Biochemical and Biophysical Research Communications xxx (2017) 1–5

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Hypotonicity activates a voltage-dependent membrane conductance in N2a neuroblastoma cells

Akiyuki Taruno [a, *], Yoshinori Marunaka [a, b, **]

[a] Department of Molecular Cell Physiology, Kyoto Prefectural University of Medicine, 465 Kajiicho Kamigyo-ward, Kyoto 602-8566, Japan
[b] Department of Bio-Ionomics, Kyoto Prefectural University of Medicine, 465 Kajiicho Kamigyo-ward, Kyoto 602-8566, Japan

ARTICLE INFO

Article history:
Received 19 January 2017
Accepted 22 January 2017
Available online xxx

Keywords:
Cell swelling
Hypotonic stress
Voltage-gated
Ion channel

ABSTRACT

To maintain cellular and bodily homeostasis, cells respond to extracellular stimuli including osmotic stress by activating various ion channels, which have been implicated in many physiological and pathophysiological conditions. However, cellular osmosensory mechanisms remain elusive. Here, we report a novel voltage-dependent current in N2a cells activated by exposure to hypotonic stress. After a hypotonic challenge, N2a cells sequentially develop two distinct currents. The volume-regulated anion channel (VRAC) current emerges first and, after a delay, activation of a previously uncharacterized strongly outwardly rectifying current follows. The latter, delayed current (I_d) is insensitive to NPPB, a nonspecific blocker of Cl^- channels, and intracellular Mg^{2+}, which inhibits VRAC and swelling-activated TRPM3 and TRPM7 channels. Replacement of extracellular Na^+ with $NMDG^+$ reduces inward tail currents, suggesting that I_d is mediated by cations. Finally, I_d shows voltage-dependent activation with slow activation kinetics and half-maximal activation at +76 mV. These pharmacological and biophysical characteristics of I_d are distinct from those of known osmotic cell swelling-activated ion channels. In conclusion, our data identify and characterize a novel osmotically-activated, voltage-dependent ion channel in N2a cells.

© 2017 Elsevier Inc. All rights reserved.

1. Introduction

Cells respond to changes in chemical and physical environments in order to maintain both cellular and bodily homeostasis. Ion channels play central roles in such functions. Because the lipid bilayer of the cell membrane is permeable to water but not to solutes, alterations in intra- and extracellular osmolarity lead to cell volume changes. Cell volume regulation is known to be crucial for cellular homeostasis, including but not limited to cell growth, cell proliferation, and cell death [1]. Therefore, dysfunction of cell volume regulation is associated with many pathophysiological conditions. To compensate for cell volume changes upon osmotic stresses, osmosensitive ion channels and transporters that regulate the passage of ions across cell membranes are activated directly via mechanical tension in the cell membrane or indirectly via intracellular signaling components. Cells can also sense and respond to direct mechanical stimuli via mechanically-activated ion channels [2,3]. Higher organisms have evolved mechanosensory systems in which specialized sensor cells detect and respond to a wide range of mechanical stimuli such as membrane stretch/vibration, hydrostatic pressure, and sheer stress. Examples are found in the somatosensory, cardiovascular, and renal systems to name a few. Perturbation of mechanosensation is devastating because touch and pain sensation as well as control of blood pressure and fluid balance in the body would be lost.

Various endogenous ionic currents activated by osmotic and mechanical stresses have been demonstrated in many cell types, and their physiological and pathophysiological relevance has been intensively studied. Conversely, osmotic and mechanical stress sensitivities have also been identified in many ion channels. Some

Abbreviations: G-V, conductance-voltage; I_d, delayed current; I_{tail}, tail current; NMDG, N-methyl-d-glucamine; NPPB, 5-nitro-2-(3-phenylpropylamino)benzoic acid; VRAC, volume-regulated anion channel.

* Corresponding author. Department of Molecular Cell Physiology, Kyoto Prefectural University of Medicine, 465 Kajiicho Kamigyo-ward, Kyoto 602-8566, Japan.
** Corresponding author. Department of Molecular Cell Physiology, Kyoto Prefectural University of Medicine, 465 Kajiicho Kamigyo-ward, Kyoto 602-8566, Japan.
E-mail addresses: taruno@koto.kpu-m.ac.jp (A. Taruno), marunaka@koto.kpu-m.ac.jp (Y. Marunaka).

http://dx.doi.org/10.1016/j.bbrc.2017.01.118
0006-291X/© 2017 Elsevier Inc. All rights reserved.

Please cite this article in press as: A. Taruno, Y. Marunaka, Hypotonicity activates a voltage-dependent membrane conductance in N2a neuroblastoma cells, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.01.118

of these ion channels have been shown to mediate those endogenous currents. Despite recent advances in the field, our understanding of cellular osmo- and mechano-sensing mechanisms is still incomplete. Here, we report a previously uncharacterized hypotonicity-activated voltage-dependent ion channel in N2a neuroblastoma cells. Our data demonstrate that this ion channel is pharmacologically and biophysically distinct from known osmosensitive ion channels, providing evidence for a novel osmotically activated ion channel.

2. Materials and Methods

2.1. Cell culture

Mouse neuroblastoma N2a cells (ATCC® CCL-131) were grown in Minimum Essential Medium (Thermo Fisher Scientific, Waltham, USA) containing 10% fetal bovine serum (Thermo Fisher Scientific) and 1x Antibiotic-Antimycotic (100 μg/mL streptomycin, 100 units/mL penicillin and 0.25 μg amphotericin B) (Thermo Fisher Scientific) in a humidified atmosphere at 37 °C with 5% CO_2. Cells were plated on glass coverslips (Matsunami Glass, Osaka, Japan) coated with poly-L-lysine (Sigma-Aldrich, St. Louis, USA) at least 2 days prior to electrophysiological recording.

2.2. Electrophysiological recording

Whole cell membrane currents were recorded under a voltage clamp using the whole cell patch-clamp configuration with 3.0–4.5 MΩ borosilicate glass pipettes. Cells were continuously perfused (~3 mL/min) at room temperature (24 ± 2 °C) with bath solutions. The isotonic bath solution (pH 7.4) contained 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM tetraethylammonium-Cl, 1 μM tetrodotoxin, 10 mM glucose, and 10 mM HEPES. To reduce the extracellular osmotic pressure, 50 mM NaCl was removed from the isotonic bath solution (hypotonic bath solution (pH 7.4): 90 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM tetraethylammonium-Cl, 1 μM tetrodotoxin, 10 mM glucose, and 10 mM HEPES). 100 μM 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) was added to the bath solutions where indicated. To replace Na^+ with N-methyl-d-glucamine ($NMDG^+$) in the hypotonic bath solution, NaCl was replaced with equimolar NMDG-Cl. The intracellular pipet solution contained 140 mM CsCl, 5 mM NaCl, 1 or 7 mM $MgCl_2$, 10 mM tetraethylammonium-Cl, 5 mM EGTA, and 10 mM HEPES (pH 7.4). tetraethylammonium and tetrodotoxin were added to block endogenous K^+ and Na^+ channels, respectively. The voltage across the plasma membrane was controlled and whole currents were recorded with an Axopatch 1D amplifier and a Digidata 1321A interface using pCLAMP 8 acquisition software (Molecular Devices, Sunnyvale, CA, USA). Currents were low-pass filtered at 500 Hz with a four-pole Bessel characteristic and sampled at 5 kHz. Cells were held at −60 mV and ramp voltage pulses (−100 to +100 mV over 1 s) were applied every 5 s. In some experiments, cells were held at −60 mV and stepped to various test voltages every 3 s ranging from −100 to +100 mV in 10-mV increments for 200 ms, followed by a short (150 ms) hyperpolarization to −100 mV. The inward tail current (I_{tail}) at each test voltage was calculated as a positive value by subtracting the holding current at −100 mV from the peak inward current immediately after the end of the test pulse. To define the conductance-voltage (*G-V*) relationship for channel activation, I_{tail} at various test voltages (*Vm*) was fitted with a two-state Boltzmann equation: $G/G_{max} = I_{tail}/I_{tail,\ max} = 1/\{1 + exp[(V_{1/2} - Vm)/k]\}$, where $I_{tail,\ max}$ is the maximum tail current amplitude, $V_{1/2}$ is the half-activation voltage, and k is the slope factor. G/G_{max}, which represents the normalized tail current amplitude, was plotted against the membrane voltage for presentation of the *G-V* relationship.

3. Results and discussion

3.1. Hypotonic stress activates two membrane currents in N2a cells

Whole cell voltage clamp recordings were made in N2a cells with continuous perfusion of the extracellular solution. The membrane potential was held at −60 mV and voltage ramp pulses (−100 to +100 mV over 1 s) were applied every 5 s. Under an isotonic condition where endogenous voltage-gated Na^+ and K^+ channels are blocked (see Materials and Methods), whole cell currents induced by the voltage ramp were negligible, indicating minimal residual currents (Fig. 1A). After bath perfusion was switched to a hypotonic solution, which causes a 100-mOsmol reduction in osmolarity, two distinct membrane currents were sequentially induced (Fig. 1A). A modestly outwardly rectifying current with no tail currents at −60 mV initially developed. Subsequently, a strongly outwardly rectifying current with a large tail current at −60 mV appeared 3–5 min after the onset of hypotonic stress. Hereafter, the delayed current is referred to as I_d. The application of 200 ms-voltage step pulses (from −100 to +100 mV with an increment of 10 mV, followed by holding at −100 mV for 150 ms) revealed that the initial current showed no time-dependent activation but rather slight inactivation at depolarized potentials, whereas I_d superimposed to the initial current was slowly activated by depolarization and exhibited a large tail current at −100 mV (Fig. 1B). The different properties of the two currents suggest that they are mediated by different ion channels. The initial current was previously reported by Carpaneto et al. [4]. They demonstrated that exposure to a hypotonic stress of −60 mOsmol activates an ionic current in N2a cells similar to the initial current that we observed, and established that the current is mediated by anion-selective ion channels. The anion selectivity and shape of this current agree with the properties of the volume-regulated anion channel (VRAC) current whose molecular identity was recently identified as LRRC8 heteromers [5,6]. However, I_d has never been reported in any cells including N2a cells. We suggest that the stronger hypotonic stress we employed in this study revealed this previously unknown current. While the VRAC current was observed in all cell lines we tested, including N2a, HeLa, and HEK293 cells, I_d was activated only in N2a cells, suggesting that it plays roles in specific cell types, including neuronal cells. Thus, in N2a cells, hypotonic stress induces the initial VRAC current and the previously uncharacterized I_d.

3.2. I_d is insensitive to intracellular Mg^{2+} and NPPB

Several ion channels have been reported to be activated by osmotic cell volume changes. Hypotonic swelling-activated ion channels include: anion-selective channels such as VRAC [7,8], Ca^{2+}-activated Cl^- channels, and the pannexin 1 channel [9]; K^+ channels such as SK, IK, BK, K2P, and various K_v channels [7,10]; and non-selective cation channels such as TRP channels [3]. Among them, VRAC and swelling-activated TRPM3 [11] and TRPM7 [12,13] channels are inhibited by millimolar concentrations of intracellular free Mg^{2+} ($Mg^{2+}{}_i$) [14–16]. When whole cell currents from N2a cells were recorded with the same protocol used in Fig. 1 except that a pipet solution containing 7 mM Mg^{2+} was utilized, the activation of the initial current induced by a hypotonic challenge was markedly suppressed, while I_d was still induced (Fig. 2A–C), ruling out the involvement of VRAC, TRPM3, and TRPM7 channels in I_d. Furthermore, NPPB (100 μM, a non-specific blocker of Cl^-

Please cite this article in press as: A. Taruno, Y. Marunaka, Hypotonicity activates a voltage-dependent membrane conductance in N2a neuroblastoma cells, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.01.118

Download English Version:

https://daneshyari.com/en/article/5505373

Download Persian Version:

https://daneshyari.com/article/5505373

Daneshyari.com