, including mouse fibroblasts [15]. To reveal possible Ca^{2+}-dependent interplay between SACs and K^+ channels we used the protocol combining cell-attached and subsequent inside-out patch configurations in the same experiment. Standard pipette solution contained 2 mM $CaCl_2$. In cell-attached mode we recorded the activation of SACs followed by the outward K^+ currents in the plasma membrane (Fig. 3A). Then, we gently pulled the patch pipette to form a cell-free

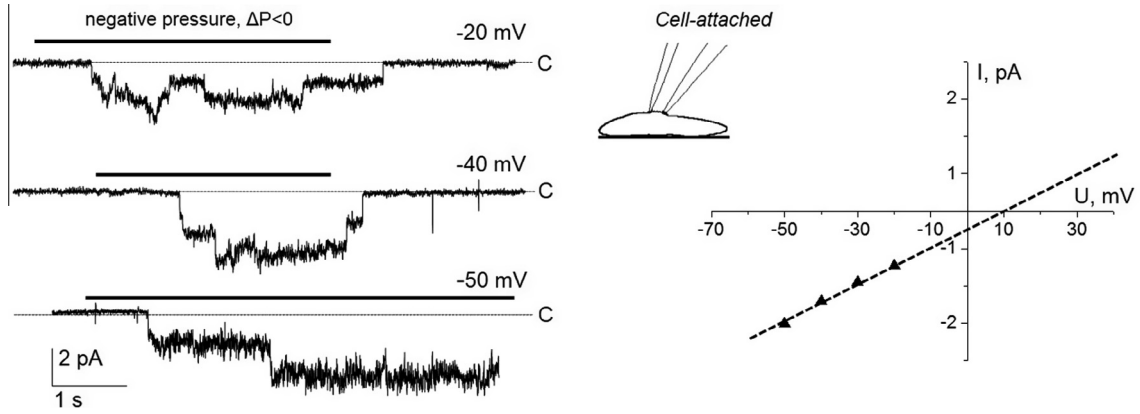

Fig. 1. Activation of native SAC channels in the plasma membrane of 3T3B-SV40 fibroblasts. Representative cell-attached current records and corresponding I–V relationship. Interval of applied "negative pressure" (suction) is marked by line above current traces. Holding membrane potentials are indicated near. Closed state is denoted by (C).

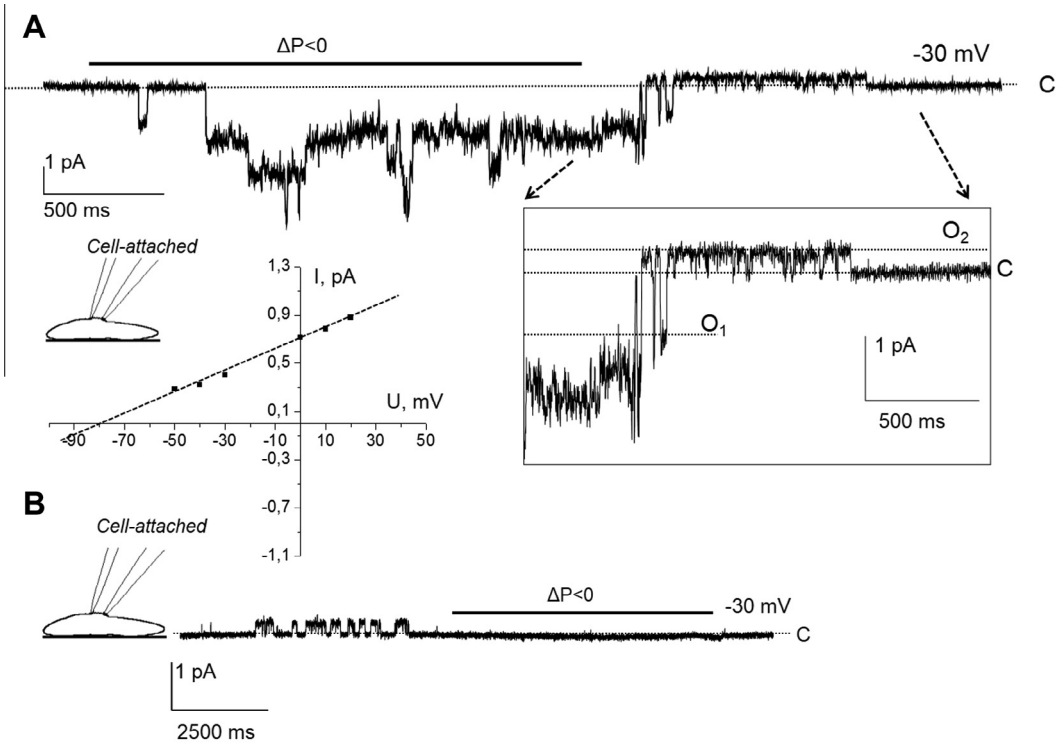

Fig. 2. Stretch-coupled activation of SACs followed by outward K^+ currents. (A) Representative cell-attached records at −30 mV, C – closed state, O_1 and O_2 – openings of SACs and K^+ channels, respectively. Typical *I–V* relationship of K^+ channels is shown below. (B) K^+ channels are not directly gated by membrane stretch.

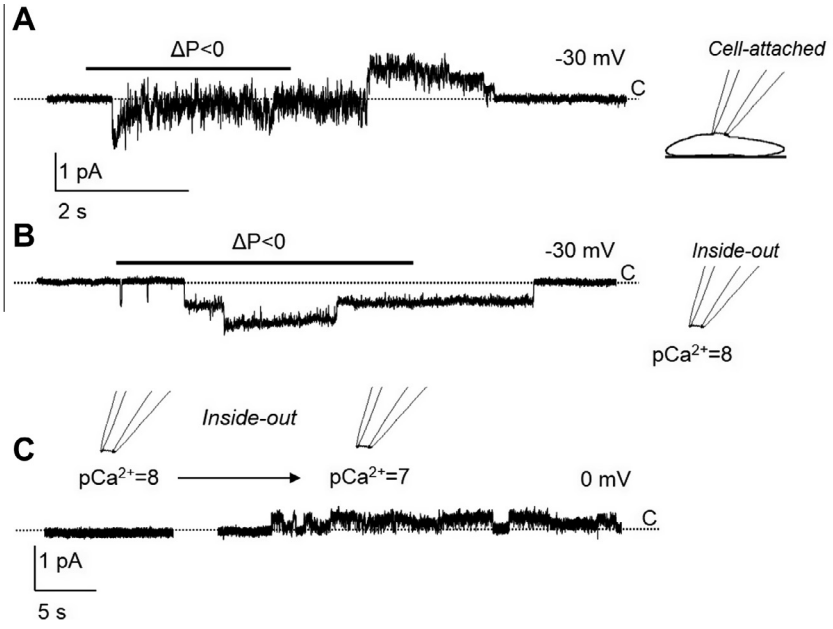

Fig. 3. Coupled activation of SACs and K^+ currents is abolished after formation of cell-free inside-out patch. Shown are representative traces in cell-attached (A) and inside-out (B) modes from the same experiment. Internal Ca^{2+} concentration was set at $[Ca^{2+}]_i = 10^{-8}$ M (pCa = 8). (C) K^+ currents could be activated by the rise of $[Ca^{2+}]_i$ to 10^{-7} M (pCa = 7).

inside-out membrane fragment exposed to cytosol-like low-Ca^{2+} solution buffered with EGTA ($[Ca^{2+}]_i = 10^{-8}$ M, pCa = 8). Under these conditions, the coupling between two different channel types was abolished. Specifically, only SAC activity without subsequent activation of K^+ currents was observed in response to stretch (Fig. 3B). These observations indicate that K^+ channels are activated by Ca^{2+} influx via co-localized SACs in plasma membrane of native cell. After patch excising, calcium chelator EGTA efficiently buffers SAC-mediated local rise in $[Ca^{2+}]_i$ thus preventing co-localized Ca^{2+}-dependent K^+ channel activation. At the same time K^+ currents could be activated by the rise of $[Ca^{2+}]_i$ thus demonstrating Ca^{2+} sensitivity of the channels (Fig. 3C). Particularly, the elevation of Ca^{2+} concentration from 0.01 μM (pCa = 8) to 0.1 μM (pCa = 7) induced K^+ channel activity. Based on single-channel properties

one can assume that the possible molecular correlate of Ca^{2+}-activated K^+ channels that are indirectly involved in SAC-dependent mechanotransduction in transformed fibroblasts belong to small conductance K^+ channel (SK) family. SK channels are characterized by high sensitivity to Ca^{2+} with IC_{50} starting from 0.07 μM [16]. As it was reported earlier, the appearance of Ca^{2+} activated K^+ currents correlated with transformation of mouse fibroblasts with Src or Ras oncogene [17,18]. The functional link between SACs and Ca^{2+}-activated K^+ channels may contribute into increased metastatic activity, migration and invasive potential of malignant cells.

In several studies the activation of Ca^{2+}-dependent K^+ currents was reported to be coupled with spatially co-localized Ca^{2+} sources including voltage-gated Ca-channels (Ca_v), ryanodine, NMDA, acetylcholine and IP_3 receptors [19]. K_{Ca} channels of different conductance (SK and BK) were proposed to participate in mechano-dependent cellular reactions in prostate cancer cells [20]. Here, we found that Ca^{2+} influx via native SACs induced the activity of Ca^{2+}-activated K^+ channels in plasma membrane of transformed fibroblasts. Moreover, our data unequivocally show that SACs provide physiologically relevant pathway for Ca^{2+} entry under natural ionic conditions. Ca^{2+} ions may serve as a functional link between SACs and different Ca^{2+}-dependent molecules thus orchestrating the interplay between various cellular reactions associated with processes of membrane mechanotransduction.

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