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Actin cytoskeleton disassembly affects conductive properties of stretch-activated cation channels in leukaemia cells

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Abstract

Mechanosensitive channels in various eucaryotic cells are thought to be functionally and structurally coupled to the cortical cytoskeleton. However, the results of electrophysiological studies are rather controversial and the functional impact of cytoskeleton assembly–disassembly on stretch-activated channel properties remains unclear. Here, the possible involvement of cytoskeletal elements in the regulation of stretch-activated Ca^{2+}-permeable channels was studied in human leukaemia K562 cells with the use of agents that selectively modify the actin or tubulin system. F-actin disassembly resulted in a considerable reduction of the amplitude of stretch-activated currents without significant change in channel open probability. The effects of treatments with cytochalasins or latrunculin were principally similar, developed gradually and consisted a strong decrease of single channel conductance. Microtubule disruption did not affect stretch-activated channels. The data presented here are in principal agreement with the general conclusion that mechanosensitive channel functions are largely dependent on the integrity of the cortical actin cytoskeleton. Specifically, changes in conductive properties of the pore may provide an essential mechanism of channel regulation underlying functional modulation of membrane currents. Our results allow one to speculate that microfilament organization may be an important determinant in modulating biophysical characteristics of stretch-activated cation channels in cells of blood origin.

Keywords: Stretch-activated channel; Actin cytoskeleton; Cytochalasin; Latrunculin

1. Introduction

All cells, whether individually or in tissue, experience and respond to intracellular and extracellular mechanical stimuli. It is generally believed that mechanotransduction may involve stretch-activated channels (SACs) in the plasma membrane and the cytoskeleton consisting of actin microfilaments, microtubules and intermediate filaments. Cortical cytoskeleton has long been known to play a fundamental role in the mediation of cell motility and shape changes [1]. Mechanosensitive (MS) channels in various eucaryotic cells are thought to be functionally and structurally coupled with cortical cytoskeleton [2,3]. Specific reports have documented that cytoskeleton disassembly increases the channel's sensitivity to stretch and thus promotes MS channel activation [4,5]. Accordingly, cytochalasins are often considered as channel activators (see Ref. [2]). Cytochalasin-induced increase of the activity of cation SACs in myotubes has been recently reported by Nakamura and coauthors [6]. Cytochalasin or colchicine treatments were shown to promote the mechanosensitive activation of TRAAK K^+ channel [7] and of large-conductance Ca^{2+}-activated K^+ channels in coronary artery smooth muscle cells [8]. Actin cytoskeleton depolymerization was proposed to activate stretch-sensitive K^+ channels involved in volume regulation in enterocytes [9]. It has been hypothesized that intact cytoskeleton prevent MS channel

Abbreviations: MS, Mechanosensitive; SAC, Stretch-activated channel; ENaC, Epithelial Na^+ channel; CytB or D, Cytochalasin B or D; LatB, Latrunculin B; P_o, Channel open probability

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activity [10]. However, the data are contradictory since some authors have shown inhibitory effects of cytochalasins and colchicine on MS channels [11–13]. An important role of intact cytoskeleton in the mechanisms of MS channel inactivation was suggested [14]. Moreover, several lines of evidence allow one to suggest intrinsic mechanisms underlying channel mechanosensitivity that is unlikely to be linked with submembranous cytoskeleton (see Refs. [2,15]).

Over the last decade, patch clamp studies together with fluorescence imaging data have revealed an apparent ubiquity of MS (or mechanogated) channels [2,15,16]. These data challenged two different views with regard to the functional significance of these channels in living cells. On the one hand, MS channels including SACs are thought to mediate a variety of functions in excitable and nonexcitable tissues. On the other hand, specific reports have questioned the reality of mechanogated channels as biological transducers and propose that such channels are artefacts of patch-clamp recording (see Ref. [17]). It has been suggested that the effect varies with ion channel and cell type and presumably arises because of disruption of membrane–cytoskeleton interactions. It is worth noting that functional coupling between Na^+ channels and actin cytoskeleton has been clarified in our previous studies on leukaemia K562 cells [18–21]. Patch-clamp data obtained in different configurations with the use of cytochalasin, gelsolin, capping protein and different forms of G-actin support the idea that microfilament organization was not essentially damaged in the course of single-current recordings on K562 cells. Besides, it is known that the cytoskeleton is mostly presented as a cortical layer in erythroleukaemia cells and erythrocytes. Therefore, K562 cells may provide a suitable model to search for possible involvement of cytoskeleton structures in the regulation of MS cation channels and mechanotransduction.

Actin filament disassembly has been previously shown to induce the activity of non-voltage-gated Na^+ channels in K562 cells [18–20]. However, these channels proved to be insensitive to mechanical stimulation. Later, cation SACs have been identified and characterized in plasma membrane of human leukaemia K562 cells [22]. The blocking actions of gadolinium and amiloride, ion selectivity and calcium permeability of these channels were examined. Mechanically gated currents were activated in response to application negative (but not positive) pressure implying possible involvement of membrane-associated structures. In the current work, a number of agents known as selective cytoskeleton disrupters were applied to assess the role of actin and tubulin system integrity in the regulations of SACs in K562 leukaemia cells. We found that drug-induced actin cytoskeleton disassembly caused a decrease of single currents and conductance of SACs implying that cortical microfilaments may be a key determinant in modulating channel functions. Importantly, the present data demonstrate that changes in conductive properties of the pore may contribute to physiological regulations of membrane currents in non-excitable cells. Our observations show that cytoskeleton disruption does not promote MS channel activation in myeloid cells.

2. Materials and methods

2.1. Cells

Human myeloid leukaemia K562 cells obtained from Cell Culture Collection (Institute of Cytology, St. Petersburg, Russia) were maintained in glass flasks in RPMI-1640 containing 10% fetal bovine serum and 80 μg/ml gentamycin at 37 °C. Cells were plated on coverslips (0.4 × 0.4 cm) 1–3 days before experiment.

2.2. Electrophysiology

We used patch clamp method in cell-attached and inside-out configurations [23]. Pipettes were pulled from soft glass capillaries to a resistance 7–14 MΩ when filled with normal external solution. The recordings were performed at room temperature (21–23 °C) on the stage of inverted microscope with Nomarsky optics (magnification of × 256). Single channel currents were measured essentially as described earlier [21,22]. Membrane voltage was the potential of the intracellular membrane side minus the potential of the extracellular one. Unless otherwise stated, data were filtered at 200 Hz and sampled at a rate of 1 kHz by 12-bit ADC for analysis and display.

2.3. Mechanical stimulation

We used well-known method of mechanical stimulation as a pressure applied to a patch pipette [4]. A “gentle” seal was essential for forming of patch as well as for application of stimulus to activate SACs. The pipette interior was connected to a manometer with a valve to allow either application of negative (positive) pressure or equilibration to atmospheric pressure. Mechanically-gated ion channels were activated in response to the negative pressure application. Pressure range was 10–20 mm Hg. For correct comparison of channel properties, the same pressure value was applied during control recording and then after the treatment with cytoskeleton-modifying drugs. We tried to use the low level of pressure to minimize possible injury. The suction used during experiments was usually applied for less than 10 s.

2.4. Statistics

All data are presented as means ± S.E. Paired and unpaired data were compared using appropriate t tests. Single channel unitary current (i) was determined from the best-fit Gaussian distribution of amplitude histograms. Channel activity (NP_o) was $NP_o = I/i$, where I is the mean

total current in the patch and i is unitary current at this voltage. By definition then, current at the closed state is 0. Where appropriate, open probability (P_o) was calculated by normalizing NP_o for the total number of estimated channels (N) in the patch.

2.5. Solutions

The bath solution for cell-attached measurements contained (in mM): 145 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES/KOH. Patch pipettes were filled with normal external solution containing (in mM): 145 NaCl, 2 $CaCl_2$, 1 MgCl2, 10 HEPES/TrisOH. pH of all solutions was set at 7.3. Cytochalasin D (CytD) or B (CytB) (20 μg/ml), latrunculin B (LatB, 20 μM), and nocodazol (20 μg/ml) were dissolved in DMSO and added to the bath as aliquots of stock solutions. Finally, test solutions contained 0.4–2% DMSO. Colchicine (500 μM) was dissolved in water–salt solution. Latrunculin B was obtained from Biomol. All other chemicals were obtained from Sigma.

3. Results

3.1. Cytochalasin treatment induced no increase in the activity of SACs in K562 cells

Our patch clamp experiments were designed to study the effect of the disruption of cytoskeleton structures on the activity and properties of SACs in plasma membrane of human leukaemia K562 cells. In control experiments mechanogated currents were observed in 55% ($n=479$) of stable cell-attached patches. Fig. 1A,B shows typical recordings from two experiments representing cell-attached patches with and without SAC activation. The first question to be addressed was whether actin disassembly results in any change of SAC activity and gating. Fig. 1A demonstrates that application of mechanical stimulus did not induce channel activity before and after treatment with CytD (20 μg/ml). The same effect was observed in all those cell-attached patches which displayed no mechanogated currents before treatments ($n=25$); CytD or CytB addition to the bath solution did not promote activation of SACs. The application of cytochalasins to the intracellular surface of excised inside-out membrane patches ($n=6$) induced no activation of SACs either. These results revealed that F-actin disruption did not induce or promote SAC activity in "silent" patches; or in other words, the integrity of cortical microfilaments did not prevent stretch activation in leukaemia cells. Fig. 1B shows typical recordings of SAC currents at membrane potential − 50 mV before and after application of CytD (20 μg/ml). Stretch-induced channel activation and gating behaviour did not significantly alter after treatments with cytochalasins. As seen from Fig. 1B and also from current traces in all other Figures, SACs in leukaemia cells were not characterized by inactivation or adaptation to

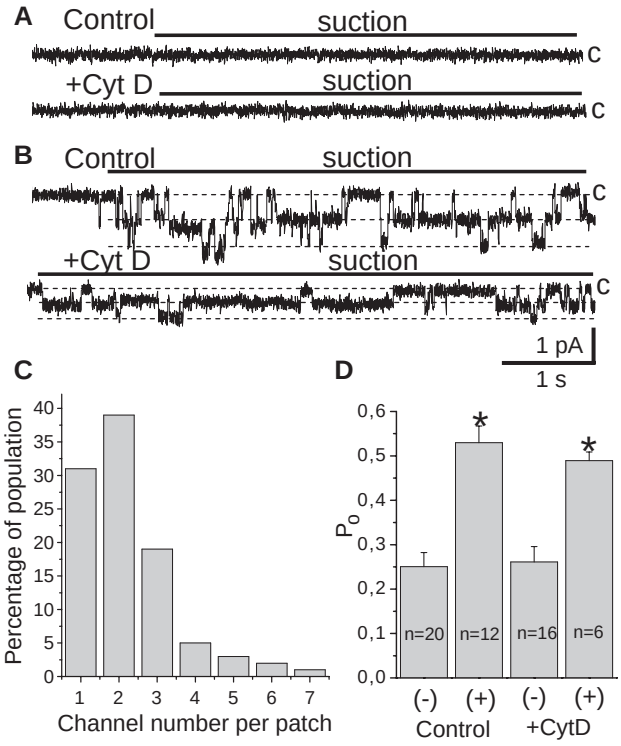

Fig. 1. Cytochalasin treatment does not alter the activity of stretch-activated channels in leukaemia K562 cells. Cell-attached recordings from two representative experiments display no SAC activity (**A**) or typical single channel activity (**B**) to applied mechanical stimulation (suction) before (control) and after application of CytD (20 μg/ml). Membrane potentials were − 50 mV; closed states are denoted by c. (**C**) The number of SACs per patch estimated as the maximum number of concurrently open channels; data collected from control patches. (**D**) A summary graph comparing channel open probability (P_o) in cells before (two left columns) and after (two right columns) CytD treatment at negative (−) and positive (+) potentials. The number of observations in each group is shown. *$P<0.05$ vs. experiments before and after CytD treatment at negative potentials.

stimulus. This is in agreement with the previous results [22], showing that mechanogated currents did not inactivate during prolonged (up to 300 s) stimulation.

We next tested whether disruption of actin network had effect on SAC activity by increasing the number of open channels in the plasma membrane and/or channel open probability (P_o). When the channels were present, the number of SACs was $2.20 \pm 0.12.9$ channels per patch. The distribution of channel number per patch in control experiments is shown in Fig. 1C. Treatment with cytochalasins did not influence this distribution for SACs. In CytD- or CytB-treated cells average of channels per patch was 2.05 ± 0.13. Fig. 1D summarizes P_o values at negative and positive potentials before and after treatment with CytD. Interestingly but not further pursued in the current study that the activity of SACs estimated as P_o displays voltage sensitivity: open probability at positive voltages was some higher than that at negative voltages (there was no essential monotonic dependence inside these ranges). Actin disrupting treatment had no effect on channel open probability and its voltage dependence. P_o values were 0.25 ± 0.03 and

0.53 ± 0.04 for negative and positive potentials in control patches and 0.26 ± 0.03 and 0.49 ± 0.02 for cells treated with CytD, correspondingly. These results allowed us to conclude that actin disassembly had no effect on activity and gating of SACs in K562 cells. We did not observe inactivation of SACs in control as well as after treatments of the cells; active channels displayed monotonic properties. It was shown that inactivation of MS channels in astrocytes required an intact cytoskeleton whereas cytoskeleton disruption led to a loss of inactivation [14]. The absence of inactivation of SACs in K562 cells was unlikely to be a result of an irreversible damage of cytoskeleton during patch formation. Earlier studies of Na^+ channel activity controlled by actin assembly–disassembly confirmed that cortical filaments were not disrupted in course of gentle suction, patch formation and excision in our experiments on K562 cells.

It is worth noting that an activation of epithelial-like Na^+-selective channels of 12 pS conductance was found in response to cytochalasin application in several experiments. This observation is in agreement with the previous data on K562 cells [18,20]. Na^+-channels were not responsive to mechanical stimulation by suction. Unlike SACs in K562 cells, Na^+-channels activated by actin disassembly have been shown to be insensitive to Gd^{3+}, a well-known inhibitor of MS channels. Besides, these results may be considered as an evidence supporting an idea that F-actin arrangement was not essentially damaged in our patch clamp measurements before selective treatments.

3.2. Actin cytoskeleton disassembly caused a significant decrease of SAC conductance

To search for the possible involvement of cortical cytoskeleton in control of intrinsic properties of single channels, the effects of selective F-actin disrupters were further tested in cell-attached experiments. CytB, CytD and LatB were applied on the patches displaying sufficient level of SAC activity (Figs. 1B and 2). Fig. 2 shows SAC currents induced by suction before (Fig. 2A) and after the CytD (20 μg/ml) addition to the bath solution (Fig. 2B). Representative recordings demonstrate that CytD application resulted in evident decrease of the amplitude of single channel currents. Fig. 2C shows all-points histograms before and after CytD. In the course of each experiment, mechanogated currents were recorded before and after drug application in response to the same level of negative pressure. It should be noted that no significant alteration of single channel properties (specifically, no decrease of the amplitude of currents) occurred in control patches without drug addition and in experiments when we added DMSO (2 v/v %) to the bath solution (data not shown).

Fig. 3A shows the development of CytB effect measured in typical cell-attached experiment. No difference in the action of CytD or CytB on mechanogated currents was

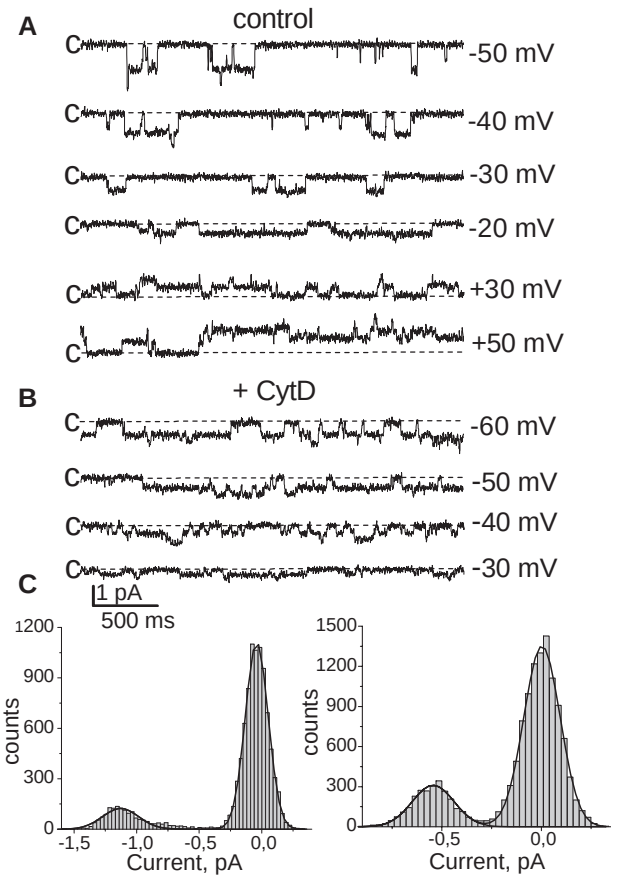

Fig. 2. The effect of cytochalasin D on single channel mechanogated currents in K562 cells. Representative cell-attached recordings show SAC currents before (**A**) and after (**B**) the addition of 20 μg/ml CytD to the extracellular solution. Membrane potentials are indicated near the traces; c—indicates the closed state. (**C**) All-points amplitude histograms constructed for current fragments recorded at −50 mV before (on the left) and after (on the right) CytD application. Populations fit with Gaussian curves.

observed. The reduction of currents was gradual; we have not found step transitions of current to the any lower level. A decrease of single channel currents and conductance in response to cytochalasin addition developed during 9–12 min and then amplitude parameters apparently reached steady state. As summarized in Fig. 3B, CytD (or CytB) significantly decreased conductance of single channel: from 17.1 ± 0.5 pS in control to 9.5 ± 0.6 pS ($n = 9$) after drug application. In a few experiments, cytochalasin addition was followed by the development of irreversible damage of electrically stable patch thus preventing single current recording. Notably, in all stable patches, we found that the addition of cytochalasins to the extracellular bath solution resulted in a considerable decrease of the unitary conductance of SACs in K562 cells. In the next experiments, cell-attached patches were formed after preliminary incubation of the cells with CytD or CytB (20 μg/ml). The incubation period varied from 35 to 105 min. After incubation of the cells with cytochalasins, SACs could be also activated by suction and they were characterized by lower level of

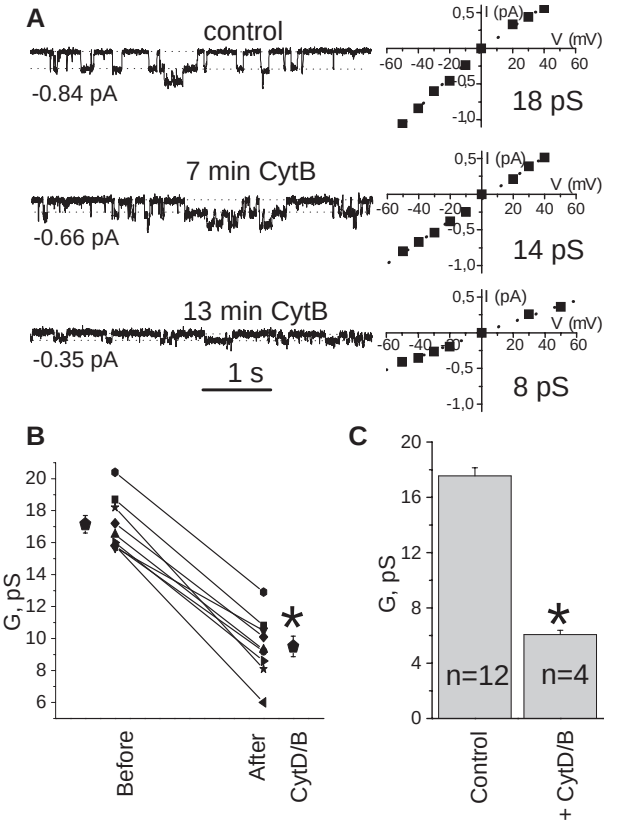

Fig. 3. Cytochalasin treatment resulted in a strong decrease of unitary conductance of stretch-activated channels. (**A**) SAC currents recorded at holding potential −40 mV (left) and corresponding I–V curves (right) in control (top), then in 7 min (middle) and in 13 min (bottom) after 20 μg/ml CytB application. Amplitudes of currents are shown near traces. The unitary conductance values were equal to 18, 14 and 8 pS, respectively. (**B**) Summary graph showing conductances in individual cells and as a population mean ± SE before and after treatment with CytD or Cyt B. *$P<0.05$ vs. control with a paired t-test. (**C**) Comparison of single channel conductances in control experiments and after preincubation with CytD/B. The number of observations in each group is shown. *$P<0.05$ vs control.

conductance. The final level of unitary conductance after treatment with CytD or CytB proved to be equal to 6.1 ± 0.3 pS ($n=4$) (Fig. 3C). This value is much lower than control one (17.1 ± 0.5) and rather similar to the "final" conductance level reached after cytochalasin addition to the bath in course of cell-attached recordings (Fig. 3B).

We have previously shown that SACs characterized by different conductance values displayed very similar gating properties and selective characteristics [22]. Two conductance levels of about 17 and 25 pS were predominant representing two major populations of channels. Data analysis gave no evidence that the multiple conductances represent transitions between different substates of the same channel. These observations were confirmed in the current study. In the most part of control patches, the channels of 17 pS were observed. Importantly, there was no difference in the effects of cytochalasins on SACs of higher (25 pS) and lower (17 pS) conductance (Figs. 2 and 3). Specifically, SAC activity of major conductance level is represented in Fig. 3A (18 pS). Fig. 2A shows mechanogated currents before cytochalasin addition (control) corresponding to the level of 24 pS. Notably, this experiment gave rather seldom example of the coexistence of two different conductances in the same patch under the control conditions: before CytD treatment, an infrequent openings corresponding to the lower conductance level (16 pS) were also recorded. In 8 min after CytD addition, SAC activity corresponded to the unitary conductance of 9.2 pS, any additional lower level could not be reliably determined.

Results in Fig. 4 further supported the idea that actin disruption caused a decrease of single currents and conductance of the SACs in K562 cells. Treatment of cells with 20 μM LatB resulted in a significant decrease in conductance of mechanogated currents when period of incubation was long enough (40 min or longer). Fig. 4A shows that after 25 min incubation with LatB, SAC activity corresponded to the 16 pS conductance that is similar to the major conductance level (17 pS). Following the longer incubation of the cells with LatB, SAC currents induced by suction in cell-attached experiments were characterized with the reduced conductance values (Fig. 4B,C). No significant alteration in activation parameters and kinetics of SACs in K562 cells was found after LatB treatment. P_o at negative potentials (0.30 ± 0.07 ($n=17$)) was not significantly different from correspondent value measured in control patches (see Figs. 1D and 5D). These data are consistent with our CytD and CytB findings.

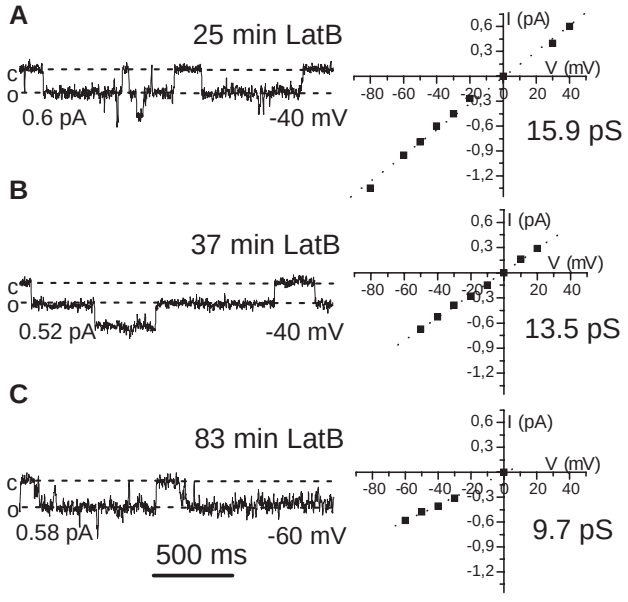

Fig. 4. The treatment of cells with latrunculin reduced the amplitude of single currents and unitary conductance of stretch-activated channels. Single channel activity induced by suction and corresponding I–V relationships measured in different patches after 25 (**A**), 37 (**B**) and 83 (**C**) min incubations with 20 μM latB; the unitary conductance values were equal to 15.9, 13.5 and 9.7 pS, respectively. Holding membrane potentials and the amplitudes of single opening are shown near traces.

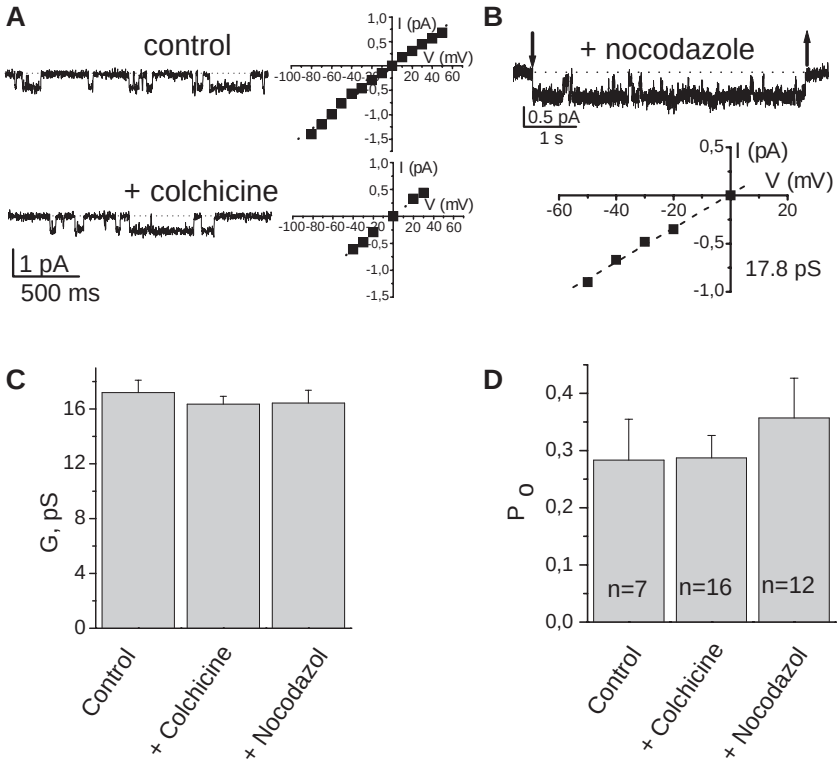

Fig. 5. Disruption of microtubule system does not affect properties of stretch-activated channels in K562 cells. (**A**) SAC currents in cell-attached patch before and after the addition of 500 μM colchicine to the bath solution. On the left—representative traces recorded at applied negative pressure at the membrane potential − 30 mV. On the right—I–V relationships measured on the same patch; the unitary conductance was equal to 15.8 and 15.3 pS before and after the treatment by colchicine, respectively. (**B**) Single channel currents recorded from cell-attached patch (top) and corresponding I–V relationship (bottom) after preincubation of cells with 20 μg/ml nocodazole. The application and removal of suction are indicated by arrows. Membrane potential was-30 mV. Unitary conductance was 17.8 pS. (**C**) Summary graph of unitary conductances in control and after treatment of the cells with colchicine and nocodazol. Data were the mean ± SE of five to seven experiments. (**D**) Summary of the effect of colchicine and nocodazol on channel open probability measured at negative voltages. The number of observations in each group is shown.

3.3. SAC properties were not affected by microtubule disassembly

To search for the effects of colchicine (500 μM) and nocodazol (20 μg/ml) on SAC properties in cell-attached experiments, two different protocols were used similarly to the testing F-actin disrupters. First, the drugs were added to the bath extracellular solution during cell-attached recording. This allowed us to analyze and to compare single channel characteristics on the same patch before and after cytoskeleton modification. Fig. 5A shows mechanogated currents and correspondent I–V curve before and after colchicine application in the representative experiment. The results of these experiments indicate that treatments of cells neither with colchicine ($n=7$) nor with nocodazol ($n=9$) alter the activity and properties of SACs in K562 cells. Second, to prolong time of treatment, cells were patched after the incubation with microtubule disrupters. Time of incubation varied from 10 to 140 min. Mechanogated currents and correspondent current–voltage relationships were not modified due to longer exposure of cells with colchicine or nocodazol. Shown in Fig. 5B are representative current trace and corresponding *I*/*V* relationship after prolonged treatment with nocodazol; single channel conductance was equal to 17.8 pS. The summary graph in Fig. 5C demonstrates that unitary conductances after long treatment with colchicine (16.4 ± 0.6 pS) or nocodazol (16.4.13 ± 0.9 pS) were not significantly different from control values (17.2 ± 0.9 pS). P_o also displayed no alteration after microtubule disruption. Fig. 5D summarizes P_o at negative potentials for control experiments (0.28 ± 0.07) and after treatments with colchicine (0.29 ± 0.03) or nocodazol (0.36 ± 0.07). Taken together, these data show that microtubule disruption caused no significant changes in the functional characteristics of SACs in K562 leukaemia cells.

4. Discussion

In the present work, a number of agents known as selective cytoskeleton disrupters were applied to assess the role of actin and tubulin system integrity in the functions of MS Ca^{2+}-permeable channels in cells of blood origin. In our previous findings, gadolinium-blockable cationic channels activated by stretch have been characterized in K562 human leukaemia cells [22]. The main outcome of the present study

is that drug-induced actin disruption caused a decrease of single currents and conductance of the SACs; this effect may provide an essential mechanism of channel regulation. In our experiments, the effects of F-actin destructors cytochalasins and latrunculin B were monitored on single channel level showing no significant alteration of channel kinetics and open probability. Obviously, no activation of SACs occurred in response to cytoskeleton disassembly in leukaemia cells unlike an enhancement of MS channel activity described earlier in several studies [4–8]. Among different mammalian tissues, cytochalasin-induced activation appeared to be more specific for SACs in muscle cell membranes. However, the data obtained for different cell types are rather controversial (see Refs. [2,15]). Particularly, stretch-activated currents were reported to be suppressed by cytoskeleton disruption in cultured sensory neurons [12] and in ventricular myocytes [13]. Whether cytoskeleton disrupters would activate or inhibit the channel activity may be due to different indirect mechanisms involved [11]. A variability of channel properties are often considered as a result of irreversible damage of intact cytoskeleton due to patch formation or excision [14,16]. Furthermore, different status of microfilament and microtubule systems in specialized native cells may play a dominant role in determination of the functional impact of drug-induced cytoskeleton disassembly. Our observations show that gating behaviour of SACs did not significantly alter due to cytoskeleton disassembly in K562 cells. The absence of inactivation of SACs in K562 leukaemia cells was unlikely to be a result of an irreversible damage of cytoskeleton during patch formation. The current study and earlier results obtained in different patch configurations on K562 cells support this opinion.

Earlier investigations of Na^+-selective channel regulation controlled by actin dynamics [18,20,21] allow us to consider K562 leukaemia cell line as an adequate experimental cell model to elucidate channel–cytoskeleton coupling. The data presented here are in principal agreement with the general conclusion that MS channel activity is dependent largely on the integrity of the cytoskeleton. Actin–spectrin submembranous layer is believed to constitute an essential part of cytoskeleton in a number of cells of blood origin, particularly in erythrocytes and erythroleukaemia K562 cells. Colchicine and nocodazole treatment displayed no effect on SACs whereas cytoplasmic microtubule system is expressed and actually involved in cycle regulations in K562 cells [24]. Cytochalasin B and D or latrunculin B treatments affected intrinsic conductive properties of SACs in K562 cells. Inside-out experiments revealed that Na^+ channel activity was directly controlled by actin dynamics [20,21]. One can speculate that the reduction of single currents and conductance of SACs may also represent primarily a direct effect of cortical F-actin rearrangement on membrane channels. Alternatively, the effects may be due to possible change in the mechanical status of the cell coupled with altered cytoskeleton integrity. An involvement of other intracellular pathways modulated by F-actin disruption also could not be excluded. An application of drugs, actin-binding protein and globular actin in inside-out experiments would be useful tools to search for intrinsic mechanism underlying changes in MS channel functions caused by actin disassembly in leukaemia cells. [20,21]. However, excised patches on K562 cells displayed no sufficient stability in response to repetitive mechanical stimulation to measure reliably single currents during successive treatments.

Cation-permeable MS channels are thought to be closely related to the ENaC/DEG family of channel proteins [25–27]. Studying cation-transporting pathways in non-excitable cells, we and others found non-voltage-gated Na^+-selective channels in macrophages [28], carcinoma [29], leukaemia [18,30] and mast cell lines [31,32]. Amiloride-sensitive Na^+ channels in other cells of blood origin have been also reported [33–35]. Several lines of evidence show the similarity of these channels with those of the well-known epithelial Na^+ channels [25]. It is reasonably to assume that SACs studied here and Na^+ channels described previously in leukaemia cells [18,30] belong to the same superfamily of channel proteins mediating cation membrane permeability and characterized by principal similarity of molecular organization and functional properties. Interestingly, an activation of Na^+-channels in K562 cells due to actin disassembly resembled by its phenomenology the effect of cytochalasins on SACs described earlier by Guharay and Sachs (1984) and then by others [5–7]. However, Na^+-channels coupled with submembranous actin assembly–disassembly proved to be insensitive to stretch under the control condition as well after drug-induced activation. Thus, our observations led us to conclude that actin disassembly strongly affects the properties of both channel types in leukaemia cells—Na^+-selective channels and SACs—but in different manner. Namely, actin rearrangements affected drastically the level of activity of Na^+-selective channels while their core conductive properties remained unaltered. In the present work, we have shown for the first time that F-actin disrupting treatments resulted in a strong decrease in the unitary conductance of SACs in K562 cells, while open probability and gating did not significantly alter.

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