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Mechanosensitive channel activity and F-actin organization in cholesterol-depleted human leukaemia cells

Elena Morachevskaya, Anastasiya Sudarikova, Yuri Negulyaev*

Institute of Cytology, Russian Academy of Sciences, Tikhoretsky Avenue 4, St. Petersburg 194064, Russian Federation

Abstract

This study focuses on the functional role of cellular cholesterol in the regulation of mechanosensitive cation channels activated by stretch in human leukaemia K562 cells. The patch-clamp method was employed to examine the effect of methyl-β-cyclodextrin (MβCD), a synthetic cholesterol-sequestering agent, on stretch-activated single currents. We found that cholesterol-depleting treatment with MβCD resulted in a suppression of the activity of mechanosensitive channels without a change in the unitary conductance. The probability that the channel was open significantly decreased after treatment with MβCD. Fluorescent microscopy revealed F-actin reorganization, possibly involving actin assembly, after incubation of the cells with MβCD. We suggest that suppression of mechanosensitive channel activation in cholesterol-depleted leukaemia cells is due to F-actin rearrangement, presumably induced by lipid raft destruction. Our observations are consistent with the notion that stretch-activated cation channels in eukaryotic cells are regulated by the membrane–cytoskeleton complex rather than by tension developed purely in the lipid bilayer.

Keywords: Mechanosensitive channel; Human leukaemia cell; Actin cytoskeleton; Cholesterol; Methyl-β-cyclodextrin

1. Introduction

Mechanically gated channels have been shown to be ubiquitously present in mammalian cells of various origin and specialization (Hamill and McBride, 1996; Sachs and Morris, 1998). With the use of the patch-clamp technique, cation channels activated by membrane stretch have been found in a number of tissues, including skeletal muscle, smooth muscle cells, neurons, epithelial, endothelial and blood cells, and osteoblasts. This intriguing ubiquity stimulates growing interest to mechanosensitive channels and their integration in mechanotransduction in living cells. An involvement of lipid bilayer and submembranous cytoskeleton structures in control of mechanosensitive channels in eukaryotic cells is currently being discussed (Hamill and Martinac, 2001). The integrity of an actin network is considered to be essential for channel activation and mechanical coupling (Janmey, 1998). Moreover, rather high mechanosensitivity of stretch-activated channels in vertebrates (that is, compared with bacterial MS channels) is assumed to derive from interactions with ancillary structures that focus and transmit mechanical forces to the channel (Hamill and McBride, 1996; Hamill and Martinac, 2001). An alternative hypothesis suggests intrinsic mechanisms underlying channel mechanosensitivity that is unlikely to be linked with the submembranous cytoskeleton (Zhang et al., 2000). Recently, Maroto et al. (2005) identified frog oocyte membrane protein fraction and showed that the mechanosensitive channel activated by stretch retains its mechanosensitivity after detergent solubilization and liposome reconstitution. These results suggest that TRPC-1 is a component of mechanosensitive cation channel which is gated by tension developed purely in the lipid bilayer.

Sensitivity to membrane tension was observed earlier in bacterial mechanosensitive channels (Sukharev, 1999) and in the pore-forming antibiotic, alamethicin, inserted into the bilayer (Opsahl and Webb, 1994). Activation by mechanical forces that develop in the lipid bilayer of the membrane was suggested for volume-regulated anion channels in endothelial

* Corresponding author. Tel./fax: +7 812 297 1497.
E-mail address: yurineg@mail.cytspb.rssi.ru (Y. Negulyaev).

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cells (Levitan et al., 2000; Romanenko et al., 2004b). A general mechanism of channel protein regulation by bilayer elasticity has been proposed (Hamill and Martinac, 2001; Lundbaek et al., 2004). According to this macroscopic approach, the dependence of various channel types on lipid organization is due to changes in the mechanical properties of the bilayer rather than specific lipid–protein interactions. (Lundbaek et al., 2004, 2005).

It is known that physical properties of the membranes depend on lipid composition; specifically, stiffness and fluidity of the bilayers are essentially determined by cholesterol content (Needham and Nunn, 1990; Hamill and Martinac, 2001). Cholesterol is the most abundant lipid component of the cell membranes, and it plays an important role in lipid organization, lateral heterogeneity and dynamics of plasma membrane (Brown and London, 2000; Xu and London, 2000). Growing evidence suggests that cholesterol-enriched membrane microdomains called lipid rafts concentrate and segregate membrane proteins and may serve as scaffolds for signaling complexes (Simons and Ikonen, 2000; Edidin, 2003). The integrity of lipid rafts is thought to be required for numerous cell functions in haematopoietic cells (Simons and Toomre, 2000). One of the emerging fields is studies to understand functional and structural link between lipid rafts and the cortical cytoskeleton (Harder et al., 1997; Grimmer et al., 2002; Nebl et al., 2002; Kwik et al., 2003; Byfield et al., 2004). Lipid rafts are considered to be dispersed when cell cholesterol is extracted (Brown and London, 2000). Therefore responses to cholesterol depletion are often taken as evidence of a role for lipid rafts in cell function. Although this approach simplifies the real situation, it could be applied as the first step to test an involvement of cholesterol-based microdomains in channel regulation.

Changes in the level of cellular cholesterol content were reported to affect several types of ion channels (e.g., Bolotina et al., 1989; Chang et al., 1995; Levitan et al., 2000; Lundbaek et al., 2004; Romanenko et al., 2004a,b) and for a long time the effects were considered as specific or non-specific lipid–protein interactions. Currently, the possible association of channel proteins with cholesterol-rich microdomains is being intensively studied. The data appear to indicate that the TRPC1 channel protein compartmentalized in cholesterol-rich caveolae accounts for the sensitivity of vascular store-operated calcium influx to cholesterol depletion (Bergdahl et al., 2003). It has also been reported that TRPC1 may respond to membrane stretch and is gated by bilayer tension without submembranous cytoskeleton (Maroto et al., 2005). Very little, however, is known about cholesterol sensitivity of endogenous stretch-activated channels in eukaryotic cells. The functional impact of cholesterol depletion on mechanosensitive cation channels is an open question. Previously, mechanosensitive cation channels activated by stretch have been identified and characterized in leukaemia cells (Staruschenko and Vedernikova, 2002; Staruschenko et al., 2005, 2006). Here, mechanosensitive channel function and F-actin organization in human leukaemia K562 cells were examined after cholesterol-depletion with methyl-β-cyclodextrin (MβCD). We found that cholesterol depletion strongly suppressed channel activation and this effect is likely to be dependent on the F-actin rearrangement.

2. Materials and methods

2.1. Cells

Human myeloid leukaemia K562 cells obtained from Russian Cell Culture Collection (Institute of Cytology, St. Petersburg) were grown in RPMI-1640 medium containing 10% fetal bovine serum and 80 mg/ml gentamycin in 5% CO_2 incubator at 37 °C. Cells were plated on glass coverslips (0.4 × 0.4 cm) 2–3 days before the electrophysiological experiment. For cholesterol depletion, cells were exposed to 2.5 or 5 mM methyl-β-cyclodextrin (MβCD) according to a standard protocol approved for K562 cells (Levitan et al., 2000; Mannechez et al., 2005). Cells were washed with serum-free RPMI medium and then incubated with MβCD-containing serum-free RPMI at 37 °C for 60, 90 or 120 min. Control cells were treated similarly and incubated in serum-free medium without MβCD.

2.2. Electrophysiology and data analysis

Single channel currents were recorded using standard cell-attached mode of the patch-clamp technique (Hamill et al., 1981). Pipettes were pulled from soft glass capillaries to a resistance of 7–15 MΩ when filled with solution. Membrane currents were measured essentially as described earlier (Staruschenko and Vedernikova, 2002; Staruschenko et al., 2006). 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. The recordings were performed at room temperature (22–23 °C) on the stage of an inverted microscope with Nomarsky optics (magnification of 256×). We used the well-known method of mechanical stimulation of applying pressure to a patch pipette. 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 (suction, about 20 mmHg = 2666 Pa in control). Channel open probability (P_o) was determined 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. Data are presented as mean ± SEM (n = number of experiments). Data were compared using unpaired Student's t-test.

2.3. Solutions for patch-clamp measurements

The bath solution for cell-attached measurements contained (in mM): 145 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES/KOH. Potassium-contained bath solution was used to nullify resting membrane potential in the course of cell-attached recording. Pipettes were filled with normal external solution (in mM): 145 NaCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES/TrisOH. pH of all solutions was set at 7.3. MβCD, cytochalasin D and other chemicals were purchased from Sigma–Aldrich.

2.4. Fluorescent analysis

Filamentous actin in control and cholesterol-depleted cells were stained with phalloidin conjugated to rhodamine Before incubation with MβCD and actin labelling procedures, the cells were plated onto the coverslips coated with L-polylysine. F-actin staining was performed using a standard procedure. Briefly, control and MβCD-treated cells were washed with phosphate-saline buffer (PBS), fixed in 3.7% paraformaldehyde for 8 min, permeabilized with 0.1% Triton X-100 for 10 min, and incubated at 37 °C (15 min) with 0.2 μM rhodamine–phalloidin (TRITC, Sigma). Samples were analysed using a confocal microscope LSM 5 PASCAL (Zeiss Inc., Germany) or epifluorescence microscope IM 35 (Zeiss Inc.). Fluorescence images were acquired

using an Alta U2000 CCD camera (Apogee Instruments Inc., USA) controlled via MaximDL/CCD software (Diffraction Ltd, USA). Images were processed in MaximDL or ImageJ (freeware from http://rsb.info.nih.gov/ij). The scale bar and Pixel Intensity Profile from MaximDL were transferred into the ImageJ program along with each image. Images were assembled in ImageJ.

3. Results

3.1. Cholesterol-depleting treatment with MβCD suppressed the activity of mechanosensitive channels in K562 leukaemia cells

To study the effect of cholesterol depletion on functional properties of mechanosensitive channels activated by stretch in plasma membrane of leukaemia cells, cell-attached measurements were performed. Pipettes contained sodium as the main cation, thus channel activity was monitored under quasi-physiological ionic conditions. In parts of the experiments, cells were plated onto the coverslips coated with L-polylysine. Experiments were designed to compare single channel behaviour in control cells and after cholesterol-depleting treatment with MβCD, a well-known acceptor of sterols (Christian et al., 1997). In control, we observed typical stretch-induced activation of cation channels in response to application of negative pressure. Fig. 1A shows single currents of inward direction reversibly activated by suction at the holding membrane potential of −40 mV. An application of negative pressure of 20–30 mmHg was necessary and sufficient to induce mechanosensitive channel activity, in agreement with our previous results on K562 cells (Staruschenko and Vedernikova, 2002; Staruschenko et al., 2005, 2006). Mechanogated currents were observed in 55% ($n = 76$) of stable patches. It should be noted that under the control conditions, activation threshold never exceeded the level of 40 mmHg and, following increase of pressure, did not induce channel activity in "silent" patches.

After incubation of cells with MβCD (2.5 or 5 mM), we did not observe activation of currents in response to equivalent levels of negative pressure (20–30 mmHg) in all patches tested ($n = 38$). However, we found that application of higher pressure (60–70 mmHg) induced a reversible activation of mechanosensitive channels in cholesterol-depleted cells. Cell-attached records from the representative experiment (Fig. 1A, right) show inward currents activated by suction

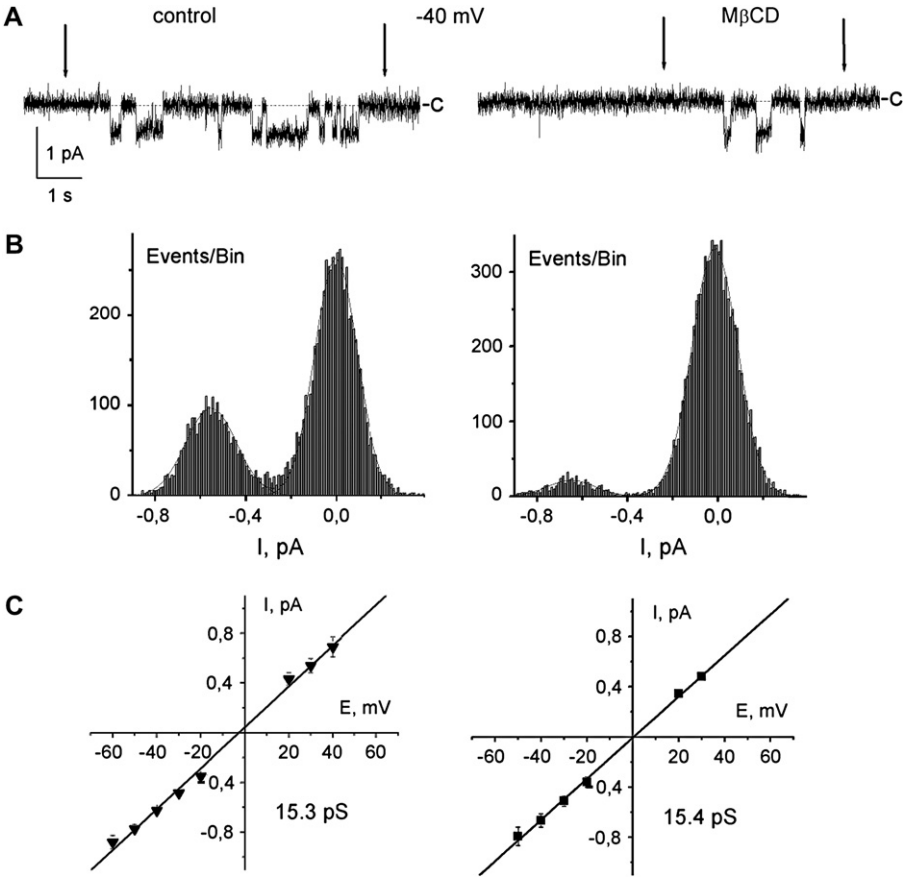

Fig. 1. Activation of mechanosensitive cation channels in control (on the left) and cholesterol-depleted (on the right) K562 leukaemia cells. (A) Representative cell-attached current records at the membrane potential −40 mV; closed states are denoted by "C". The application and removal of suction are indicated by arrows: the level of negative pressure applied to the patches was 20 mmHg in control and 70 mmHg after 60 min incubation of the cells with 2.5 mM MβCD. (B) Corresponding all-point amplitude histograms. Populations fit with Gaussian curves. (C) Mean current–voltage relationships of mechanosensitive channels activated by stretch in K562 cells. Mean conductance was 15.3 ± 0.5 pS ($n = 12$) in control and 15.4 ± 1.3 pS ($n = 9$) after cholesterol depletion.

(70 mmHg) after 60 min incubation of cells with 2.5 mM MβCD. The removal of stimulus caused a rapid abolishment of single currents induced by stretch in control as well as in cholesterol-depleted cells (Fig. 1A). After cholesterol extraction using MβCD treatment, mechanogated currents were observed in 50% ($n = 38$) of stable patches. Thus, we can conclude that cholesterol depletion resulted in a significant increase of activation threshold of mechanosensitive channels.

Comparison of single currents, amplitude histograms and current-voltage relationships (Figs. 1 and 2A) indicated that channel conductance and selective properties of mechanosensitive channels did not alter after MβCD treatment. Mean values of unitary conductance corresponded to 15.3 ± 0.5 and 15.4 ± 1.3 for control and cholesterol-depleted cells, respectively. These values are very close to the main conductance level obtained previously for the cation channels activated by stretch in K562 cells under control conditions (Staruschenko and Vedernikova, 2002; Staruschenko et al., 2005). Fig. 2A shows representative current traces recorded at applied negative pressure (suction) in control and after MβCD treatment. In both cases, the channels were not characterized by inactivation or adaptation to stimulus. The comparison of single currents in cholesterol-depleted and in control cells demonstrates an evident difference in the level of channel activity. As a measure of it, open probability values (P_o or NP_o) are commonly used (see Section 2). Mean P_o values measured for membrane potential of −40 mV corresponded to 0.15 ± 0.02 and 0.05 ± 0.02 for control and MβCD-treated cells, respectively. Fig. 2 shows that cholesterol depletion resulted in evident decrease of the probability of channel being open. It is worth noting that P_o values presented in Fig. 2B were obtained at different pressure levels: 20–30 mmHg was applied in control experiments and 60–70 mmHg was necessary after MβCD treatment. Formally, it would be more correct to compare P_o values measured at the same level of mechanical stimulation. However, at 20–30 mmHg, no activation occurred in MβCD-treated cells, that is, P_o was equal to zero. On the other hand, in response to negative pressure

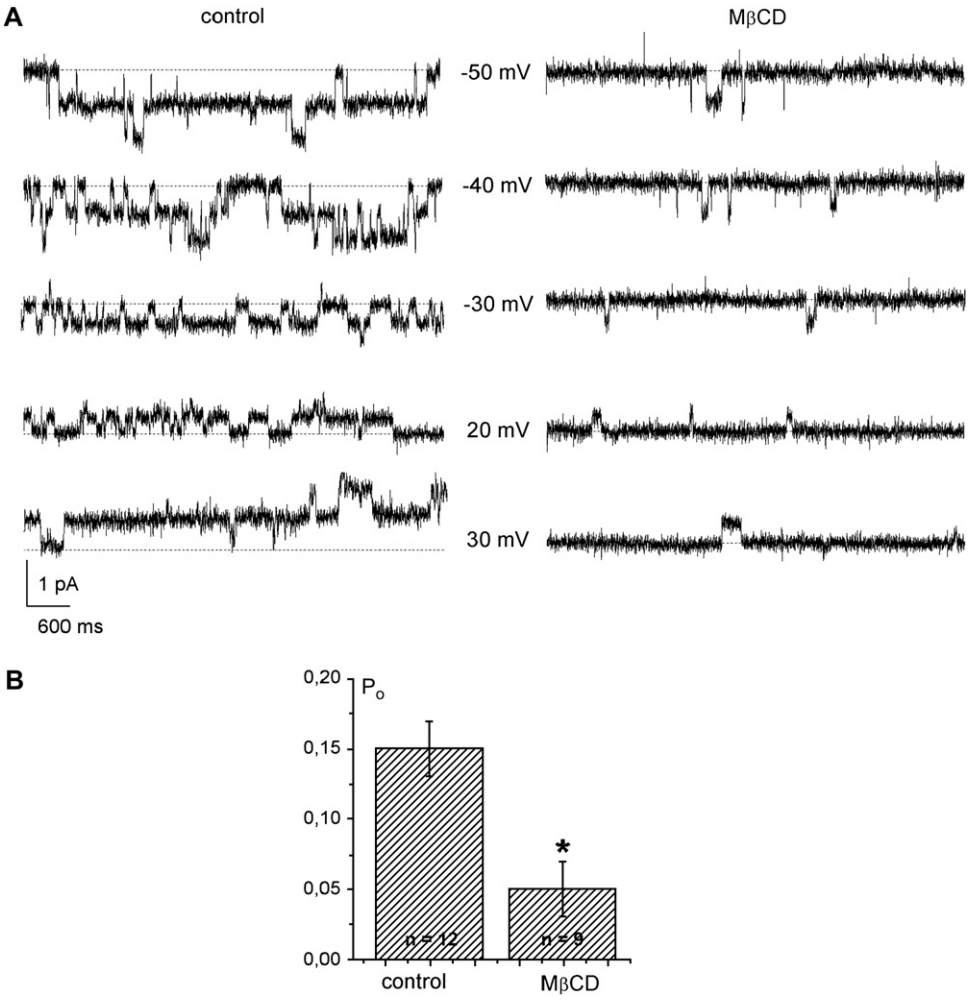

Fig. 2. Cholesterol-depleting treatment suppressed the activity of mechanosensitive channels in plasma membrane of leukaemia cells. (A) Mechanogated currents recorded at applied negative pressure from representative cell-attached patches in control (on left) and after MβCD treatment (on right). Membrane potentials are indicated near the traces. Pressure level was 20 mmHg in control and 60 mmHg after 60 min incubation of the cells with 2.5 mM MβCD. (B) Summary graph comparing channel open probability (P_o) measured at membrane potential −40 mV in control (left column) and after cholesterol depletion (right column). The number of observations in each group is shown. *$P < 0.05$ vs. control.

application of ~60–70 mmHg to control patches, the channel activity (and corresponding NP_o values) enhanced greatly to be analysed quantitatively. This means that the dependence of NP_o on applied stimulus (activation curve) shifted to a higher pressure level in cholesterol-depleted cells. The channel activity was strongly inhibited after incubation of the cells with 2.5 or 5 mM MβCD. In sum, our patch-clamp experiments indicated that cholesterol-depleting treatment strongly suppressed stretch-induced activation of cation channels in plasma membrane of K562 cells. These effects seem to make the membrane become stiffer, whereas cholesterol depletion is known to decrease the stiffness of lipid bilayers. Thus, suppression of mechanosensitive channel activation in cholesterol-depleted cells could not be explained by changes in physical properties of lipid bilayers. A question arises as to what additional structures may be involved in modulation of mechanosensitive channel gating due to cholesterol depletion. It is known that in eukaryotes mechanical properties of cell surface are primarily dependent on the membrane–cytoskeleton complex. Therefore, it is reasonable to suppose that the F-actin network may be an important determinant, mediating changes in channel gating in K562 cells.

3.2. F-actin reorganization in leukaemia cells after MβCD treatment

To study the effect of cholesterol depletion on cortical cytoskeleton in leukaemia cells, F-actin organization in control and after MβCD treatment was examined using fluorescent analysis. Filamentous actin was labelled with rhodamine–phalloidin. Before incubation with MβCD and F-actin staining procedures, the cells were plated onto the coverslips coated with L-polylysine. Under the control conditions, a cortical F-actin layer was observed using confocal or epifluorescence microscopy (Figs. 3A and 4A). Fig. 3 demonstrates the effect of cytochalasin D, visualized in our experiments on K562 cells. In agreement with known effects of actin disrupters, cytochalasins or latrunkulin (not shown) caused evident disorganization of cortical cytoskeleton structures and appearance of amorphous aggregates. Fig. 4 shows F-actin labelling in control and after 60 min incubation of the cells with 5 mM MβCD. Images show that MβCD treatment strongly affected actin organization in leukaemia cells. After cholesterol depletion, diffuse distribution of F-actin throughout the cytoplasm was observed while the cortical layer became less prominent. Fluorescent staining was quantified by taking images through a plane at the centre of the cell diameter. Pixel intensity profiles (Fig. 4) indicated actin redistribution in cholesterol-depleted cells compared to control images. F-actin seemingly formed a thin network in the cytoplasm, as was shown for some other treatments of leukaemia cells (Grzanka et al., 2003). Our fluorescence measurements showed that cholesterol depletion caused cytoskeleton reorganization in leukaemia cells, presumably, an accumulation of F-actin in the cytoplasm. We suggest that F-actin rearrangement underlies changes in mechanical properties of K562 cells and thus mediates modulations in mechanosensitive channel gating and activity due to cholesterol depletion.

4. Discussion

Several lines of evidence suggest that ion channels are regulated by their lipid environment in cell membranes. A number of channels were reported to be modulated by changes in the level of cellular cholesterol, one of the main lipid components of the plasma membrane in all mammalian cells. The present study aimed to elucidate the possible involvement of cellular cholesterol and lipid raft integrity in the function of mechanosensitive cation channels in mammalian cells. The effects of MβCD, synthetic acceptor of sterols (Christian et al., 1997) on stretch-activated channels and F-actin organization in human leukaemia K562 cells were examined. We have found that cholesterol depletion significantly suppressed the activity of mechanosensitive channels and strongly affected F-actin organization in K562 cells. Channel behaviour was

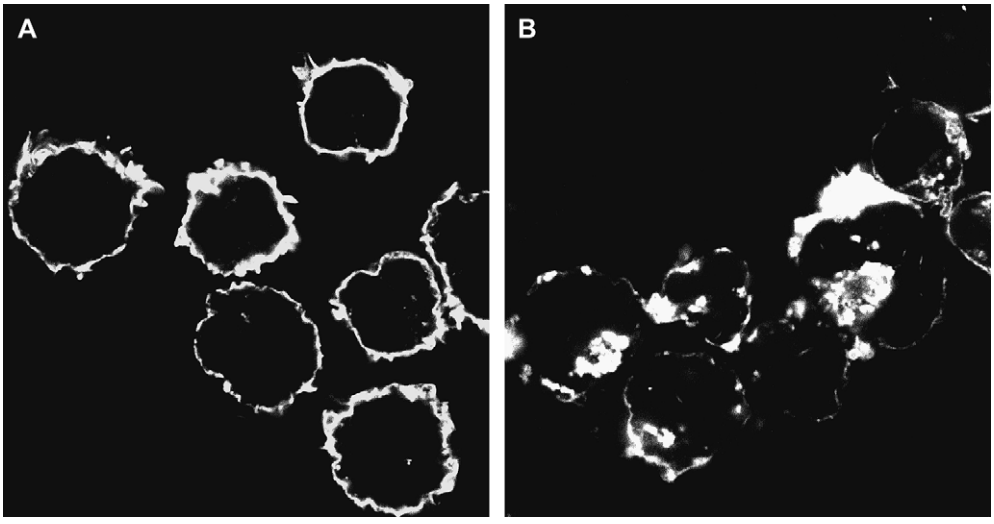

Fig. 3. K562 leukaemia cells labelled for F-actin with rhodamine–phalloidin (A) in control and (B) after incubation with cytochalasin D (10 μg/ml, 15 min). Images were obtained using confocal microscope.

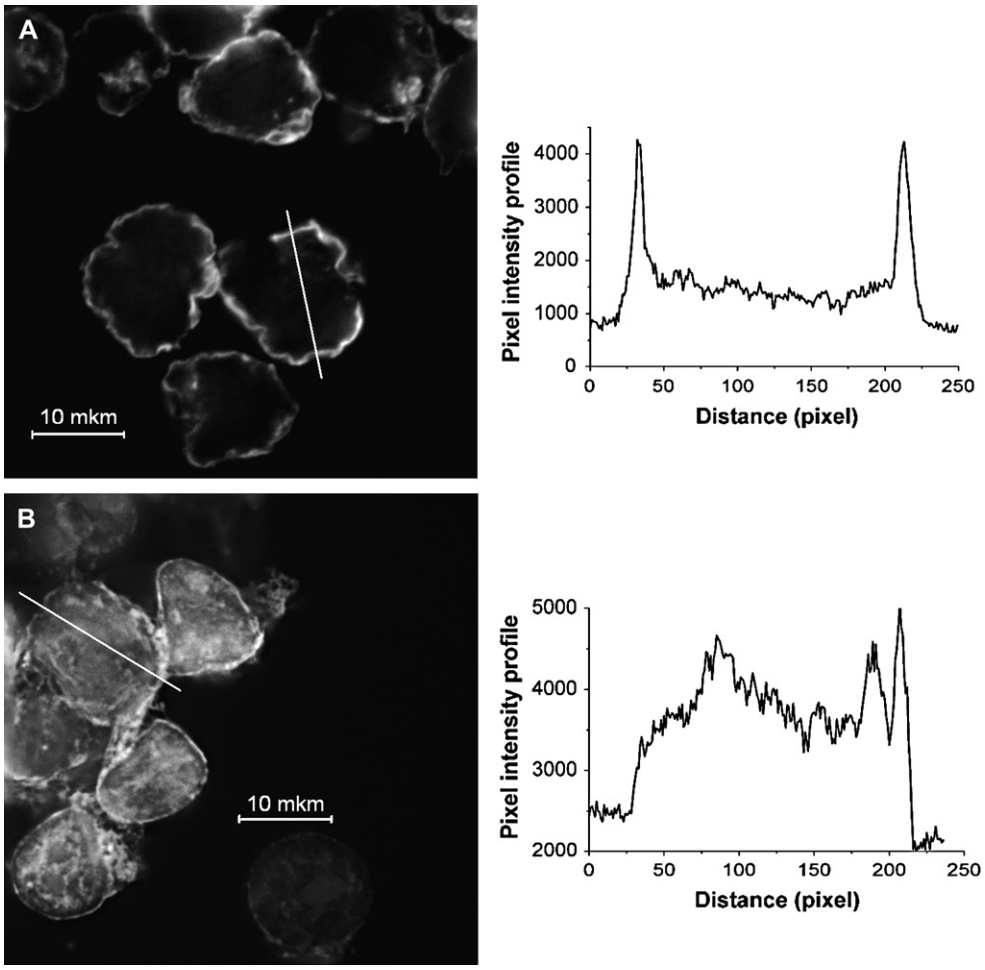

Fig. 4. Effect of cholesterol depletion on F-actin organization in leukaemia cells observed under epifluorescence microscopy system. Images (on left) were acquired using an Alta U2000 CCD camera. The distributions of pixel intensity (Pixel Intensity Profile, on right) were determined over cell diameter using MaximDL software. (A) Control cells. (B) Cells were incubated for 60 min with 5 mM MβCD.

monitored at single current level with the use of patch-clamp method. No change in conductive properties of mechanosensitive channels was observed after treatment with MβCD. Interestingly, as was shown earlier, actin-disrupting treatments resulted in a decrease of the unitary conductance without significant changes in gating and activity of stretch-activated channels in K562 cells (Staruschenko et al., 2005).

Mechanosensitive channels have been found in a variety of tissues. Currently, they are characterized as ''ubiquitously present''. However, there is very little information on how cellular cholesterol may affect mechanosensitive channels activated by stretch in eukaryotic cells. Modulations of volume-regulated anion channels by cholesterol content in endothelial cells were investigated (Levitan et al., 2000; Romanenko et al., 2004b). In contrast to suppression of channel activation observed in our experiments, cholesterol depletion was reported to cause an enhancement of volume-regulated current activation. These observations have been explained by affecting membrane deformation energy, that is, by ''non-specific'' changes in bilayer elasticity/stiffness.

For a long time, the effects of cholesterol were considered as specific or non-specific lipid–protein interactions. To explain the latter, a general mechanism of channel protein regulation by bilayer elasticity has been proposed (Hamill and Martinac, 2001; Lundbaek et al., 2004). These authors postulate a dominant role for hydrophobic coupling between a membrane-spanning proteins and surrounding bilayer. According to this model, the dependence of various channels on lipid organization is due to the changes in physical properties of the bilayer. Particularly, non-physiological amphiphiles as well as cholesterol depletion reduced bilayer stiffness, and were shown to cause similar shifts in characteristics of different unrelated channels (Lundbaek et al., 2004, 2005). This macroscopic approach seems to be appropriate for mechanically gated channels. However, the suppression of mechanosensitive channel activation after MβCD treatment observed in our patch-clamp experiments implies that cholesterol depletion increases rather than decreases the stiffness of plasma membrane, in contrast to the known effects of cholesterol on lipid bilayer stiffness and deformability (Needham and Nunn, 1990). Thus, the effects of cholesterol-depleting treatment have not resulted from changes in bilayer elasticity. We can speculate that mechanosensitive channel activity is primarily dependent on mechanical properties of the membrane–cytoskeleton complex in leukaemia cells.

Indeed, fluorescent staining of K562 cells for F-actin revealed essential reorganization of cortical cytoskeleton after treatment with MβCD.

Our observations do not support the notion that stretch-activated channels in vertebrates are gated by tension developed purely in lipid bilayers (Maroto et al., 2005), as is the case in various prokaryotic mechanosensitive channels (Sukharev, 1999). It is of peculiar interest to reveal some distinguishing features of the behaviour of mechanosensitive cation channels in eukaryotic cells for a comparison with putative molecular correlates. Maroto et al. (2005) concluded that TRPC-1 is a component of mechanosensitive cation channels. Besides, it was also shown that TRPC1 channel protein compartmentalized in cholesterol-rich caveolae that accounted for sensitivity of vascular store-operated calcium influx to cholesterol depletion (Bergdahl et al., 2003). An association of TRPC1 with platelet lipid raft domains has been reported (Brownlow and Sage, 2005).

Evidently, the suppression of stretch-induced activation after MβCD treatment could not be explained directly by changes in physical properties of bilayers. Consequently, cholesterol depletion affects mechanosensitive channel gating via some indirect mechanisms and an involvement of lipid rafts and actin cytoskeleton is plausible. It is accepted that extraction of cholesterol from plasma membrane causes disruption or disorganization of cholesterol-based microdomains (lipid rafts) (Brown and London, 2000; Edidin, 2003). The integrity of lipid rafts is thought to be required for numerous cell functions in cells of blood origin (Simons and Toomre, 2000). One can hypothesize that the destruction of cholesterol-rich microdomains modulates some intrinsic properties of the channel protein or their specific interactions with the lipid environment. This assumption could not be excluded but nowadays we have no evidence in favour of it. Fluorescence data allow us to assume that F-actin reorganization in cholesterol-depleted K562 cells may account for, at least partially, changes in gating behaviour of stretch-activated channels.

Growing evidence suggests that lipid rafts play an important role in the interactions between the membrane and the cortical cytoskeleton (Grimmer et al., 2002; Nebl et al., 2002; Kwik et al., 2003). Some observations have shown that disruption of lipid rafts due to cholesterol-depleting treatments results in a dissociation of cytoskeleton proteins from the membrane and in a corresponding decrease of cell surface stiffness (Harder et al., 1997). More recently, various effects of cholesterol depletion on membrane–cytoskeleton coupling have been documented (Grimmer et al., 2002; Kwik et al., 2003; Byfield et al., 2004). Our results are, in principle, in agreement with the recent data on endothelial cells (Byfield et al., 2004) indicating that stiffness of cellular membranes increases upon cholesterol depletion, and this stiffening effect depends on the integrity of F-actin. The data together with our observations support the notion that, in living cells, membrane deformability depends strongly on cortical cytoskeleton. It is worth noting that no alteration in the actin-specific fluorescence after cholesterol depletion was found in endothelial cells (Byfield et al., 2004). In contrast, fluorescent microscopy showed that MβCD treatment strongly affected actin organization in leukaemia cells. After cholesterol depletion, we observed F-actin redistribution, presumably involving an accumulation of F-actin throughout the cytoplasm. Intact actin cytoskeleton in K562 erythroleukaemia cells, as well as in their congeners, represents a thin cortical layer (Kurisaki et al., 1993; Levitan et al., 1995; Grzanka et al., 2003). It was reported earlier that in untreated K562 cells almost all (>90%) of actin was monomer, that is, a significant pool of cytoplasmic G-actin, is available for polymerization (Kurisaki et al., 1993). Our data allow us to assume that cholesterol depletion induces fast actin assembly in leukaemia cells; lipid raft destruction is likely to participate or even to be a key step in initiation of actin remodelling. This assumption is consistent with the recent evidence indicating that cholesterol efflux is prerequisite of actin polymerization during sperm capacitation (Brener et al., 2003). Accumulation of F-actin appeared to be more significant in leukaemia cells as compared with endothelial cells (Byfield et al., 2004) because the intact endothelial cells have preformed filament structures.

Our patch-clamp data together with fluorescent imaging support the idea that cholesterol depletion affects coupling between cortical cytoskeleton and plasma membrane (Kwik et al., 2003; Byfield et al., 2004). Various pathways have been proposed; specifically, possible involvement of phosphoinositides (PIP_2) (Kwik et al., 2003) and small G-protein-dependent mechanisms has been documented (Grimmer et al., 2002). We suggest that F-actin rearrangement underlies changes in mechanical properties of the cell surface and thus mediates the modulation of mechanosensitive channel activity due to cholesterol depletion. Consistent with this notion, depletion of cell cholesterol was reported to cause global effects on cell and plasma membrane architecture and function (Kwik et al., 2003). The authors have shown that acute or chronic cholesterol depletion resulted in a decrease of lateral mobility of membrane proteins in fibroblasts that was a consequence of the reorganization of cell actin.

Previously, the effects of cytoskeleton disassembly on mechanosensitive channels activated by stretch in K562 human leukaemia cells have been studied (Staruschenko et al., 2005). F-actin-disrupting treatments with cytochalasins or latrunculin B were found to result in a considerable decrease of channel conductance without significant change in channel opening probability. Notably, cytoskeleton disruption did not promote an activation of mechanosensitive channels in K562 cells. On the other hand, the results presented here imply that F-actin rearrangement, possibly involving actin assembly, may suppress mechanosensitive channel activity. The rationale of this discrepancy is that the status of the cytoskeleton in intact cells plays a dominant role in determining an effect of induced assembly or disassembly on cellular mechanotransduction.

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