Biochimica et Biophysica Acta xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Identification of voltage-gated K^+ channel beta 2 (Kvβ2) subunit as a novel interaction partner of the pain transducer Transient Receptor Potential Vanilloid 1 channel (TRPV1)☆

Carlo Bavassano [a], Letizia Marvaldi [b], Michiel Langeslag [c], Bettina Sarg [d], Herbert Lindner [d], Lars Klimaschewski [b], Michaela Kress [c], Antonio Ferrer-Montiel [e], Hans-Günther Knaus [a,*]

[a] Division of Cellular and Molecular Pharmacology, Medical University Innsbruck, Peter-Mayr strasse 1, 6020 Innsbruck, Austria
[b] Division of Neuroanatomy, Medical University Innsbruck, Müllerstrasse 59, 6020 Innsbruck, Austria
[c] Division of Physiology, Medical University Innsbruck, Fritz-Pregl-Straße 3, 6020 Innsbruck, Austria
[d] Division of Clinical Biochemistry, Medical University Innsbruck, Innrain 80, 6020 Innsbruck, Austria
[e] IBMC, Universidad Miguel Hernandez Elche, Av. de la Universidad s/n., Edif. Torregaitán, E-03202, Spain

ARTICLE INFO

Article history:
Received 20 June 2013
Received in revised form 2 September 2013
Accepted 3 September 2013
Available online xxxx

Keywords:
Signaling complex
Dorsal root ganglia
Accessory subunit
TRPV1

ABSTRACT

The Transient Receptor Potential Vanilloid 1 (TRPV1, vanilloid receptor 1) ion channel plays a key role in the perception of thermal and inflammatory pain, however, its molecular environment in dorsal root ganglia (DRG) is largely unexplored. Utilizing a panel of sequence-directed antibodies against TRPV1 protein and mouse DRG membranes, the channel complex from mouse DRG was detergent-solubilized, isolated by immunoprecipitation and subsequently analyzed by mass spectrometry. A number of potential TRPV1 interaction partners were identified, among them cytoskeletal proteins, signal transduction molecules, and established ion channel subunits. Based on stringent specificity criteria, the voltage-gated K^+ channel beta 2 subunit (Kvβ2), an accessory subunit of voltage-gated K^+ channels, was identified of being associated with native TRPV1 channels. Reverse co-immunoprecipitation and antibody co-staining experiments confirmed TRPV1/Kvβ2 association. Biotinylation assays in the presence of Kvβ2 demonstrated increased cell surface expression levels of TRPV1, while patch-clamp experiments resulted in a significant increase of TRPV1 sensitivity to capsaicin. Our work shows, for the first time, the association of a Kvβ subunit with TRPV1 channels, and suggests that such interaction may play a role in TRPV1 channel trafficking to the plasma membrane.

© 2013 Published by Elsevier B.V.

1. Introduction

TRPV1 is a Ca^{2+} and Na^+-permeable ion channel responding to heat (>42 °C), acidosis (pH < 6), endovanilloids and a variety of chemicals of which capsaicin, the pungent component of red hot chili, is best known [1,2]. This ion channel is predominantly expressed in the peripheral sensory system, and therefore is in a strategic position to determine specificity, speed and modulation of sensory and nociceptive neurotransmission [2]. TRPV1 channels are associated with inflammatory pain and thermal hyperalgesia [3]: mice lacking TRPV1 channels are impaired in the detection of noxious heat, and show impaired thermal hypersensitivity [3].

TRPV1 is the ancestor of the transient receptor potential vanilloid channel family, which structurally resembles voltage-gated potassium channels [4]. Accordingly, these channels are presumed to be constituted of four identical subunits, each of which having six membrane-spanning domains (S1–S6) and intracellular carboxyl and amino termini [1].

Recent research indicates that many ion channels are organized in a multiprotein assembly, termed signaling complexes, where channel regulation is critically dependent on protein–protein interactions [5]. For instance, proteomic studies on NMDA receptors, or voltage-gated calcium channels, demonstrated close interaction of multiple polypeptides which modulate the ion channel function, some of which have previously not been identified of being associated with ion channels [6,7].

Abbreviations: AKAP, A kinase anchor protein; ATP, adenosine tris-phosphate; BSA, bovine serum albumin; cDNA, complementary deoxyribonucleic acid; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; CHO, Chinese hamster ovary; CGRP, calcitonin gene-related peptide; CMC, critical micelle concentration; DAPI, 4′,6-diamidino-2-phenylindole; DNA, deoxyribonucleic acid; DRG, dorsal root ganglia; DTT, di-tiothreitol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Kvβ2, voltage-gated K^+ channel subunit beta 2; Icaps, capsaicin-activated current; IgG, immunoglobulin G; PMSF, phenylmethylsulfonyl fluoride; PBS, phosphate buffer saline; SDS, sodium dodecyl sulfate; TRPV1, Transient Receptor Potential Vanilloid 1; WT mouse, wild type mouse

☆ This work was supported by the Austrian Research Foundation PhD program SPIN B05, Project Number ZFW1206i0-05.

* Corresponding author. Tel.: +43 512 9003 70440; fax: +43 512 9003 73440.
E-mail addresses: carlo.bavassano@i-med.ac.at (C. Bavassano), letizia.marvaldi@i-med.ac.at (L. Marvaldi), michiel.langeslag@i-med.ac.at (M. Langeslag), bettina.sarg@i-med.ac.at (B. Sarg), herbert.lindner@i-med.ac.at (H. Lindner), lars.klimaschewski@i-med.ac.at (L. Klimaschewski), michaela.kress@i-med.ac.at (M. Kress), aferrer@umh.es (A. Ferrer-Montiel), hans.g.knaus@i-med.ac.at (H.-G. Knaus).

0167-4889/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.bbamcr.2013.09.001

Please cite this article as: C. Bavassano, et al., Identification of voltage-gated K^+ channel beta 2 (Kvβ2) subunit as a novel interaction partner of the pain transducer Transient Receptor Potential..., Biochim. Biophys. Acta (2013), http://dx.doi.org/10.1016/j.bbamcr.2013.09.001

UNCORRECTED PROOF

ARTICLE IN PRESS

Recombinant TRPV1 channels retain functional properties similar to those of their native counter-parts, for instance, from sensory neurons [8]. However, the pharmacological profile of these recombinant channels differs from native channel, giving room for a possible co-association of accessory regulatory proteins in native channels [8]. Therefore, the peculiarity of TRPV1 channel signaling could be partly due to association of thus far uncharacterized proteins. In this context, a limited number of previous studies focused on protein–protein interaction of the pore-forming subunit TRPV1 with potential interaction partners [9–15]. For instance, tubulin was shown to be capable to associate with TRPV1 protein [9,10], demonstrating direct association of TRPV1 with cytoskeletal elements. In addition, co-immunoprecipitation and co-staining experiments indicated an intimate and physiologically relevant interaction of TRPV1 with TRPV2 subunits in rodent dorsal root ganglia (DRG), as well as in cell lines after recombinant channel expression [12,14]. The protein kinase A anchoring protein 150 (AKAP150) and the phosphoinositide-binding protein Pirt were also found to associate with TRPV1 channels [11,15]. The alternative approach of yeast two-hybrid screening identified two interacting proteins, snapin and synaptotagmin IX, which play a role in SNARE-dependent exocytosis, suggesting that their interaction with TRPV1 may modulate aspects of TRPV1 trafficking and/or delivery to the plasma membrane [13].

In order to deepen our insight into TRPV1 channel assembly, we embarked on a systematic approach of isolating native TRPV1 channel complexes through immunoprecipitation with anti-TRPV1 antibodies and identifying the isolated complex components by mass spectrometry. We report the identification of the voltage-gated K^+ channel accessory subunit beta 2 (Kvβ2) as a novel TRPV1 interacting protein. Thus far, Kvβ2 was believed to be an exclusive ancillary subunit of voltage-gated K^+ channels (Kv) which is associated with a cytoplasmic domain on pore-forming Kv1 (Shaker) alpha subunits [16]. Their functional contribution was shown to be either conferring an inactivation particle to the Kv1 channel family [17], or facilitating the trafficking of the Kv1 channel to the plasma membrane [18]. This is the first study that demonstrates that Kvβ2 is found in association with structurally different pore-forming subunits other than Kv channels.

2. Experimental procedures

2.1. Reagents

For immunoprecipitation experiments, two polyclonal sera were raised and affinity purified as previously published [19]. The sequences of the synthetic peptides employed and their location along the rat TRPV1 sequence are: EDA EVF KDS MVP GEK (anti-TRPV1$_{(824–838)}$) and EDP GNC EGV KRT LSF SLR (anti-TRPV1$_{(761–778)}$). The amino acid numbering refers to the rat TRPV1 clone isoform 1 (accession number: O35433). An additional rabbit anti-TRPV1 antibody was purchased from Sigma-Aldrich, directed against amino acids 817–838 of rat and mouse TRPV1. This antibody was used for immunostaining and Western blotting experiments. Monoclonal anti-Kvβ2 antibody (clone K17/70) was purchased from NIH Neuromab. Monoclonal anti-c-*myc* (clone 9E10) was from Sigma-Aldrich. Monoclonal anti-GAPDH-hrp (clone GAPdh-71.1) was from Sigma-Aldrich. Monoclonal anti-Na^+/K^+ ATPase α1 (clone C464.6) was from Upstate Biotechnology.

Fluorescently-labeled secondary antibodies, anti-rabbit Alexa fluor-488 and anti-mouse Alexa fluor-594, were from Invitrogen. Anti-fade mounting media were from Vectashield. High glucose DMEM for cell culture, poly-L-lysine and laminin were from Sigma-Aldrich. Fetal bovine serum, trypsin–EDTA, L-glutamine, streptomycin sulfate, and protein A coated Dynabeads were from Invitrogen. Liberase Blendzyme 1 was from Roche. TNB medium and Protein–Lipid-Complex were from Biochrom. Streptavidin magnetic beads and Sulf-NHS-LC-LC-Biotin were from Thermo Scientific.

Primers for subcloning were from Sigma, pcDNA3.1 and pcDNA3 plasmids were from Invitrogen, and pGFP-c1 plasmid was from Clontech.

Dodecanoyl sucrose was purchased from Merck (Darmstadt, Germany), mass spectrometry grade trypsin from Promega, and cell transfection reagent Metafectene Pro was from Biontex.

2.2. Animals

TRPV1 deficient mice (TRPV1$^{-/-}$) were purchased by Charles River–Jackson Laboratories (strain B6.129X1-Trpv1^{tm1Jul}/J). TRPV1$^{-/-}$ and C57Bl/6J wild type (WT) mice were housed and handled in accordance with the guidelines of the Directive 2010/63/EU for the use of laboratory animals.

2.3. Cell line culture

The mammalian cell line tsA201 is derived from human embryonic kidney HEK-293 cells by stable transfection with SV40 large-T antigen and has been reported to produce high levels of recombinant proteins [20]. N1E-115-1 cells are derived from mouse neuroblastoma and have been utilized in a wide range of functional studies [21]. tsA-201 cells were maintained at 37 °C and 5% CO_2 in high glucose DMEM supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin (PAA). Cells were transiently transfected with Metafectene Pro following manufacturers's guidelines. N1E-115-1 cells were maintained and transfected in the same conditions used for tsA-201 cells.

2.4. DRG membrane preparation and TRPV1 complex solubilization

DRG membrane fraction was prepared according to Berkefeld et al. [22] with minor modifications. Briefly, whole DRG from adult mice were dissected and collected in PBS containing 3 mM EDTA and 0.5 mM PMSF. Subsequently, they were homogenized with a glass potter in lysis buffer (10 mM Tris pH7.4, 1 mM EDTA, 1 mM iodoacetamide, 1 mM PMSF and protease inhibitors: 2 μM leupeptin, 1.5 μM aprotinin and 0.15 μM pepstatin). Low-speed centrifugation (1000 ×*g*) was performed twice to remove cellular debris, resuspending the pellet in lysis buffer in between. The resulting supernatant was centrifuged at high-speed (100,000 ×*g*) 15 min, the membrane fraction pellet was resuspended in 20 mM Tris, and protein concentration was determined. In order to solubilize the TRPV1 protein complex, the membrane fraction was incubated in solubilization buffer (1% dodecanoyl sucrose (w/v), 150 mM NaCl, 20 mM Tris pH7.4, 1 mM EDTA, 1 mM PMSF and protease inhibitors, in the same concentration as mentioned above), 30 min on ice. Subsequent high-speed centrifugation (100,000 ×*g*) 30 min led to the separation of non-soluble pellet from a solubilized supernatant. This fraction was used as starting material for the immunoprecipitation experiment described below.

2.5. Immunoprecipitation of TRPV1 protein complex

Antibodies immobilized on protein A-coated Dynabeads were incubated with the detergent-solubilized membrane fraction obtained from mouse DRG (see above) 2 h at 4 °C. 10 μg of antibody was used to precipitate 300 μg of total membrane extract for analytical experiments. For mass spectrometric sequencing, 3 mg of total membrane protein was used as starting material. After immunoprecipitation, the TRPV1 complex was eluted under denaturing conditions (Laemmli buffer without reducing agent). Thereafter, reducing agent dithiothreitol (DTT) was added to the sample (final concentration of 120 mM). For MS-sequencing, eluates were shortly run on SDS-PAGE minigels, stained by silver staining [23], divided into three molecular-weight ranges and separately subjected to in-gel trypsin digestion according to Shevchenko et al. [24], with the only modification that the gel pieces were incubated overnight at 37 ° C with trypsin (5 μg/ml).

UNCORRECTED PROOF

Please cite this article as: C. Bavassano, et al., Identification of voltage-gated K^+ channel beta 2 (Kvβ2) subunit as a novel interaction partner of the pain transducer Transient Receptor Potential..., Biochim. Biophys. Acta (2013), http://dx.doi.org/10.1016/j.bbamcr.2013.09.001

Download English Version:

https://daneshyari.com/en/article/8303935

Download Persian Version:

https://daneshyari.com/article/8303935

Daneshyari.com