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Gating the mechanical channel Piezo1

A comparison between whole-cell and patch recording

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Piezo1 is a eukaryotic cation-selective mechanosensitive ion channel. To understand channel function in vivo, we first need to analyze and compare the response in the whole cell and the patch. In patches, Piezo1 inactivates and the current is fit well by a 3-state model with a single pressure-dependent rate. However, repeated stimulation led to an irreversible loss of inactivation. Remarkably, the loss of inactivation did not occur on a channel-by-channel basis but on all channels at the same time. Thus, the channels are in common mechanical domain. Divalent ions decreased the unitary conductance from ~68 pS to ~37 pS, irrespective of the cation species. Mg and Ca did not affect inactivation rates, but Zn caused a 3-fold slowing. CytochalasinD (cytoD) does not alter inactivation rates or the transition to the non-inactivating mode but does reduce the steady-state response. Whole-cell currents were similar to patch currents but also had significant differences. In contrast to the patch, cytoD inhibited the current suggesting that the activating forces were transmitted through the actin cytoskeleton. Hypotonic swelling that prestressed the cytoskeleton and the bilayer greatly increased the sensitivity of both control and cytoD cells so there are two pathways to transmit force to the channels. In contrast to patch, removing divalent ions decreased the whole-cell current. The difference between whole cell and patch properties provide new insights into our understanding of the Piezo1 gating mechanisms and cautions against generalization to in situ behavior.

Introduction

Piezo1 is a member of the newly identified family of mechanosensitive ion channels (MSCs) found in higher eukaryotic cells.[1,2] The channel was identified in the mouse cell line Neuro2A where microRNA inhibited whole-cell responses to mechanical stimulation. The gene that was subsequently isolated encoded a protein of approximately 2,500 amino acids with over 30 predicted trans-membrane regions and with no obvious homology to other channel proteins.[1] Heterologous expression of Piezo1 conferred mechanosensitive whole cell currents to cells that did not normally respond.

The channel monomer associates in the membrane as homotetramers with no auxiliary proteins. It is not yet known whether the channels function as monomers or tetramers. The pore is cation selective, reversing near 0 mV in normal saline.[2] The channel is active in outside-out patches and has properties similar to currents from the cell-attached mode. The currents in outside-out patches are inhibited by the extracellular peptide, GsMTx4, the only known specific inhibitor for for cationic mechanical channels and it has a K_D of ~150 nM. The sensitivity to GsMTx4 is similar to the value reported for other endogenous cationic MSCs.[3] This result and the similar kinetics to endogenous cationic MSCs indicate that the endogenous channels may be members of the Piezo family. Knocking out the gene for the Piezo1 homolog in Drosophila larvae suggested it had a physiological role in the response to noxious stimuli. The knockout significantly reduced the sensitivity to the rollover response by pressing the larvae with a fiber.[4]

Since patch formation involves applying huge mechanical stresses to the cortex that can change the channel's environment,[5] we wanted to know how well patch behavior can be used to predict in situ behavior. As a first approximation to the in situ condition, we used whole-cell recordings and many features were similar to the properties in cell-attached patches, but there were significant differences that warrant caution about extrapolating the response channels in the patch to those on the cell.

Results

Piezo1 currents have transients with a time-dependent desensitization.[1,6] We first asked whether the desensitization arises from a channel-dependent property (inactivation) or from adaptation of the force transmission system, a time-dependent decrease in the local stimulus typically because of viscoelastic coupling. To test for adaptation we used the classic stair step stimulus. In patches we first activated all the channels with a saturating

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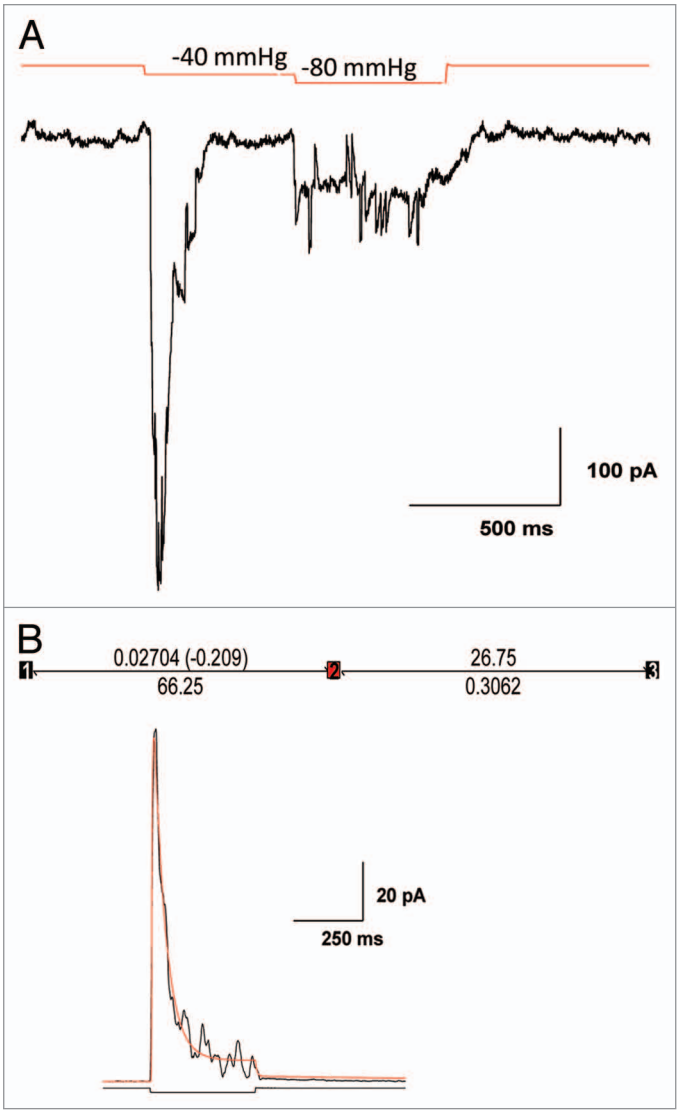

Figure 1. Inactivation in cell-attached patches. Panel (**A**) shows the results of a two-step staircase stimulus where the multichannel currents are shown to inactivate rather than adapt. The first step activates the channels and they proceed to desensitize. If desensitization were caused by adaptation of the stimulus the second step would produce a transient current similar to the first step. In general the second step produced very little current and in the example shown, the second step produced a complete conversion of the transient current to a non-inactivating current. Panel (**B**) shows the state model used to fit the transient and the optimal rate constants were obtained from the MAC routine in QUB. The rate constant $k_{12} = k^{12} * \exp(q*P)$ where q is the pressure sensitivity shown in parentheses after the constant k_{12} and k^{12} is the rat at zero pressure. For the fitted data, note that once the stimulus ends the recorded current trace is smaller than that of the model for many seconds as drop in pressure wrinkled the membrane and it took time to reanneal.[6]

pressure. When the desensitized current approached baseline levels, we doubled the stimulus amplitude. If adaptation caused desensitization, the response to the second step should look like the first. If it were inactivation, we should get almost no current since most channels were inactivated by the first stimulus. **Figure 1A** shows desensitization of multichannel currents in a cell-attached patch in response to a two-step staircase stimulus. In this example, the response to the second step no longer desensitized. Note that the response to both steps is homogeneous across all the channels rather than exhibiting a mix of desensitizing and non-desensitizing responses. The fact that there was no significant response to the second step implies that desensitization is not the result of adaptation of the local stimulus but is channel based inactivation. However, all the channels are in the same mechanical domain and subject to the same forces within the domain.

To quantify the kinetics we modeled multichannel currents using the MAC routine[7] in QUB software (www.qub.buffalo.edu) (**Fig. 1B**). The data were well fit by a linear 3 state model: closed, open and inactivated. The only pressure-dependent rate constant was from closed to open. To account for the known viscoelastic properties of patch geometry, the idealized pressure stimulus was modified to incorporate those relaxations. QuB software uses the idealized data to drive an ordinary differential equation that describes the translation of applied pressure to geometric changes.[5,8] This equation effectively applied a damping factor to the stimulus (the damping factor was 1.1).

To explore the dynamic range of the response, we applied large pressures and voltages. Repeated stimuli of either kind produced an irreversible transition to a non-inactivating response (**Fig. 2A and B**). Recordings made with single channel resolution mimicked these observations (**Fig. 2C and D**). However, the channels still activated sigmoidally with pressure. Moderate amplitude stimuli that were as much as 10 times longer than those shown in **Figure 2** retained inactivation (data not shown). The probability of transition isn't linear with the stimulus amplitude. The irreversible loss of inactivation does not seem to arise from occupancy in any particular state of the model. We were able to produce non-inactivating channels with positive or negative pressure indicating that the transition was associated with membrane stress not hydrostatic pressure.

To look for the involvement of the cytoskeleton in gating, we measured the response after treatment with cytoD. In patches, the inactivation time constant with cytoD was τ = 46 ± 6 ms (n = 5) similar to that of untreated cells (τ = 45 ± 17 ms, n = 5), suggesting actin is not involved in inactivation. The transition to a non-inactivating form also occurred in cells treated with cytoD (**Fig. 2E and F**). The only observable effect of cytoD on patch currents was to reduce the steady-state current during inactivation, i.e., cytoD made inactivation more complete.

We had previously observed that divalent ions affected the currents, so to extend the comparison with whole-cell recordings we did a more careful study. We defined the base conductance to be that in divalent free saline. Single channel recordings in cell-attached patches demonstrated that $MgCl_2$ in the pipette reduced the conductance by ~50% (**Fig. 3**) as previously reported.[2] With no divalents and high K saline (see Methods), the conductance was 67.8 ± 4.3 pS and in the presence of added 1 mM $MgCl_2$ the conductance fell to 37.1 ± 2.5 pS. We observed similar responses with Ca^{2+} and Zn^{2+} (data not shown). We also measured the sensitivity of the inactivation rate to divalents (**Fig. 3C**). For 1 mM Mg^{2+} and Ca^{2+}, the inactivation rates were nearly identical to that

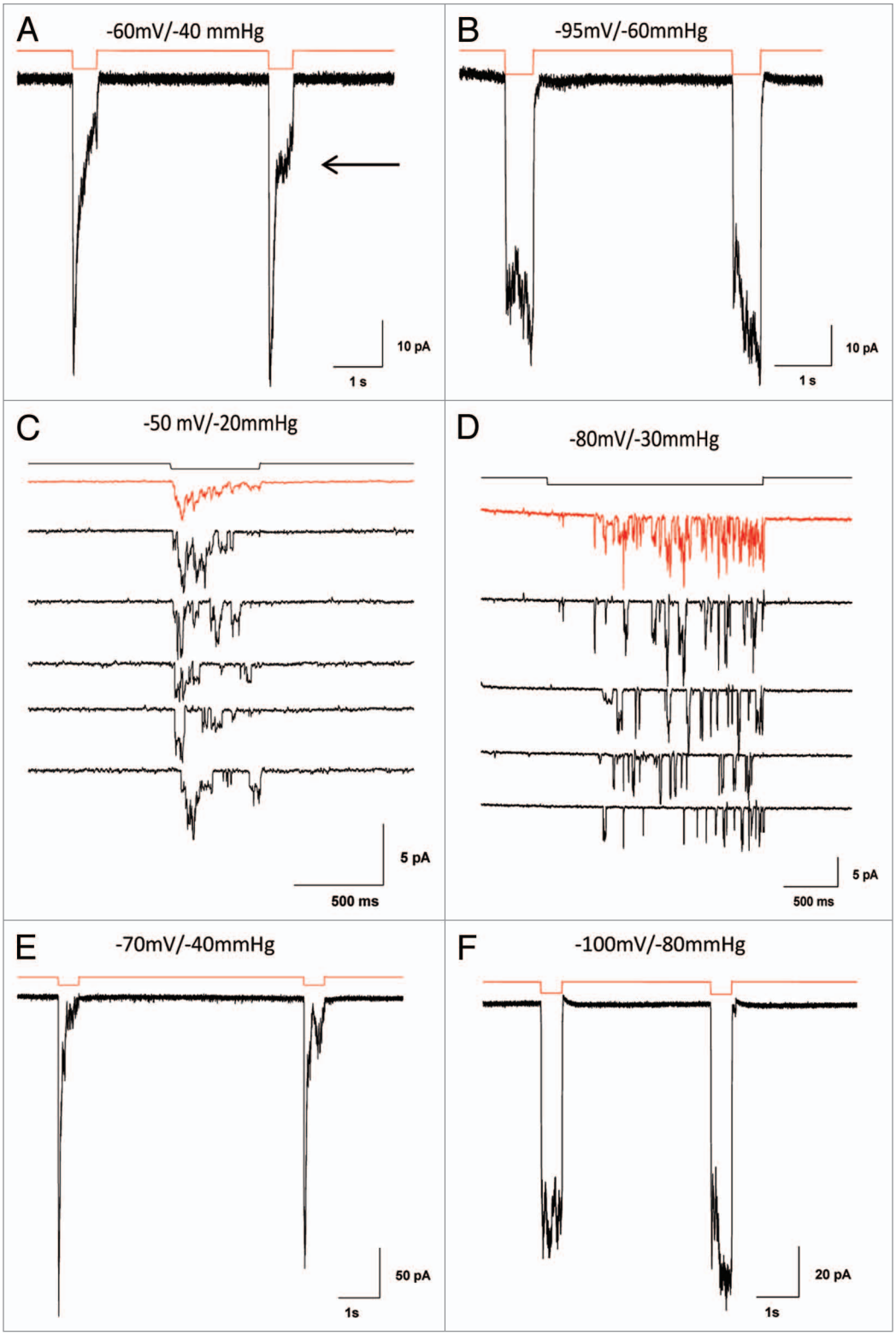

Figure 2. Irreversible transition of inactivating Piezo1 to a non-inactivating form. Panel (**A**) is the initial response in cell-attached mode showing a transient with no steady-state response while the next stimulus has a significant steady-state indicated by an arrow. After repeated stimuli of high pressure and voltage, all channels lose inactivation simultaneously and irreversibly (**B**). Panels (**C**) and (**D**) are single channel recordings of the inactivating and non-inactivating forms, respectively (average data of individual pulses are shown just below the stimulus trace). Treatment with cytoD (4–6 μM) reduces the ratio of peak to steady-state current (**E**) but does not change the transition from inactivating to non-inactivating (**F**), and the inactivation rate is unaltered.

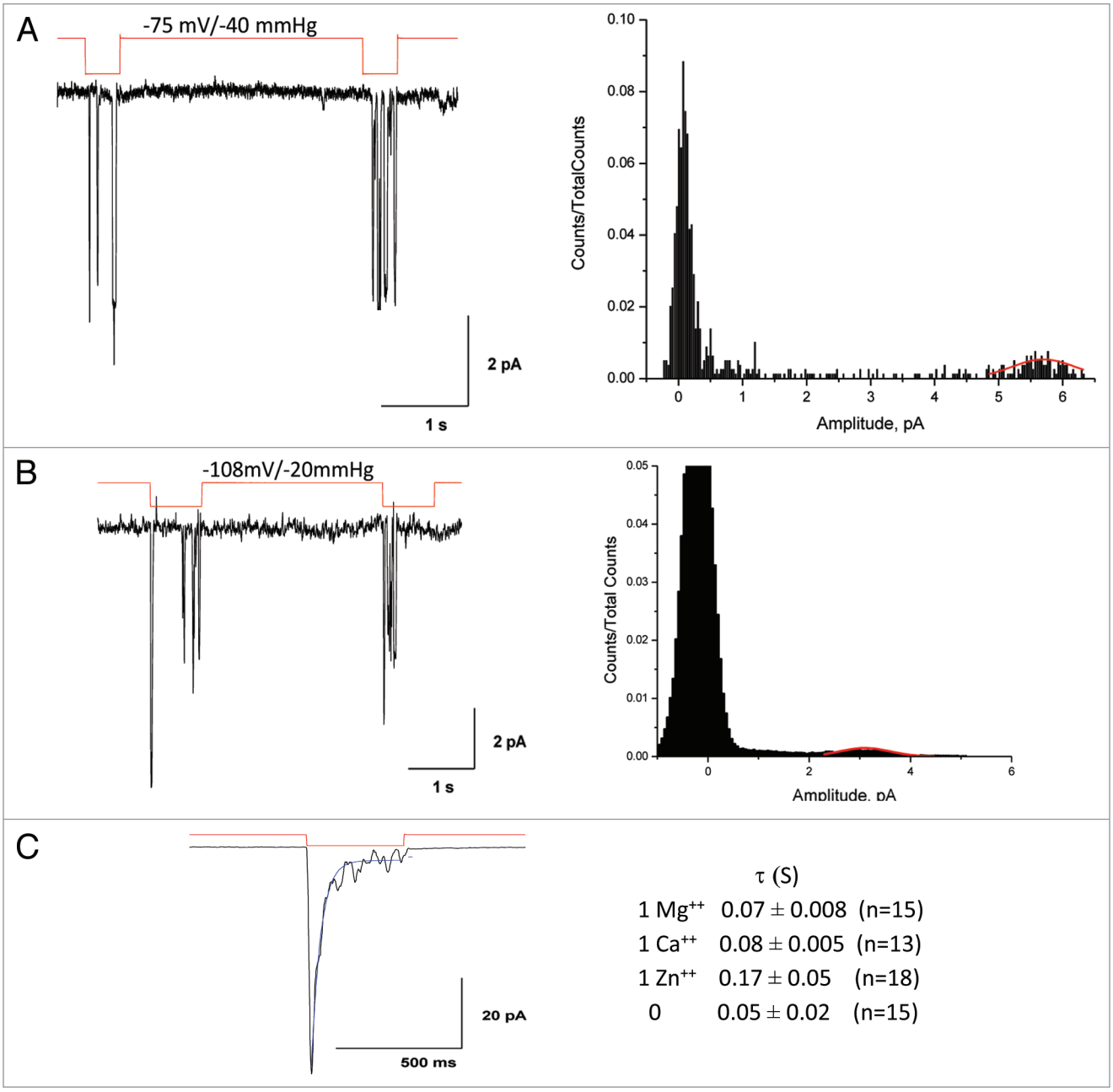

Figure 3. Conductance of Piezo1 in the presence and absence of divalent ions. (**A**) Without divalent ions the unitary conductance is approximately 68 pS. (**B**) In the presence of 1 mM Mg the conductance is approximately 37 pS. Panel (**C**) shows inactivation with the falling phase fit to a single exponential indicated. The time constant of the fit (τ) is shown for different divalent ions. $Mg^{2+}$and Ca^{2+} have a small effect on the inactivation rate but Zn slows it 3-fold.

seen in divalent free solution. However, Zn^{2+} slowed inactivation 3-fold (**Fig. 3B**).

We compared the cell-attached patch responses to whole-cell responses elicited by indentation with a fire polished probe.[1,3] When divalent ions were removed from the bath, the whole-cell response was reduced, whereas in a patch the single channel conductance was larger in divalent free saline (**Fig. 4**). **Figure 4A** shows the washout of Mg^{2+} reduced the whole-cell currents and reperfusion with Ca^{2+} restored the current. Thus, in whole-cell mode and presumably in situ, extracellular divalent ions are required for activity. A summary of the divalent responses is shown in **Figure 4B and C** as the mean response (± SD) averaged over multiple stimuli.

To test the involvement of cytoskeleton in whole-cell currents, we pretreated cells with 5 μM cytoD. In contrast to cell-attached patch recordings where the drug only decreased the steady-state current, CytoD produced a pronounced reduction in amplitude (**Fig. 5B and C**) as though f-actin were in series with the channel stressors. To prestress the channels in a different way, we swelled cells that with a 50% hypotonic solution.[9] Swelling produced a

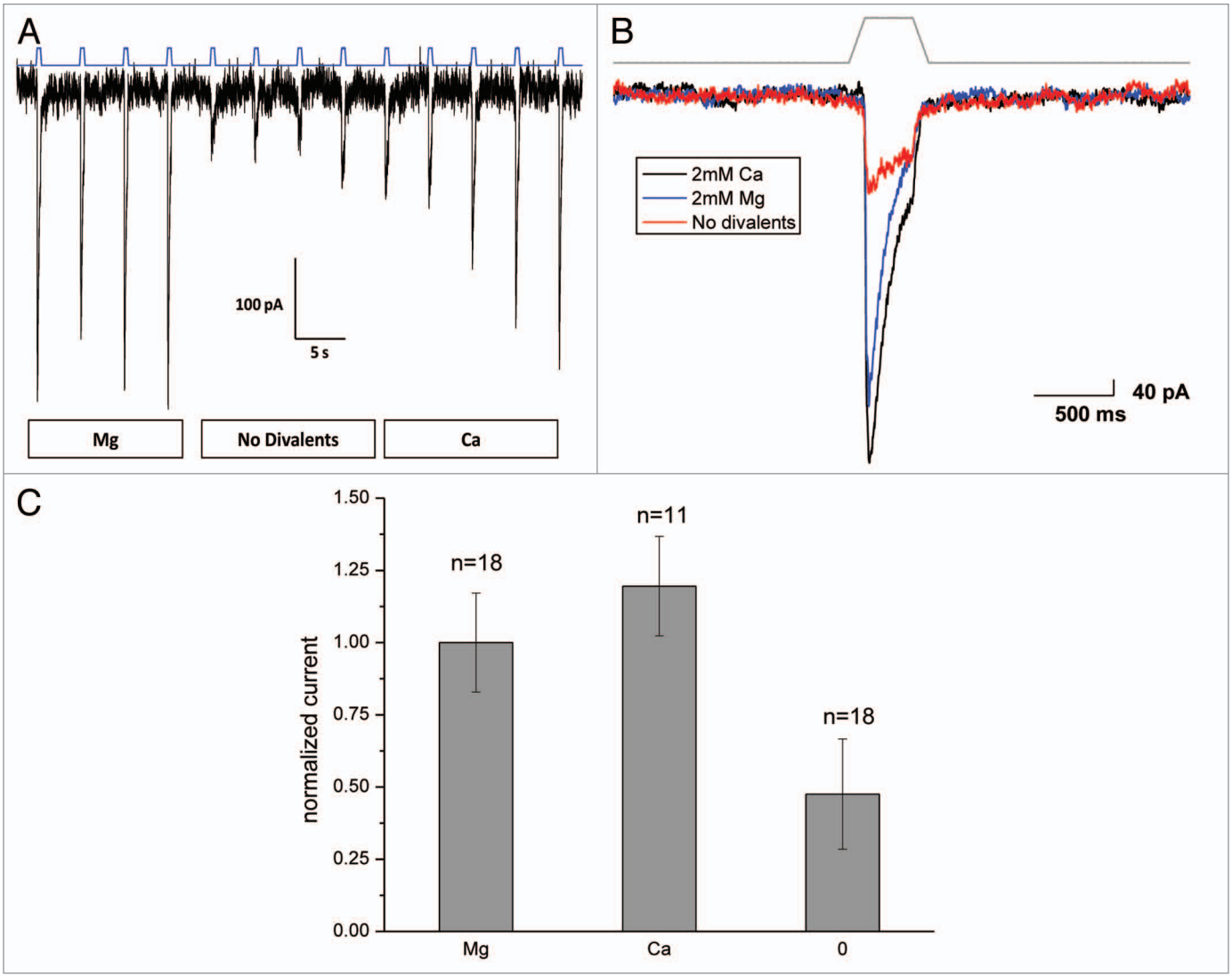

Figure 4. Whole-cell currents require divalent ions. (**A**) Cells were perfused with bath solution containing Mg^{2+}. Holding potential was -60 mV and the stimulus is the indicated ramp. Removal of divalent ions reduced the whole-cell currents and the addition of Ca^{2+} restored them. Panel (**B**) shows the average response of the data from (**A**), and (**C**) shows the aggregate response (mean ± SD).

dramatic increase in the sensitivity to indentation (**Fig. 5B and C**). Swelling also sensitized indentation in cells that had been treated with cytoD also suggesting that the stress in actin is not the key stimulus link for the channel (**Fig. 5C**).

Discussion

Do patch recordings of Piezo1 provide reliable predictors for in situ behavior? In a patch these channels are activated by cortical tension whether produced by positive or negative pressure so the response is insensitive to membrane curvature, as is the case for other MSCs.[3] Patch currents usually inactivated with time although the inactivation proved labile. In whole-cell mode the currents usually inactivated following indentation (**Figs. 4 and 5**) although it too was labile. The channel kinetics in the patch were well fit by a linear 3-state model of closed, open and inactivated, with a pressure dependent opening rate. The kinetic modeling was usually performed on currents from cells treated with cytoD that made inactivation more complete.

For reasons that remain unclear, repeated stimulation produced non-inactivating currents. The transition to non-inactivating currents was irreversible and did not arise selectively from the inactivated state. The transition did not occur on a channel-by-channel basis, but collectively as though groups of channels were in a single domain that broke apart under repeated stress. We have yet to define the stimulus conditions that reliably cause the loss of inactivation,[10,11] but it is associated with higher stress. Both high voltage and mechanical stress increase energy density in the bilayer that in extreme cases leads to membrane pore formation.[12] If the bilayer reforms in a domain that includes the channels, they would feel a new stress. The sensitivity of activation to pressure was not very different with inactivating and non-inactivating channels suggesting that the stress field after breaking the domain was not very different from that before. Single channel

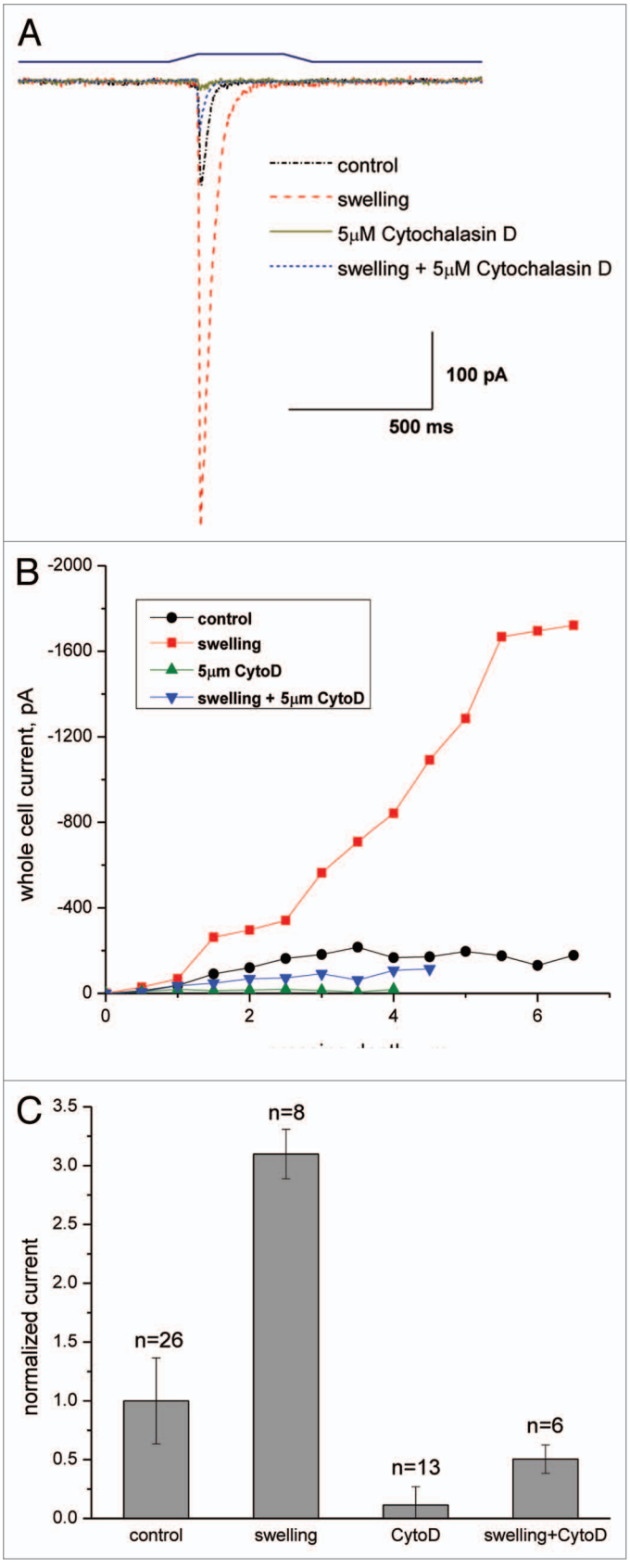

Figure 5. Disruption of the cytoskeleton and swelling alters Piezo1 sensitivity. Panel (**A**) shows typical current traces from two cells (control and cytoD) with and without swelling. Hypotonic swelling greatly enhanced the indentation sensitivity (-60 mV holding potential and 4 μm indentation) resulting in a 6-fold increase in current compared with control (**B**). Treatment with cytoD reduced the mean response by 11-fold but that response was potentiated by hypotonic swelling suggesting that there are at least two modes of stress transfer. Panel (**B**) summarizes these results (squares) and shows that treatment of cells with cytoD suppresses the activity of Piezo1 (triangles) compared with the control (circles). Panel (**C**) is the mean peak current (± SD) from many cells subjected to swelling or cytoD.

recordings show that inactivating and non-inactivating channels have similar activation and deactivation kinetics implying that inactivation does not reflect channels moving into and out of the seal.[5,13] Our current hypothesis is that inactivation involves interactions of the channel with the cytoskeleton and the global loss of inactivation represents a cytoskeletal breakdown. The loss of inactivation with repeated stimuli is remarkably similar to what Hamill's lab observed with cationic MSCs in Xenopus oocytes suggesting that the oocyte channel is a member of the Piezo family.[14]

We tested the involvement of the cytoskeleton in the inactivation by using cytoD to disrupt f-actin, and the only effect in patch recordings was a decrease in steady-state current, i.e., a smaller probability of being open, consistent with the data that actin can increase patch tension.[15] In a representative group of control cells from the same passage number, the peak to steady-state ratio was 18 ± 3.2 (n = 5) and 6.6 ± 3.1 (n = 5) for cells treated with cytoD. We found that ratio for cells from different passages were different as possibly reflecting changes in cytoskeletal properties.

Kinetic analysis showed that the inactivation rate in patches was insensitive to pipette pressure. The inactivation rate is similar to that observed for endogenous MSCs in astrocytes.[16] Extracellular Mg^{2+} and Ca^{2+} had little effect on the inactivation rate relative to a divalent ion free solution, but Zn^{2+} caused a 3-fold decrease in the inactivation rate.

Divalents decreased the single channel conductance as expected for a cation channel. However, the lack of chemical specificity among the divalents suggested a surface charge effect where the divalents were bound near the pore and repelled incoming cations.[17] Based upon the ability of divalents to reduce conductance, we expected that removal of divalents from the bath in a whole-cell recording would increase the currents, but it had the opposite effect. The loss of divalents apparently caused a breakdown of the stress transmitting network in the cell but not the patch. This suggests there are at least two types of divalent binding sites in the cell that affect the channel.[18,19]

The most striking difference between patch and whole-cell currents was the effect of cytoD. cytoD had minor effects on patch currents, but it significantly inhibited the whole cell currents. This inhibition could be partially relieved by swelling the cells with hypotonic solutions. If the stress caused by indentation passed through actin on its way to the channel, that might explain the results. Bacteria MSCs are activated by bilayer

tension,[20,21] and if we assume the same for Piezo1, indentation of the cell should cause an increase in bilayer tension by actin pulling on membrane embedded proteins.[15,22] A breakdown of actin would relax the stress and reduce channel activation. That model permits osmotic swelling to potentiate activation by increasing stress in the bilayer. The rapid inactivation of Piezo1 suggests that it evolved to be a sensor for transient not steady-state stress. However, we know that with repeated stress the channels can lose inactivation and that makes them sensitive to steady-state stress. That sensitivity can lead to calcium loading of mechanically stimulated cells and that may have clinical consequences. In muscular dystrophy, for example, loss of the dystrophin lattice transfers stress to other parts of the cortex leading to activation of MSCs.[15,23,24] Similarly, the stresses in sickled red cells could be sustained and lead to calcium loading.[25] The potentiating effect of cell swelling of Piezo1 gating may have major implications for pathology as cell swelling is one of the most common markers of inflammation and cell damage.

Remarkably, the loss of inactivation with extended stress occurred in all channels at the same time. If channels lost inactivation one at a time we would observe a time-dependent mix of two populations: transients plus plateaus. As illustrated in **Figures 1 and 2**, however, we rarely observed currents that were a mix of both populations. This implies that the channels exist in discrete domains, and that breakdown of the domain boundary can change the properties of all the channels in the domain. Domain breakage is more likely with large and/or repetitive stimulation.[12,26-28] The physical domain could be lipids such as a raft, or a more complicated structure such as a caveolus,[29] a protein corral[28] or the patch dome and seal. We can make some crude estimates of the domain size. We often measured patch currents from 50–100 channels so the domain is clearly smaller than the patch and much larger than one channel. A close packed array of channels could probably fit in a domain of ~100nm scale. We don't yet know whether inactivation is a property of the channel protein itself or involves other components of the localizing domain such as the cytoskeleton.

In summary, while Piezo1 responses in a patch emulate many of the responses seen in whole-cell mode, there are significant differences, presumably in the force transfer structures. Extrapolation from the patch to functionality in situ deserves caution.

Experimental

Electrophysiology. The extracellular solution contained 145 NaCl, 5 KCl, 3 $MgCl_2$, 10 HEPES and 0.1 $CaCl_2$ (in mM, pH adjusted to 7.3 with NaOH). For both patches and whole cell, the common pipette solution was 140 KCl, 10 HEPES and 0.5 EGTA. For each experiment we have reported the divalent concentration, patch pressure and potential. For experiments with cytoD, cells were treated with 4–6 μMcytoD in bath solution for 30 min at 37°C and patched in the presence of cytoD. The mechanical stimulus in the patch was applied as pipette pressure using a high speed pressure clamp[30] (HSFC-1, ALA Scientific Instruments) controlled by QuBIO software (www.qub.buffalo.edu). HEK293 cells were transfected with Fugene according to manufacturer's specifications (Roche Diagnostic) using 0.5–1.0 μg of plasmid (Gift of A. Patapoutian, Scripps Institute) and were tested 24–48 h later. All experiments were done at room temperature. Currents were sampled at 20 kHz, filtered at 2 kHz and collected using QuBIO software. Whole-cell and patch-clamp experiments used an Axopatch 200B amplifier (Axon Instruments). Hypotonic swelling with 50% of normal osmolarity was initiated by adding distilled water in equal volume to the bath solution.

Whole-cell mechanical stimulation[1] utilized a fire-polished glass pipette (tip diameter 2–4 μm) positioned at an angle of 45° to the cell. A computer controlled micromanipulator (MP-285, Sutter Instruments Co.) using LabVIEW software provided coarse positioning of the probe to ~10 μm from the cell. From that position, further rapid downward movement was driven by a piezoelectric stage (P-280.20 XYZ NanoPositioners, PhysikInstrumente) until the pipette crossed a "threshold," defined as the depth at which the probe visibly deformed the cell. The probe velocity was 0.2 μm/ms during the upward and downward movement and the stimulus was maintained constant for 400 ms forming a trapezoidal stimulus. Stimulation was applied every 4 sec and currents were recorded most commonly at a holding potential of -60 mV.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, Petrus MJ, et al. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. Science 2010; 330:55-60; PMID:20813920; http://dx.doi.org/10.1126/science.1193270.
2. Coste B, Xiao B, Santos JS, Syeda R, Grandl J, Spencer KS, et al. Piezo proteins are pore-forming subunits of mechanically activated channels. Nature 2012; 483:176-81; PMID:22343900; http://dx.doi.org/10.1038/nature10812.
3. Bae C, Sachs F, Gottlieb PA. The mechanosensitive ion channel Piezo1 is inhibited by the peptide GsMTx4. Biochemistry 2011; 50:6295-300; PMID:21696149; http://dx.doi.org/10.1021/bi200770q.
4. Kim SE, Coste B, Chadha A, Cook B, Patapoutian A. The role of Drosophila Piezo in mechanical nociception. Nature 2012; 483:209-12; PMID:22343891; http://dx.doi.org/10.1038/nature10801.
5. Suchyna TM, Markin VS, Sachs F. Biophysics and structure of the patch and the gigaseal. Biophys J 2009; 97:738-47; PMID:19651032; http://dx.doi.org/10.1016/j.bpj.2009.05.018.
6. Honoré E, Patel AJ, Chemin J, Suchyna T, Sachs F. Desensitization of mechano-gated K2P channels. Proc Natl Acad Sci USA 2006; 103:6859-64; PMID:16636285; http://dx.doi.org/10.1073/pnas.0600463103.
7. Milescu LS, Akk G, Sachs F. Maximum likelihood estimation of ion channel kinetics from macroscopic currents. Biophys J 2005; 88:2494-515; PMID:15681642; http://dx.doi.org/10.1529/biophysj.104.053256.
8. SuchynaT, SachsF. The link between patch capacitance and mechanosensitive channel function probed with MSC blockers and activators. Biophysical Journal 2004; 86:548.
9. Spagnoli C, Beyder A, BeschS, Sachs F. Atomic force microscopy analysis of cell volume regulation. Phys Rev E Stat Nonlin Soft Matter Phys 2008; 78:031916; PMID:18851074; http://dx.doi.org/10.1103/PhysRevE.78.031916.

10. Evans E, Smith BA. Kinetics of hole nucleation in biomembrane rupture. New J Phys 2011; 13:095010; PMID:21966242; http://dx.doi.org/10.1088/1367-2630/13/9/095010.
11. Chen C, Smye SW, Robinson MP, Evans JA. Membrane electroporation theories: a review. Med Biol Eng Comput 2006; 44:5-14; PMID:16929916; http://dx.doi.org/10.1007/s11517-005-0020-2.
12. Akinlaja J, Sachs F. The breakdown of cell membranes by electrical and mechanical stress. Biophys J 1998; 75:247-54; PMID:9649384; http://dx.doi.org/10.1016/S0006-3495(98)77511-3.
13. Bae C, Markin V, Suchyna T, Sachs F. Modeling ion channels in the gigaseal. Biophys J 2011; 101:2645-51; PMID:22261052; http://dx.doi.org/10.1016/j.bpj.2011.11.002.
14. Hamill OP, McBride DW Jr. Mechanogated channels in Xenopus oocytes: different gating modes enable a channel to switch from a phasic to a tonic mechanotransducer. Biol Bull 1997; 192:121-2; PMID:9057280; http://dx.doi.org/10.2307/1542583.
15. Suchyna T, Sachs F. Mechanosensitive channel properties and membrane mechanics in mouse dystrophic myotubes. J Physiol 2007; 581:369-87; PMID:17255168; http://dx.doi.org/10.1113/jphysiol.2006.125021.
16. Suchyna TM, Besch SR, Sachs F. Dynamic regulation of mechanosensitive channels: capacitance used to monitor patch tension in real time. Phys Biol 2004; 1:1-18; PMID:16204817; http://dx.doi.org/10.1088/1478-3967/1/1/001.
17. Suchyna TM, Tape SE, Koeppe RE 2nd, Andersen OS, Sachs F, Gottlieb PA. Bilayer-dependent inhibition of mechanosensitive channels by neuroactive peptide enantiomers. Nature 2004; 430:235-40; PMID:15241420; http://dx.doi.org/10.1038/nature02743.
18. Ermakov YuA, Averbakh AZ, Arbuzova AB, Sukharev SI. Lipid and cell membranes in the presence of gadolinium and other ions with high affinity to lipids. 2. A dipole component of the boundary potential on membranes with different surface charge. Membr Cell Biol 1998; 12:411-26; PMID:10024973.
19. Ermakov YuA, Averbakh AZ, Sukharev SI. Lipid and cell membranes in the presence of gadolinium and other ions with high affinity to lipids. 1. Dipole and diffuse components of the boundary potential. Membr Cell Biol 1997; 11:539-54; PMID:9553941.
20. Sukharev SI, Martinac B, Blount P, Kung C. Functional reconstitution as an assay for biochemical isolation of channel proteins: application to the molecuar identification of a bacterial mechanosensitive channel. Methods: A Companion to Methods in Enzymology 1994; 6:51-9.
21. Sukharev SI, Blount P, Martinac B, Blattner FR, Kung C. A large-conductance mechanosensitive channel in E. coli encoded by mscL alone. Nature 1994; 368:265-8; PMID:7511799; http://dx.doi.org/10.1038/368265a0.
22. Ayalon G, Hostettler JD, Hoffman J, Kizhatil K, Davis JQ, Bennett V. Ankyrin-B interactions with spectrin and dynactin-4 are required for dystrophin-based protection of skeletal muscle from exercise injury. J Biol Chem 2011; 286:7370-8; PMID:21186323; http://dx.doi.org/10.1074/jbc.M110.187831.
23. Gailly P, De Backer F, Van Schoor M, Gillis JM. In situ measurements of calpain activity in isolated muscle fibres from normal and dystrophin-lacking mdx mice. J Physiol 2007; 582:1261-75; PMID:17510188; http://dx.doi.org/10.1113/jphysiol.2007.132191.
24. Yeung EW, Whitehead NP, Suchyna TM, Gottlieb PA, Sachs F, Allen DG. Effects of stretch-activated channel blockers on [Ca2+]i and muscle damage in the mdx mouse. J Physiol 2005; 562:367-80; PMID:15528244; http://dx.doi.org/10.1113/jphysiol.2004.075275.
25. Vandorpe DH, Xu C, Shmukler BE, Otterbein LE, Trudel M, Sachs F, et al. Hypoxia activates a Ca2+-permeable cation conductance sensitive to carbon monoxide and to GsMTx-4 in human and mouse sickle erythrocytes. PLoS One 2010; 5:8732; PMID:20090940; http://dx.doi.org/10.1371/journal.pone.0008732.
26. Sachs F. Mechanical transduction by ion channels: how forces reach the channel. [Review] [52 refs]. Soc Gen Physiol Ser 1997; 52:209-18; PMID:9210231.
27. Bett GCL, Sachs F. Whole-cell mechanosensitive currents in rat ventricular myocytes activated by direct stimulation. J Membr Biol 2000; 173:255-63; PMID:10667920; http://dx.doi.org/10.1007/s002320001024.
28. Sachs F. Stretch-activated ion channels: what are they? Physiology (Bethesda) 2010; 25:50-6; PMID:20134028; http://dx.doi.org/10.1152/physiol.00042.2009.
29. Shevchuk AI, Hobson P, Lab MJ, Klenerman D, Krauzewicz N, Korchev YE. Endocytic pathways: combined scanning ion conductance and surface confocal microscopy study. Pflugers Arch 2008; 456:227-35; PMID:18180951; http://dx.doi.org/10.1007/s00424-007-0410-4.
30. Besch SR, Suchyna T, Sachs F. High-speed pressure clamp. Pflugers Arch 2002; 445:161-6; PMID:12397401; http://dx.doi.org/10.1007/s00424-002-0903-0.