Cell Calcium 55 (2014) 208–218

Contents lists available at ScienceDirect

Cell Calcium

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

Plasma membrane stretch activates transient receptor potential vanilloid and ankyrin channels in Merkel cells from hamster buccal mucosa

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ARTICLE INFO

Article history:
Received 17 October 2013
Received in revised form 5 February 2014
Accepted 20 February 2014
Available online 28 February 2014

Keywords:
Merkel cell
TRP
Mechanotransduction
Sensory transduction

ABSTRACT

Merkel cells (MCs) have been proposed to form a part of the MC-neurite complex with sensory neurons. Many transient receptor potential (TRP) channels have been identified in mammals; however, the activation properties of these channels in oral mucosal MCs remain to be clarified. We investigated the biophysical and pharmacological properties of TRP vanilloid (TRPV)-1, TRPV2, TRPV4, TRP ankyrin (TRPA)-1, and TRP melastatin (TRPM)-8 channels, which are sensitive to osmotic and mechanical stimuli by measurement of intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) using fura-2. We also analyzed their localization patterns through immunofluorescence. MCs showed immunoreaction for TRPV1, TRPV2, TRPV4, TRPA1, and TRPM8 channels. In the presence of extracellular Ca^{2+}, the hypotonic test solution evoked Ca^{2+} influx. The $[Ca^{2+}]_i$ increases were inhibited by TRPV1, TRPV2, TRPV4, or TRPA1 channel antagonists, but not by the TRPM8 channel antagonist. Application of TRPV1, TRPV2, TRPV4, TRPA1, or TRPM8 channel selective agonists elicited transient increases in $[Ca^{2+}]_i$ only in the presence of extracellular Ca^{2+}. The results indicate that membrane stretching in MCs activates TRPV1, TRPV2, TRPV4, and TRPA1 channels, that it may be involved in synaptic transmission to sensory neurons, and that MCs could contribute to the mechanosensory transduction sequence.

1. Introduction

Sensory receptors on the primary sensory afferents, such as Meissner's, Ruffini's, and Krause's corpuscles, free nerve endings, and Merkel cells (MCs), produce somatosensation in response to various stimuli. Among them, MCs have been identified and reported by Friedrich Merkel in the mammalian skin [1]. As sensory receptors, MCs are mainly found in touch-sensitive areas of the glabrous epidermis, such as in the outer root sheaths of hair follicles and of the oral mucosa [2]. In the oral mucosa, MCs constitute a distinct cell population localized at basal layer of the stratified squamous epithelium and are characterized by dense-core granules that contain a variety of neuropeptides [3,4]. Thus, MCs, have been suggested to form a part of the MC-neurite complex with myelinated Aβ-neurons, which act as mechanoelectric transducers [5,6], and they are classified as slowly-adapting type 1 (SA1) mechanoreceptors [7–9]. In addition, their contact points morphologically resemble chemical synapses [6].

To generate sensation of touch or pressure, activation of plasma membrane sensor proteins such as ionotropic/metabotropic-receptors as well as ionic channels are necessary to drive mechanical sensory transduction and to induce generator or receptor potentials following stimulation applied to the sensory receptors. However, how MCs, as believed to be mechanotransducer cells, receive mechanical cell deformation following mechanical stimuli has remained to be clarified. In the present study, we examined expression of plasma membrane sensor proteins in MCs, which transduct stimulation into sensation.

Transient receptor potential (TRP) channels, such as Ca^{2+}-permeable cation channels, have essential roles in transduction of a wide variety of sensations, which act as polymodal cell sensors and are also involved in a diverse variety of cellular processes [10,11]. In mammals, homologs of TRP channels are grouped into six main subfamilies [12]. Among them, TRP vanilloid (TRPV) channels consist of six members (TRPV subfamily member-1 [TRPV1] to TRPV6), and at least TRPV1, TRPV2, TRPV3, and TRPV4 channels

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http://dx.doi.org/10.1016/j.ceca.2014.02.015
0143-4160/

are highly expressed in primary sensory neurons [13–15]. These TRPV channels are known to contribute to the detection of thermal, mechanical, osmotic, and/or acidic stimuli as well as endogenous and exogenous agonists [14,16,17]. The TRP ankyrin (TRPA) subfamily member-1 (TRPA1) channel is also localized in the primary sensory neurons and activated by noxious cold (<17 °C) as well as chemical and mechanical stimuli, while TRP melastatin (TRPM) subfamily member-8 (TRPM8) channels are sensitive to thermal (25–28 °C) as well as chemical stimuli [18–20].

It has been demonstrated that $[Ca^{2+}]_i$ in rat vibrissal hair follicle MCs increases by shear mechanical force [21]. Mouse skin MCs expressed swelling-activated Ca^{2+} channels, which are activated by hypotonic stimulation [22], and mechanical stimulation by fine glass rods induced increases in $[Ca^{2+}]_i$ in the rat sinus hair follicle MCs [23]. Immunohistochemical study showed that expression of TRPV1 channels occurs in the plasma membrane in the MCs of both the outer and inner root sheath of human hair follicles [24]. In addition, it has been demonstrated that pharmacological activation of TRPV4 channels in the epidermal layer of the rat footpad MCs induce neurosecretory granule exocytosis [25]. However, the detailed biophysical, pharmacological, and mechanosensitive properties of TRP channels including TRPV1 and TRPV4 channels in MCs remain to be clarified. Furthermore, little information has been obtained detailing the functional properties and expression patterns of other mechanosensitive TRP channels in oral mucosa MCs.

Clarification is needed regarding whether MCs are capable of detecting cell membrane deformation via plasma membrane sensor proteins, which constitute the underlying transduction mechanism for the mechanical stimuli applied to MCs. To address this, we investigated the biophysical and pharmacological properties of various mechanosensitive TRP channel activations and their localization patterns in MCs from hamster buccal mucosa.

2. Materials and methods

2.1. Ethical approval

All animals were treated in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences approved by the Council of the Physiological Society of Japan and the American Physiological Society. The study also followed the guidelines established by the National Institute of Health in the USA regarding the care and use of animals for experimental procedures, as well as the UK Animals (Scientific Procedures) Act 1986. The study was conducted according to the Guidelines for the Treatment of Experimental Animals at Tokyo Dental College (approval No. 242503 and 252503).

2.2. Isolation of MCs from hamster buccal mucosa

MCs were isolated from the bulge on the epithelium in the mucosa inside the buccal pouch, named the touch dome (Fig. 1A and B) of male Syrian golden hamsters (4 weeks old). These touch domes (Fig. 1B) are known to have high mechanical sensitivity with high expression of mechanoreceptors [2,3].

To identify single MCs after isolation, a standard-extracellular solution (standard-ECS) containing quinacrine dihydrochloride, an MC-specific marker [26,27], was intraperitoneally (15 mg/kg) injected 12 h prior to MC isolation procedure. For isolation, Syrian golden hamsters were deeply anesthetized with sodium pentobarbitone (50 mg/kg *i.p.*, Kyoritsuseiyaku, Tokyo, Japan). Buccal mucosa including the touch domes were excised and treated by the standard-ECS containing DL-dithreitol (3 mg/mL) at 37 °C for 10 min to separate stratified squamous epithelium from buccal mucosa (Fig. 1C). The separated stratified squamous epithelium was then treated enzymatically with Ca^{2+} free standard-ECS containing 0.025% collagenase and 0.01% trypsin at 37 °C for 8 min and then rinsed with fresh standard-ECS. We then rubbed together the enzymatically treated stratified squamous epithelium carefully in the standard-ECS to be mechanically dissociated in order to obtain single epithelial cells including MCs (Fig. 1D). The suspended epithelial cells in standard-ECS were triturated carefully and plated onto poly-L-lysine-coated dishes (CORNING, Corning, NY, USA) and then incubated and maintained at 37 °C and 5% CO_2 for 2 h (Fig. 1E) before use [28].

2.3. Immunofluorescence analysis

To identify localization of TRP channel isoforms in MCs, acutely isolated epithelial cells were seeded on the 8-well Millicell EZ slides (Millipore; Billerica, MA, USA). After fixation with 4% paraformaldehyde and blocking with 10% goat serum, which were both diluted in the phosphate-buffered saline (PBS), acutely isolated epithelial cells including MCs were incubated with a mouse monoclonal antibody against anti-human cytokeratin 20 (CK-20) (dilution 1:30; Dako, Glostrup, Denmark) [29] or rabbit polyclonal antibody against anti-mouse TRPV1 (dilution 1:400; Abcam, Cambridge, UK), anti-mouse TRPV4 (dilution 1:200; Abcam), anti-rat TRPA1 (dilution 1:100; Abcam), anti-human TRPM8 (dilution 1:200; Abcam), and anti-rat TRPV2 (dilution 1:200, Alomone, Jerusalem, Israel). These cells were then incubated with secondary antibodies (Alexa Fluor 568 goat anti-mouse IgG, or Alexa Fluor 568 goat anti-rabbit IgG, dilution 1:100; Invitrogen, Carlsbad, CA, USA) for fluorescence staining at room temperature for 30 min, and with 4′,6-diamino-2-phenylindole dihydrochloride (Invitrogen) for nuclear staining at room temperature for 5 min. Quinacrine-fluorescence was measured at 525 nm in response to an excitation wavelength of 385 nm. Negative controls were prepared by using non-immune IgGs diluted at an equivalent concentration to primary antibodies. Cells were examined using a conventional fluorescence microscope (Axiophot 2; Zeiss, Jena, Germany).

2.4. Solutions

Standard-ECS was comprised of the following (in mM): 136 NaCl, 5 KCl, 2.5 $CaCl_2$, 0.5 $MgCl_2$, 1.2 NaH_2PO_4, 11 glucose, and 12 $NaHCO_3$ (328 mOsm/L). The pH was adjusted to 7.4 by Tris. To adjust osmolality in the test-solution, we prepared a low-NaCl solution by reducing extracellular Na^+ in the standard-ECS to 36 mM (128 mOsm/L). By adding various concentrations of mannitol, isotonic, or hypotonic test solutions were prepared. To obtain isotonic test solutions (328 mOsm/L), 172 mM mannitol was added to 128 mOsm/L of low-NaCl solution. To obtain hypotonic solutions, various concentrations of mannitol ranging from 12 mM (which gives 140 mOsm/L in total) to 172 mM (which gives 328 mOsm/L in total) were added to the low-NaCl solution. To prepare extracellular Ca^{2+}-free test- or standard-ECS, extracellular Ca^{2+} was removed (0 mM) from these solutions. To obtain high-KCl solutions, we increased extracellular K^+ concentrations in the standard-ECS to 100 mM and reduced extracellular Na^+ in the standard-ECS to 41 mM.

2.5. Reagents

Fura-2-acetoxymethyl ester was obtained from Dojindo Laboratories (Kumamoto, Japan), and pluronic acid F-127 was purchased from Invitrogen. Pharmacological agents, capsazepine and A784168 (1-[3-(trifluoromethyl)pyridin-2-yl]-*N*-[4-(trifluoromethylsulfonyl)phenyl]-1,2,3,6-tetrahydropyridine-4-carboxamide) (TRPV1 channel antagonists), capsaicin (TRPV1