FEBS Letters 588 (2014) 3047–3054

journal homepage: www.FEBSLetters.org

Activation of TRPV1 mediates thymic stromal lymphopoietin release via the Ca^{2+}/NFAT pathway in airway epithelial cells

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ARTICLE INFO

Article history:
Received 11 March 2014
Revised 8 May 2014
Accepted 4 June 2014
Available online 12 June 2014

Edited by Michael R. Bubb

Keywords:
TRP channel
Transient receptor potential vanilloid 1
Protease-activated receptor-2
Thymic stromal lymphopoietin
Asthma
Nuclear factor of activated T-cells

ABSTRACT

The airway epithelium is exposed to a range of irritants in the environment that are known to trigger inflammatory response such as asthma. Transient receptor potential vanilloid 1 (TRPV1) is a Ca^{2+}-permeable cation channel critical for detecting noxious stimuli by sensory neurons. Recently increasing evidence suggests TRPV1 is also crucially involved in the pathophysiology of asthma on airway epithelium in human. Here we report that in airway epithelial cells TRPV1 activation potently induces allergic cytokine thymic stromal lymphopoietin (TSLP) release. TSLP induction by protease-activated receptor (PAR)-2 activation is also partially mediated by TRPV1 channels.

1. Introduction

Inflammatory airway diseases such as asthma are a prevailing global public health problem [1,2]. The airway epithelium is a frontline barrier responding to environmental stimuli including cold air, pollution, viral infection, allergens and such stimuli are well known to be associated with the risk of developing asthma [2]. These stimuli damage the lung epithelium by activating specific receptors on the cellular surface [3,4] like transient receptor potential (TRP) ion channels, which will induce inflammatory responses [5–8].

The transient receptor potential vanilloid type 1 (TRPV1) channel is a polymodal transducer activated by noxious physical and chemical stimuli [9,10]. TRPV1 channel is a temperature- and ligand-sensitive Ca^{2+}-permeable ion channel highly expressed in dorsal root ganglia (DRG) nociceptor neurons to detect pungent extracts, proton, heat and membrane depolarization [11–13]. However, the expression profile of TRPV1 channels is broad, particularly, these channels are functionally expressed in non-sensory tissues including the human epidermal keratinocyte and airway epithelium [14–16]. Recently, population-based genetic epidemiological studies provided evidence for the involvement of TRPV1 in symptoms typically associated with inflammation such asthma [17]. For instance, expression of TRPV1 is significantly up-regulated in the airway epithelia of patients with refractory asthma [18]. Moreover, environmental risk factors such as airborne 2- and 10-μM particulate matter and coal fly ash particulate matter cause lung epithelial toxicity through activation of TRPV1 channel. In addition, respiratory rhinovirus up-regulates TRPV1 expression level to trigger airway hypersensitivity [19–21]. Herein, we sought to elucidate the mechanisms how airway inflammation such as asthma is regulated by TRPV1 channel in airway epithelium.

TSLP is a protein belonging to the cytokine family, whose expression is linked to human allergic airway disease including asthma [22,23]. Mice overexpressing TSLP in the lungs showed spontaneous airway inflammation and hyperreactivity with features similar to asthma, suggesting TSLP is necessary and sufficient for the development of airway inflammation [22,24]. TSLP is also implicated to play a key role in a number of other disorders, such as atopic dermatitis, allergic rhinitis and autoimmunity [23,25]. The factors that can promote TSLP release include intracellular Ca^{2+} increase and protease-activated receptor-2 (PAR-2) activation [26,27]. PAR-2 is a protease-sensing G protein-coupled receptor and has been found to involve in airway inflammation and airway hyperresponsiveness, both are the prominent features of asthma. To date, the molecular mechanisms that control the expression and release of TSLP are still not unveiled.

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http://dx.doi.org/10.1016/j.febslet.2014.06.018
0014-5793/

In this report, we provide evidence that TRPV1 channel mediates an asthma-associated physiological response via inducing TSLP production. TRPV1 activation increases intracellular Ca^{2+} and then triggers nuclear factor of activated T-cells (NFAT) action to further enhance cytokine TSLP secreted from BEAS-2B cells. Our results suggest that TSLP release by PAR-2 activation also depends on Ca^{2+} influx and is partially dependent on TRPV1 channel activation. These findings suggest that TRPV1 and other Ca^{2+}-permeable ion channels are potential therapeutic targets for the treatment of airway inflammation such as asthma.

2. Materials and methods

2.1. Reagents

The PAR-2 agonist peptide, SLIGKV-NH_2 was purchased from Alomone Labs. Cyclosporin A was purchased from Santa Cruz. Ruthenium red (RR), capsaicin and capsazepine were purchased from Sigma–Aldrich. GCaMP6s-encoding plasmid was obtained from Addgene (Plasmid 40753). The siRNA against human TRPV1 (sc-36826A, F: gaagaccugucugcugaaatt, R: uuucagcagacaggucuuctt) and negative control siRNA were designed and synthesized by Santa Cruz.

2.2. Cell culture

BEAS-2B human bronchial epithelial cells were cultured in RPMI-1640 medium (Invitrogen) supplemented with 10% FBS (Gibco) at 37 °C with 5% CO_2.

2.3. Electrophysiology

BEAS-2B cells grown on glass coverslips were used for recording currents. Whole-cell membrane currents were recorded using a HEKA EPC10 amplifier. Patch pipettes were pulled by P-97 puller (Sutter) and then fire polished to a resistance of about 4 MΩ. Membrane potential were held at 0 mV and membrane currents were elicited by a 300-ms step to +80 mV followed by a 300-ms step to −80 mV at 1-s intervals (Fig. S1A). Mean current amplitude was analyzed at ±80 mV. During whole cell recording the series resistance was compensated by 80%. To record TRPV1 currents, both the external and internal solution contained 130 mM NaCl, 3 mM EGTA, and 10 mM HEPES (pH 7.2 with NaOH) as TRPV1 channel is sensitive to divalent cations including Ca^{2+} and Mg^{2+} from the intracellular and extracellular sides [17,28,29]. To record PAR2 activation-induced currents we employed an external solution containing 130 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl, 10 mM HEPES (pH 7.2 with NaOH); and an intracellular solution containing 135 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES (pH 7.2 with KOH). Chemical solutions were applied to the patch with gravity perfusion system (VC3-8C, ALA Scientific Instruments). All experiments were conducted at 22–24 °C.

2.4. GCaMP6s-based Ca^{2+} imaging

BEAS-2B cells were grown on glass coverslips and transfected with GCaMP6s-encoding plasmid using 2 μg GCaMP6s-encoding plasmid at 30–50% confluence. 24 h after transfection, cells were continuously perfused with physiological buffer containing 130 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl, 10 mM HEPES (pH 7.2 with NaOH) and images was captured on a Olympus IX-81 inverted fluorescence microscope equipped with a 75-W xenon arc lamp. Fluorophores were excited at 474 nm, and fluorescence intensity was measured through a 515/30-nm bandpass emission filter. Images were collected every 3 s, and exposure time was set to 300 ms. Background correction was performed by subtracting the average pixel intensity of a non-cellular region. The basal response was recorded for 30 s and then chemical solution was applied for 2 min. The fluorescence intensity was proportional to the Ca^{2+} concentration, thus the effect was quantified as a response ratio by dividing the chemical induced response amplitude by the basal response amplitude.

2.5. RNA isolation and Real-time (RT)-PCR

Total RNA was purified from BEAS-2B with TRIzol (Invitrogen). The primers used for RT-PCR amplification were as follows: hTSLP, 5′catggaagtgctgtcgaaga3′, 5′tttccgtgaccaatcctttc3′ (231 bp); hTRP V1, 5′ ggctgtcttcatcatcctgctgct3′, 5′gttcttgctctcctgtgcgatcttgt3′ (117 bp); β-actin, 5′atcatgtttgagaccttcaaca3′, 5′catctcttgctcgaagtcca3′ (322 bp). Reactions were made up to a final volume of 20.0 μl with sterile water. Quantitative RT-PCR analysis were performed using the iQ™5 Multicolor Real-Time PCR Detection System (Bio-Rad).

2.6. ELISA analysis of TSLP released in cell supernatants

The culture supernatants from BEAS-2B cells with or without stimulation were quantitated using hTSLP ELISA Kit according to the manufacturer's instructions (R&D Systems).

2.7. Immunofluorescent microscopy and image analysis

BEAS-2B cells were seeded on sterile slides and cultured in the absence/presence of stimuli for 24 h. In the next day the slides were fixed with poly-formaldehyde for 20 min then treated with 0.1% TritonX-100 for 5 min. The slides were incubated with rabbit anti-NAFT1 antibody (1:200, CST) or Rabbit anti-TRPV1 antibody (1:100, Alomone) overnight at 4 °C. After a second incubation with appropriate secondary antibodies rhodamine-conjugated anti-rabbit IgG (1:200, ZSGB-Bio, China), the slides were counterstrained with 1 mg/ml DAPI (4′-6-diamidino-2-phenylindole, Sigma).

2.8. Western blotting

Protein pellet were isolated with TRIzol Regent (Invitrogen). Proteins were separated by 10% SDS–PAGE and transferred to a PVDF membrane (Bio-Rad). Membranes were probed overnight with rabbit anti-TRPV1 (1:1000, Alomone) and mouse anti-β-actin (1:5000, Sigma) antibodies followed by incubation with HRP-conjugated goat anti-rabbit IgG (1:5000, ZSGB-Bio, China) and HRP-conjugated goat anti-mouse IgG (1:5000, Santa Cruz), membranes were analyzed using a Hitachi Genetic Systems (Hitachi software Engineering).

2.9. Statistical analysis

Data are presented as means ± S.E.M. Two-sided differences between two samples were analyzed with Student t test. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Activation of TRPV1 increases intracellular Ca^{2+} level in BESA-2B cells

First, we investigated if TRPV1 channels are expressed on BESA-2B cells. BEAS-2B cells are an SV40-transfected, immortalized, human bronchiolar epithelial cell line that is frequently used to study the mechanisms of airway inflammation in vitro [30]. Consistent with previous reports, Immunofluorescence studies