Direct Mechano-Stress Sensitivity of TRPM7 Channel

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Key Words

TRPM7 • Mechanosensor • Shear stress • Osmotic swelling

Abstract

It has recently been shown that shear stress augments the heterologously expressed TRPM7 channel activity by exocytosis-mediated incorporation of TRPM7 into the plasma membrane. On the other hand, our recent study has shown that the TRPM7-like channel endogenously expressed in HeLa cells is activated by membrane expansion induced by membrane stretch or osmotic cell swelling. Thus, the present study was aimed at exploring the possibility that the heterologously expressed TRPM7 channel is activated directly by membrane expansion in a manner independent of exocytosis. Here, whole-cell currents of the TRPM7 channel heterologously expressed in HEK293T cells were found to be augmented not only by perfusion of bath solution but also by osmotic swelling even under the conditions where exocytotic events can hardly take place in the cytosol dialyzed with ATP-free, Ca²⁺-free and EGTA-containing pipette solution. In addition, shear stress-induced augmentation was not affected by a blocker of

vesicular protein traffic, brefeldin A. Furthermore, in cell-free patches, membrane stretch directly augmented single-channel activity of TRPM7 by increasing P_o value at ≤ 20 mV. We thus conclude that the TRPM7 channel can be directly activated by mechano-stress in a manner independent of exocytosis-mediated incorporation of this channel protein into the plasma membrane.

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Introduction

The stretch-activated cation (SAC) channel has essential roles in sensing touch, sound and gravity, as well as in detecting cell volume, cardiovascular tone and membrane tension during muscle contraction and cell motility or movement [1]. Since members of the transient receptor potential (TRP) channel family are known to be involved in sensory signal transduction for a large variety of other stimuli including light, temperature, osmolarity, sound, acid, pheromones, capsaicin and menthol [2, 3], some member of the TRP family might be a good candidate for the SAC channel in vertebrates. Actually, Oancea et al. [4] have demonstrated that shear stress

augments TRPM7 currents by increased exocytotic incorporation of TRPM7 into the plasma membrane in vascular smooth muscle cells and recombinant TRPM7-overexpressing HEK293T cells. On the other hand, we have recently shown that TRPM7-like channel endogenously expressed in HeLa cells are directly activated by membrane expansion induced by membrane stretch or osmotic cell swelling [5]. Thus, there arises questions whether the heterologously expressed TRPM7 channel is also activated by membrane expansion in cell-free excised patches and by osmotic cell swelling in the whole-cell mode and whether activation of this channel is induced by mechano-stress in a manner independent of exocytosis. Thus, the purpose of the present study is to examine mechano-sensitivity of heterologously expressed TRPM7 channel and its dependence on exocytotic events.

Materials and Methods

Cell culture and recombinant expression

HEK293T cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 40 units/ml penicillin, and 200 µg/ml streptomycin under a 95% air-5% CO₂ atmosphere at 37°C. For electrophysiological experiments, cells were detached from a plastic substrate and cultured in suspension with agitation for 15-300 min.

HEK293T cells in 6-well plates were transfected 24 h after plating with the plasmids pEGFP-N1 (Clontech, Mountain View, CA) and pCI-neo (Promega, Madison, WI) for mock-transfected (HEK/Mock) cells, or pEGFP-N1 and pCI-neo-TRPM7 (a generous gift from Dr. Y. Mori) for TRPM7-transfected (HEK/TRPM7) cells. Lipofectamine 2000 (Invitrogen, Carlsbad, CA) was used for transfections according to the manufacturer's instructions.

Patch-clamp experiments

Whole-cell and single-channel recordings were performed at room temperature (22-26°C). To minimize K⁺ and Cl⁻ currents, patch-clamp experiments were carried out under K⁺-free, Cs⁺-rich, Cl⁻-deficient and aspartate-rich conditions. The patch electrode had a resistance of around 2 MΩ for whole-cell recordings and of around 7 MΩ for single-channel recordings. Currents were recorded using an Axopatch 200B amplifier (Axon Instruments, Union City, CA). Current signals were filtered at 5 kHz and digitized at 20 kHz. pClamp software (version 9.0.2; Axon Instruments) was used for command pulse control, data acquisition and analysis.

In whole-cell recordings, series resistance (< 5 MΩ) was compensated (to 70-80%) to minimize voltage errors. The intracellular (pipette) low-Cl⁻ isotonic (300 mosmol/kg-H₂O) or hypertonic (370 mosmol/kg-H₂O) solution contained (in mM): 100Cs-aspartate, 1EGTA, 10HEPES, 0.5CsCl, and 100 or

170mannitol, respectively (pH 7.4). In some experiments, 1 or 5 mM MgSO₄ was added to the intracellular solution. The isotonic Cl⁻-free bath solution (320 mosmol/kg-H₂O) contained (in mM): 100Cs-aspartate, 1EGTA, 10HEPES and 120mannitol (pH 7.4). When required, 0.1, 1 or 10 mM MgSO₄ was added to the bath solution. To examine Mg²⁺-free effects, 1 mM EGTA was replaced with 1 mM EDTA. Experiments were performed after stopping perfusion of bath solution, except when effects of perfusion-induced mechanical stress were analyzed.

Single-channel recordings were performed in the excised inside-out configurations. The amplitude of single-channel current was measured from the peak-to-peak distance evaluated by Gaussian fits of the amplitude histogram. The open probability (*P_o*) of single-channel current was calculated by dividing the total time spent in the open state by the total time of continuous recording (30-200 s) in the patches containing one active channel. The intracellular side was perfused with Cl⁻-free bath solution (300 mosmol/kg-H₂O) consisting of (in mM) 100Cs-aspartate, 1EGTA, 10HEPES and 100mannitol (pH 7.4 adjusted with CsOH), and the extracellular (pipette) solution which contained (in mM) 100Cs-aspartate, 1EGTA, 10HEPES, 0.5CsCl, and 100 or 180mannitol (pH 7.4) was used.

Statistical analysis

Data are presented as means ± S.E.M. of *n* observations. Statistical differences of the data were evaluated by the paired or unpaired Student's *t* test and were considered significant at *P* < 0.05.

Results and Discussion

Shear stress induced augmentation of whole-cell TRPM7 currents in a manner independent of exocytosis

When whole-cell patch-clamp recordings were performed in Cl⁻-deficient, Cs⁺-rich solutions, we could consistently observe spontaneous activation of macroscopic cation currents in HEK/TRPM7 cells in the absence or presence of 1 mM Mg²⁺ in the extracellular solution (Fig. 1), as originally reported [6, 7]. In contrast, virtually no current could be recorded in HEK/Mock cells (Fig. 1) or in HEK/TRPM7 cells in the presence of 10 mM Mg²⁺ added to the extracellular solution (data not shown). Under ramp-clamp from -100 to +100 mV (4 mV/ms), the whole-cell currents exhibited steep outward rectification in the presence of extracellular Mg²⁺ (1 mM). When extracellular Mg²⁺ was removed, the macroscopic current markedly increased, and its outward rectification became less prominent. These results are in good agreement with the properties of TRPM7 current reported previously [6-10]. Under divalent cation-free conditions, however, the *I-V* relationship still exhibited outward rectification in the voltage range between -50 and +100 mV, and the current

Fig. 1. Effects of removal of extracellular Mg^{2+} on whole-cell TRPM7 currents under ramp-clamp. Representative I - V relationships of whole-cell cation currents in a HEK/TRPM7 cell in the presence ($1Mg^{2+}$) and absence ($0Mg^{2+}$) of extracellular Mg^{2+} (1mM) and in a HEK/Mock cell in the absence of Mg^{2+} (Mock). Ramp pulses (50ms duration) were applied from -100 to +100mV to the cells without applying shear stress and osmotic perturbation. Data are representative of 8 experiments.

showed inward rectification at negative potentials below -50mV. Therefore, it appears that Mg^{2+} block is a cause of outward rectification of the macroscopic TRPM7 current in the presence of extracellular Mg^{2+} (1mM). Under divalent cation-free conditions, however, an additional mechanism(s) causing outward rectification of the TRPM7 channel current should exist.

To observe the effect of shear stress, bath solution in the chamber (135 μ l volume) was perfused at a flow rate of 15 μ l/s (at striped bars) or 35 μ l/s (at filled bars), which corresponds to 30 or 70dyne/cm² calculated by assuming ideal laminar flows [11] but should correspond to lesser shear stress under actual conditions where turbulent flows took place. The whole-cell current was reversibly increased in a manner dependent on the perfusion speed in HEK/TRPM7 cells (Fig. 2A: top panel), as reported previously [4]. Augmenting effects on TRPM7 currents were more prominent at negative potentials, and perfusion-induced mechano-stress converted the outwardly rectifying I - V relationship to a more ohmic one at ≥ -50 mV and even to an inwardly rectifying one at large negative potentials (≤ -50 mV), as shown in Fig. 2A (bottom panel).

When step pulses (50ms duration) were applied from a pre-potential of -80mV to test potentials to +80mV in 20-mV increments, time-independent currents were observed, as shown in Fig. 2B (top left panel). The I - V relationship exhibited outward rectification (circles). During perfusion, inward currents were more markedly augmented than outward currents (top right panel), and the I - V relationship became more ohmic (triangles). Thus,

it is concluded that shear stress augments, in a voltage-dependent manner, whole-cell TRPM7 currents.

Since these whole-cell experiments were performed under the conditions where the cytosol was dialyzed with ATP-free, Ca^{2+} -free and EGTA-containing pipette solution, it is unlikely that exocytotic events are involved in the shear stress-induced response. In fact, essentially identical perfusion-induced augmentation of whole-cell currents were observed in the presence of 5 μ M brefeldin A (BFA), which blocks membrane traffic from the *trans*-Golgi compartment to the cell surface [12], in HEK/TRPM7 cells that had been pretreated with 5 μ M BFA for 30 - 60min (Fig. 2C). Thus, it appears that shear stress-induced augmentation of macroscopic TRPM7 current was not due to exocytotic insertion of TRPM7 into the plasma membrane.

Cell swelling also induced augmentation of whole-cell TRPM7 currents

The TRPM7-like channel endogenously expressed in HeLa cells has recently been shown to respond to membrane expansion induced by osmotic cell swelling [5]. Similarly, in HEK/TRPM7 cells, osmotic cell swelling induced by intracellular hypertonicity significantly augmented the whole-cell TRPM7 current elicited by step pulses, as shown in Fig. 3A. Since swelling-induced augmentation was more marked at negative potentials, the I - V relationship of steady-state currents became less outwardly rectified (filled circles) compared to the control currents which showed prominent outward rectification (open circles). In contrast, osmotic swelling never affected

Fig. 2. Augmentation of whole-cell TRPM7 currents by shear stress. **A.** Effects of perfusion of bath solution on whole-cell TRPM7 currents under ramp-clamp (every 6s at 4mV/ms) in HEK/TRPM7 cells. Top panel: Representative peak outward and inward currents recorded at +100 and -100mV, respectively, before, during and after perfusion at a flow rate of 15 μ l/s (at striped bars) or 35 μ l/s (at filled bars). Bottom panel: Representative I - V relationships of currents in response to ramp pulses (50ms duration) from -100 to +100mV before (at 1 in a) and during (at 2 in top panel) perfusion. Data are representative of 31 experiments. **B.** Effects of perfusion (35 μ l/s) on current responses to step pulses (50ms duration) applied from a pre-potential of -80mV to test pulses to +80mV (in 20-mV increments) in HEK/TRPM7 cells. Top panel: Representative current responses. Arrowheads represent the zero-current level. Bottom panel: I - V relationships of steady-state currents in response to step pulses before (Control) and during perfusion (Perfused: 35 μ l/s). *, significantly different from control currents recorded at the same voltages. **C.** Effects of brefeldin A (BFA) on perfusion-induced augmentation of whole-cell TRPM7 currents. Peak outward and inward currents recorded at +100mV (top panel) and -100mV (bottom panel), respectively, before (Non-perfused) and after (Perfused) perfusion of bath solution at a flow rate of 35 μ l/s in HEK/TRPM7 cells with (BFA) or without (Control) BFA treatment. BFA and Control experiments were performed with 5 μ M BFA plus vehicle (0.1% DMSO) and vehicle alone, respectively, by pretreatment for 30 to 60min and by continued exposure during experiments. Each column represents the mean $\pm$ S.E.M. (vertical bar). *, Significantly different from non-perfused currents recorded at the same voltages. No significant differences were observed between Control and BFA data for either the Non-perfused or Perfused experiment.

whole-cell currents recorded in HEK/Mock cells (triangles). Thus, it is concluded that osmotic swelling can also augment the macroscopic TRPM7 current; moreover, it shifts the I - V relationship from an outwardly rectified one to one that is more ohmic at ≥ -50 mV and inwardly rectified at more negative potentials.

Swelling-activated whole-cell TRPM7 currents were largely blocked by 1mM Mg^{2+} added to the intracellular solution in a voltage-independent manner, as summarized in Fig. 3B (Mg^{2+}_i (1mM)). The TRPM7 currents were completely blocked by intracellular Mg^{2+} at 5mM (Mg^{2+}_i (5mM)), as reported previously [9, 13]. The effect of

Fig. 3. Augmentation of whole-cell TRPM7 currents by osmotic swelling. A. Effects of osmotic swelling on whole-cell cation currents elicited by step pulses (50ms duration). Top panel: Representative current responses to step voltage pulses (in 20-mV increments or decrements) applied from a pre-potential of -80mV under control and swelling conditions in HEK/TRPM7 cells. Arrowheads represent the zero-current level. Bottom panel: $I-V$ relationships of steady-state whole-cell currents elicited by step pulses applied from a pre-potential of -80mV under control (open symbols) and swelling (filled symbols) conditions in HEK/TRPM7 (circles) and HEK/Mock (triangles) cells. Each symbol represents the mean $\pm$ S.E.M. (vertical bar). *, Significantly different from control TRPM7 currents recorded at the same voltages. B. Effects of 1 and 5mM intracellular Mg^{2+} (Mg_i^{2+}), 0.1 and 10mM extracellular Mg^{2+} (Mg_o^{2+}), 30 μ M Gd^{3+} , 100 μ M SKF and 100 μ M RR on swelling-activated cation currents under step pulse-clamp in HEK/TRPM7 cells. Data are normalized to the means of control current recorded at ± 80 mV in the absence of Mg^{2+} and drugs. All the data are significantly different from the control current.

0.1mM Mg^{2+} added to the extracellular solution was voltage-dependent. The currents were mostly blocked at negative potentials, while they were only partially suppressed at positive potentials by extracellular Mg^{2+} at 0.1mM (Mg_o^{2+} (0.1mM)). Extracellular Mg^{2+} almost completely blocked the currents recorded at ± 80 mV at 10mM (Mg_o^{2+} (10mM)). The whole-cell TRPM7 currents activated by cell swelling were voltage-independently blocked by extracellular application of 30 μ M Gd^{3+} , as previously observed in non-swollen HEK/TRPM7 cells [8, 9], and also by that of 100 μ M SKF-96365 (SKF). In

contrast, ruthenium red (RR) added to the extracellular solution at 100 μ M blocked the currents in a voltage-dependent manner. Thus, it appears that swelling-activated TRPM7 currents are sensitive to Mg^{2+} , Gd^{3+} , SKF and RR.

Membrane stretch also induced activation of single-channel currents of TRPM7 channels

Single-channel recordings performed in inside-out patches excised from HEK/TRPM7 cells (Fig. 4) demonstrated that activation of single-channel events was

Fig. 4. Activation of single TRPM7 channel currents by membrane stretch. **A.** Stretch-induced activation of single-channel currents. Top panel: representative unitary current responses to repeated applications (horizontal bars) of suction (8cm-H₂O) at +80mV. Middle panel: time-expanded trace of the designated portion of upper-panel trace. Bottom panel: P_o -pressure relationship for steady-state single-channel currents. The half-maximum activation pressure is 4.0cm-H₂O. Insets: Three representative records of unitary current responses among those plotted in the P_o -pressure relationship. **B.** Voltage dependence of the stretch-activated single TRPM7 channel activity. Top panel: representative single-channel activities induced by suction (8cm-H₂O) at different voltages. Middle panel: current-voltage (i - V) relationship for unitary currents of amplitude i . Bottom panel: P_o -voltage relationship for steady-state single-channel currents recorded during application of suction at 8cm-H₂O (filled circles) or 20cm-H₂O (open circles). The half-maximum activation voltage is 14.7mV at 8cm-H₂O.

consistently (in all of 88 patches) induced by gentle negative pressure (less than 15cm-H₂O) applied to the back of the pipette. These results are essentially similar to those observed for TRPM7-like channels endogenously expressed in HeLa cells [5]. In contrast, similar single SAC channel activities were not consistently observed in HEK/Mock cells (in 2 of 21 patches). As shown in Fig.

4A, repeated stretch-induced activation of single-channel events was observed at +80mV when negative pressure of 8cm-H₂O was repeatedly applied to the patches excised from HEK/TRPM7 cells (top panel). The P_o observed in excised patches that contained only one channel was increased with increasing negative pressures (insets), with a half-maximum activation pressure of

around 4cm-H₂O (bottom panel), which is comparable to that observed for TRPM7-like channels endogenously expressed in HeLa cells [5].

Under divalent cation-free conditions, the amplitude of single SAC channel currents (*i*) changed with voltage (*V*), and the *i*-*V* relationship was almost linear in the range of -60 to +80mV, with a slope conductance of 26.3 ± 0.01 pS (*n*=16-21) and a reversal potential of 0mV (Fig. 4B, top and middle panels). The *i*-*V* curve exhibited inward rectification at negative potentials less than -60mV, and the slope conductance increased to ~31 pS at -80 to -60mV and ~73pS at -100 to -80mV. The results are essentially similar to those observed for TRPM7-like channels endogenously expressed in HeLa cells [5]. Also, these values of single-channel conductance of heterologously expressed TRPM7 are comparable to those reported previously: 40pS at +60mV [6] and 105pS at +40 to +100mV [7] in the presence of extracellular divalent cations; as well as 40pS at -100mV [10, 14] and 28-48pS at +60mV [15] under divalent cation-free conditions.

When 5mM Mg²⁺ was added to the intracellular solution, the single-channel events of stretch-activated TRPM7 channel were almost completely abolished at both negative and positive potentials (data not shown).

At a constant level of mild membrane stretch (induced by negative pressure of 8cm-H₂O), the steady-state *P*_o was increased with depolarization under divalent cation-free conditions (Fig. 4B, bottom panel, filled circles). The voltage dependence of *P*_o would be a causal factor for outward rectification of whole-cell TRPM7 currents observed even under divalent cation-free

conditions (Figs. 1 and 2A).

On the other hand, at a constant level of strong membrane stretch (induced by negative pressure of 20cm-H₂O), the steady-state *P*_o became independent of voltage and was around 1 even at negative potentials (Fig. 4B, bottom panel, open circles). This microscopic *i*-*V* behavior matches the macroscopic *I*-*V* behavior, in which the relationship switched from being outwardly rectifying to being ohmic, due to mechanical stress caused by perfusion of bath solution (Fig. 2A, B) or osmotic cell swelling (Fig. 3A).

Taken together, it is concluded that the single TRPM7 channel is directly activated by membrane stretch which renders the channel more open by increasing the steady-state *P*_o at potentials more negative than +20mV and that the macroscopic TRPM7 channel activity is upregulated not only by shear stress but also by osmotic swelling.

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References

- Hamill OP, Martinac B: Molecular basis of mechanotransduction in living cells. *Physiol Rev* 2001;81:685-740.
- Clapham DE: TRP channels as cellular sensors. *Nature* 2003;426:517-524.
- Voets T, Nilius B: TRPs make sense. *J Membr Biol* 2003;192:1-8.
- Oancea E, Wolfe JT, Clapham DE: Functional TRPM7 channels accumulate at the plasma membrane in response to fluid flow. *Circ Res* 2006;98:245-253.
- Numata T, Shimizu T, Okada Y: TRPM7 is a stretch- and swelling-activated cation channel involved in volume regulation in human epithelial cells. *Am J Physiol Cell Physiol* 2006;in press (doi:10.1152/ajpcell.00367.2006).
- Nadler MJ, Hermosura MC, Inabe K, Perraud AL, Zhu Q, Stokes AJ, Kuroski T, Kinet JP, Penner R, Scharenberg AM, Fleig A: LTRPC7 is a Mg²⁺-ATP-regulated divalent cation channel required for cell viability. *Nature* 2001;411:590-595.

- 7 Runnels LW, Yue L, Clapham DE: TRP-PLIK, a bifunctional protein with kinase and ion channel activities. *Science* 2001;291:1043-1047.
- 8 Aarts M, Iihara K, Wei WL, Xiong ZG, Arundine M, Cerwinski W, MacDonal JF, Tymianski M: A key role for TRPM7 channels in anoxic neuronal death. *Cell* 2003;115: 863-877.
- 9 Schmitz C, Perraud AL, Johnson CO, Inabe K, Smith MK, Penner R, Kurosaki T, Fleig A, Scharenberg AM: Regulation of vertebrate cellular Mg^{2+} homeostasis by TRPM7. *Cell* 2003;114:191-200.
- 10 Kozak JA, Matsushita M, Nairn AC, Cahalan MD: Charge screening by internal pH and polyvalent cations as a mechanism for activation, inhibition, and rundown of TRPM7/MIC channels. *J Gen Physiol* 2005;126:499-514.
- 11 Bird RB, Stewart WE, Lightfoot EN: *Transport Phenomena* 1960;pp.1-780 Wiley, NY.
- 12 Miller SG, Carnell L, Moore HH: Post-Golgi membrane traffic: brefeldin A inhibits export from distal Golgi compartments to the cell surface but not recycling. *J Cell Biol* 1992;118:267-283.
- 13 Demeuse P, Penner R, Fleig A: TRPM7 channel is regulated by magnesium nucleotides via its kinase domain. *J Gen Physiol* 2006;127:421-434.
- 14 Li M, Jiang J, Yue L: Functional characterization of homo- and heteromeric channel kinases TRPM6 and TRPM7. *J Gen Physiol* 2006;127:525-537.
- 15 Runnels LW, Yue L, Clapham DE: The TRPM7 channel is inactivated by PIP_2 hydrolysis. *Nat Cell Biol* 2002;4:329-336.