Biochemistry and Biophysics Reports 4 (2015) 291–298

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

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

Identification of potential novel interaction partners of the sodium-activated potassium channels Slick and Slack in mouse brain

Sandra Rizzi [a,*], Christoph Schwarzer [b], Leopold Kremser [c], Herbert H. Lindner [c], Hans-Günther Knaus [a,*]

[a] Division of Molecular and Cellular Pharmacology, Medical University of Innsbruck, Peter-Mayr Str 1, Innsbruck, Austria
[b] Division of Pharmacology, Medical University of Innsbruck, Peter-Mayr Str 1 a, Innsbruck, Austria
[c] Division of Clinical Biochemistry, Biocenter, Medical University of Innsbruck, Innrain 80-82, Innsbruck, Austria

ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form
18 September 2015
Accepted 29 September 2015
Available online 9 October 2015

Keywords:
Slo2.1
Slo2.2
SAP 102
DPP 10
TMEM 263
Beta-synuclein

ABSTRACT

The sodium-activated potassium channels Slick (Slo2.1, KCNT2) and Slack (Slo2.2, KCNT1) are paralogous channels of the Slo family of high-conductance potassium channels. Slick and Slack channels are widely distributed in the mammalian CNS and they play a role in slow afterhyperpolarization, generation of depolarizing afterpotentials and in setting and stabilizing the resting potential. In the present study we used a combined approach of (co)-immunoprecipitation studies, Western blot analysis, double immunofluorescence and mass spectrometric sequencing in order to investigate protein–protein interactions of the Slick and Slack channels. The data strongly suggest that Slick and Slack channels co-assemble into identical cellular complexes. Double immunofluorescence experiments revealed that Slick and Slack channels co-localize in distinct mouse brain regions. Moreover, we identified the small cytoplasmic protein beta-synuclein and the transmembrane protein 263 (TMEM 263) as novel interaction partners of both, native Slick and Slack channels. In addition, the inactive dipeptidyl-peptidase (DPP 10) and the synapse associated protein 102 (SAP 102) were identified as constituents of the native Slick and Slack channel complexes in the mouse brain. This study presents new insights into protein–protein interactions of native Slick and Slack channels in the mouse brain.

© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The sodium-activated potassium channels Slick (sequence like an intermediate potassium channel, Slo2.1, KCNT2) and Slack (sequence like a calcium-activated potassium channel, Slo2.2, KCNT1) are structurally highly related and belong to the high-conductance potassium channels of the Slo family. Slick and Slack channels are widely distributed in the rat brain with partial overlap in their expression patterns [1,2]. In neurons, sodium-activated potassium channels are involved in adapting the firing pattern of neurons, in the generation of the slow afterhyperpolarization (sAHP) and depolarizing afterpotentials (DAP) and in stabilization and setting of the resting membrane potential [3–8]. The pore-forming alpha subunits of Slick and Slack channels are assembling into tetrameric channels [9,10]. Alpha subunits of Slick and Slack channels are composed of an intracellular N-terminus, six membrane spanning domains, and a long intracellular C-terminus harboring various functional domains [9,10].

Native sodium-activated potassium channels are high-conductance outward rectifying potassium channels that are activated upon sodium-influx [11]. Heterologously expressed Slick and Slack channels resemble most of the biophysical properties of native sodium-activated potassium currents. Nonetheless, there are some discrepancies regarding their unitary conductance, sensitivity to internal sodium ions, subconductance states and open probabilities of the channels as well as rundown in excised patches. Such discrepancies were not only observed when comparing heterologously expressed Slick and Slack channels with native currents. In addition, biophysical properties of native sodium-activated potassium currents varied depending on the brain region and/or the cell type examined [11,12]. Such discrepancies might possibly reflect different isoforms of the underlying channels or channels associating with different endogenous factors and/or with several (regulatory) proteins.

While for the Slick channel no isoforms have been described,

* Corresponding authors.
E-mail addresses: Sandra.Rizzi@gmx.net (S. Rizzi), Schwarzer.Christoph@i-med.ac.at (C. Schwarzer), Leopold.Kremser@i-med.ac.at (L. Kremser), Herbert.Lindner@i-med.ac.at (H.H. Lindner), Hans.G.Knaus@i-med.ac.at (H.-G. Knaus).

http://dx.doi.org/10.1016/j.bbrep.2015.09.024
2405-5808/© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

five different Slack channel isoforms were identified so far. The Slack channel isoforms only differ in their N-terminal region. The physiological relevance of these Slack channel isoforms has not been investigated yet [13], and most studies exploring various aspects of the Slack channels were analyzing the so-called Slack-B isoform.

Slick and Slack channel diversity may be further increased by the formation of heteromeric Slick and Slack channels. A previous study provided first evidence that Slick alpha-subunits are forming heteromeric channels with Slack-B alpha-subunits in the rat brain. Heterologously expressed Slick/Slack heteromeric channels were shown to have biophysical properties distinct from those of homomeric Slick and Slack channels [14]. The BK channel (big conductance calcium-activated potassium channel) is another potassium channel that is structurally highly related to Slick and Slack channels and thus dedicated to the same family of potassium channels (Slo family). *In vitro* studies revealed that BK channel alpha subunits do associate with Slack subunits, thereby forming heteromeric potassium channels. However, the existence of such channel complexes has never been proven *in vivo* [15].

There is growing evidence that Slick and Slack channel activity may be regulated by several cellular signaling pathways, including activation of G-protein coupled receptors linked to activation of protein kinase C (PKC) or protein kinase A (PKA) and by direct phosphorylation by these signaling proteins [14,16–18]. Channel activity and gating may also be regulated by binding of endogenous signaling factors to the C-terminal tail of the channel, like NAD+ [19], PIP_2 [20] and fragile *X* mental retardation protein (FMRP) [21,22] as well as by small changes in cell volume [23].

Moreover, recent studies were suggesting that the Slack channel might interact with the postsynaptic density protein 95 (PSD 95) [24], FMRP [21] and with Glu2/3 subunits of the AMPA receptor [25].

In the present study we aimed to provide new insights into protein–protein interactions of the Slick and Slack channels in mouse brain. In order to address this issue, we performed double immunofluorescence and (co-)immunoprecipitation studies followed by Western blot analysis and mass spectrometric sequencing. Here we report Slick and Slack channels co-assemble into protein complexes in native mouse fore- and midbrain purified synaptic vesicle plasma membranes. Moreover, we provide first evidence for potential novel interaction partners of native Slick and Slack channels.

2. Material and methods

2.1. Animals

C57BL/6J mice were housed and handled in accordance with the guidelines with Austrian law which is in line with the directive of the European Union (2010/63/EU) for the use of laboratory animals. All procedures involving animals were approved by the Austrian Animal Experimentation Ethics Board in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Every effort was taken to minimize the number and suffering of animals used.

2.2. Preparation of purified synaptic plasma membrane vesicles from mouse fore- and midbrain

Preparation of purified synaptic plasma membrane vesicles was performed according to [26]. In brief, 30 male and female C57BL/6J mice were killed by cervical dislocation and fore- and midbrain was excised. Tissue was homogenized in ice-cold homogenization buffer (320 mM sucrose, 10 mM Tris–HCl pH 7.4, 1 mM EDTA, 0.5 mM phenylmethylsulfonylfluoride, PMSF) supplemented with protease inhibitors (complete tablets, Roche). Subsequently, homogenized fore- and midbrain tissue was separated on 7.5%/10% Ficoll gradient. Intact synaptosomes were lysed in 5 mM Tris–HCl supplemented with protease inhibitors. Lysed synaptosomes were spun at 125,000 × *g* for 1 h at 4 °C. Purified synaptic plasma membranes were resuspended in 20 mM Tris–HCl, snap frozen in liquid nitrogen and stored at −80 °C.

2.3. Solubilization of membrane protein

Purified synaptic plasma membrane vesicles from mouse fore- and midbrain were incubated for 30 min at 4 °C in solubilization buffer (150 mM NaCl, 20 mM Tris–HCl pH 7.4, 1 mM EDTA, 1 mM PMSF, 2 μM leupeptin (Sigma-Aldrich), 1.5 μM aprotinin (Sigma-Aldrich), 0.15 μM pepstatin (Sigma-Aldrich), 0.9% n-dodecyl-β-D-maltoside (Calbiochem)) in a protein:detergent ratio of 1:9). Thereafter, soluble protein fraction was separated from unsoluble protein fraction by high-speed centrifugation at 105,000 × *g* for 30 min. Solubilization efficiency was controlled in Western blot analysis. Solubilized protein fraction was used for subsequent immunoprecipitation experiments.

2.4. Immunoprecipitation

Slick and Slack channel specific antibodies were immobilized and cross-linked onto dynabeads protein G (Life Technologies) following manufacture's guidelines and incubated with n-dodecyl-β-D-maltoside (Calbiochem) solubilized synaptic plasma membranes derived from mouse fore- and midbrain overnight at 4 °C. For each experiment, 40 μg of either anti-Slick (clone N11/33, Neuromab) or anti-Slack (clone N3/26, Neuromab) channel antibody was used and 2 mg of synaptic plasma membranes served as starting material for solubilization. Immunoprecipitation experiments using a non-immune antibody of the same IgG subtype raised in chicken (MABC002, Chemicon, Millipore) served as negative control and were run in parallel. Unbound material was removed and collected (flow through). Affinity-purified protein complexes were eluted with Laemmli buffer (reducing agent added after elution). Resulting eluates were analyzed by Western blot and/or by mass spectrometric sequencing.

2.5. Western blot analysis

Western blot analysis was performed as described earlier [26]. In brief, 20 μg of protein samples (starting material, solubilized and unsolubilized protein fractions, and flow through) or 2.5% or 20% of eluates were separated by 4–15% (precasted TGX gel, Biorad) and transferred onto PVDF-membranes. Membranes were blocked in phosphate buffered saline (PBS) containing 0.05% Tween-20 (Roth) and 3% bovine serum albumin (Roth) for 1 h. Subsequently, membranes were incubated in PBS containing 0.05% Tween-20, 3% bovine serum albumin and either mouse monoclonal anti-Slick (1:1000, IgG1, clone N11/33, NeuroMab), mouse monoclonal anti-Slack (1:3000, IgG1, clone N3/26, NeuroMab), mouse polyclonal anti-beta-synuclein (1:100, abcam), rabbit polyclonal anti-DPP 10 (1:500, abcam) or mouse monoclonal anti-SAP 102 (1:2000, IgG1, clone N19/2, Biolegend) antibody for 2.5 h at room temperature. HRP-labeld goat anti-mouse IgG1 (1:100,000, Life Technologies), goat anti-mouse IgG (1:75,000, Dako) or goat anti-rabbit (1:75,000, Dako) were used as secondary antibodies. Western blots were developed using chemiluminescent HRP substrate (Millipore) and subsequently PVDF-membranes were incubated with Amersham hyper film.

Download English Version:

https://daneshyari.com/en/article/1941829

Download Persian Version:

https://daneshyari.com/article/1941829

Daneshyari.com