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Research Report

Modulation of Kv4.2 K^+ currents by neuronal interleukin-16, a PDZ domain-containing protein expressed in the hippocampus and cerebellum

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ABSTRACT

Neuronal interleukin-16 (NIL-16) is a multi-PDZ domain protein expressed in post-mitotic neurons of the hippocampus and cerebellum. NIL-16 contains four PDZ domains, two of which are located within the neuron-specific N-terminal region. In yeast two-hybrid systems, the N-terminus of NIL-16 interacts with several ion channel proteins, including the Kv4.2 subunit of A-type K^+ channels. Here we provide evidence that NIL-16, through interactions with Kv4.2, influences Kv4.2 channel function and subcellular distribution. Specifically, coexpression of NIL-16 with Kv4.2 in COS-7 cells results in a significant reduction in whole-cell A-type current densities; however, when the Kv4.2 PDZ-ligand domain is mutated, Kv4.2 current densities are not affected by NIL-16 coexpression. Moreover, single-channel conductance was not influenced by the presence of NIL-16. In hippocampal neurons, A-type current densities are increased by conditions that inhibit interactions between NIL-16 and Kv4.2, such as overexpression of the Kv4.2 C-terminal PDZ-ligand domain and treatment with small-interfering RNA duplexes that reduce NIL-16 expression. Results of surface biotinylation assays using COS-7 cells suggest that Kv4.2 surface expression levels are reduced by coexpression with NIL-16. In addition, coexpression of NIL-16 with Kv4.2 induces Kv4.2 to form dense intracellular clusters; whereas without NIL-16, Kv4.2 channels cells are dispersed. Taken together, these data suggest that interactions between Kv4.2 and NIL-16 may reduce the number of functional Kv4.2-containing channels on the cell surface. In summary, NIL-16 may provide a novel form of A-type K^+ channel modulation that is localized specifically to neurons of the hippocampus and cerebellum.

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Abbreviations: BSA, bovine serum albumin; Co-IP, coimmunoprecipitation; FBS, fetal bovine serum; GFP, green fluorescent protein; IL-16, interleukin-16; NIL-16, neuronal interleukin-16; PDZ, PSD-95/Disc-large/ZO-1 homology; PBS, phosphate buffered saline; PVDF, polyvinylidene difluoride; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; siRNA, small-interfering RNA

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1. Introduction

Transient A-type K^+ currents modulate neuronal excitability and synaptic plasticity in the hippocampus. For example, down-regulation of A-type K^+ currents in hippocampal neurons increases the amplitude of back-propagating dendritic action potentials (Hoffman et al., 1997; Johnston et al., 1999) and enhances membrane excitability (Castro et al., 2001; Hu and Gereau, 2003; Varga et al., 2004). Kv4.2 is the predominant pore-forming subunit of A-type K^+ channels in hippocampal pyramidal neurons (Jin et al., 2003; Kim et al., 2005) and localizes to somatodendritic regions where it forms clusters at postsynaptic sites (Jinno et al., 2005; Burkhalter et al., 2006). Postsynaptic Kv4.2-containing channels may affect NMDA-receptor-dependent plasticity by influencing membrane potentials sensed by NMDA-receptors (Alonso and Widmer, 1997; Schrader et al., 2002; Watanabe et al., 2002). Consistent with an important role in modulating hippocampal synaptic plasticity, Kv4.2 gene deletion in mice largely eliminates dendritic A-type currents and reduces the threshold for induction of long-term potentiation in hippocampal CA1 pyramidal neurons (Chen et al., 2006).

Activation of PKC, PKA and/or certain neurotransmitter receptors have been shown to influence the biophysical properties of Kv4.2-mediated K^+ currents (Hoffman and Johnston, 1998, 1999). These effects are believed to be secondary to the activation of the mitogen-activated protein kinase ERK (Hu and Gereau, 2003; Watanabe et al., 2002; Yuan et al., 2002), which directly phosphorylates Kv4.2 (Adams et al., 2000; Schrader et al., 2006). In addition to phosphorylation-dependent mechanisms, proteins that specifically interact with Kv4.2 subunits can influence channel function. For example, KChiPs, which co-assemble with Kv4 family subunits, modulate the surface expression and biophysical properties of Kv4-containing channels (Rhodes et al., 2004; Schrader et al., 2006; Shibata et al., 2003). The synapse associated multi-PDZ (PSD-95/Disc-large/ZO-1 homology) domain protein, PSD-95, interacts with the C-terminal PDZ binding domain of Kv4.2 and increases Kv4.2 surface expression, cluster formation, and whole-cell current densities in heterologous expression systems (Wong et al., 2002). Neuronal interleukin-16 (NIL-16) is a multi-PDZ domain protein that has also been shown to interact with Kv4.2 (Kurschner and Yuzaki, 1999). Here we investigate the effects of the interaction between NIL-16 and Kv4.2.

NIL-16 is a cytosolic protein that is expressed exclusively in post-mitotic neurons of the hippocampus and cerebellum (Kurschner and Yuzaki, 1999). The N-terminal region (corresponding to amino acids 1–698) of NIL-16 is expressed only in neurons. The remaining C-terminal region of NIL-16 is identical to pro-IL-16, the precursor of IL-16, a cytokine with functions believed previously to be restricted to the immune system. Similar to pro-IL-16, NIL-16 can be cleaved by caspase-3 to result in the secretion of IL-16 (Kurschner and Yuzaki, 1999); thus, NIL-16 is a neuron-specific precursor of IL-16 that may serve as a neuronal signaling molecule. In addition to the role of NIL-16 as a precursor of IL-16, NIL-16 contains four PDZ domains, two of which are within the neuron-specific N-terminal region. Using a yeast two-hybrid screen, Kurschner and Yuzaki (1999) identified several neuronal ion channels (each of which contained the C-terminal PDZ-interacting consensus sequence x-S-x-V/I/L) as potential ligands for the N-terminal region of NIL-16. Ion channels identified in this screen include NMDA receptor subunits (NR2A-D), inward rectifier K^+ channels (Kir2.1, 2.3 and Kir4.1, 4.2), the Ca^{2+} channel α1C subunit, and A-type K^+ channel subunits (Kv4.1–3). Considering (1) that NIL-16 is found only in hippocampal and cerebellar neurons, (2) the role of Kv4.2 channels in influencing neuronal excitability, and (3) the identification of NIL-16 as a Kv4.2 binding protein, it is possible that NIL-16, through functional interactions with the Kv4.2 subunit, influences neuronal excitability in the hippocampus and cerebellum.

In this study, we investigate the ability of NIL-16 and Kv4.2 to interact *in vivo* and the influence of NIL-16 on the function of Kv4.2-containing channels. We show that coexpression of NIL-16 with Kv4.2 in heterologous expression systems reduces whole-cell A-type current densities but does not influence single-channel conductance, results in intracellular clustering of Kv4.2, and decreases Kv4.2 surface expression levels. Results of coimmunoprecipitation assays suggest that NIL-16 and Kv4.2 are present in the same protein complexes in the hippocampus and cerebellum. In addition, in primary cultures of hippocampal neurons, A-type current densities are increased by conditions which either disrupt binding between Kv4.2 and NIL-16 or reduce NIL-16 expression levels. In conclusion, interactions between NIL-16 and Kv4.2-containing channels may provide a novel form of A-type K^+ current modulation that specifically occurs in neurons of the hippocampus and cerebellum.

2. Results

2.1. *NIL-16 reduces Kv4.2 current density in COS-7 cells*

To test the hypothesis that NIL-16 influences Kv4.2-mediated currents, we first compared Kv4.2 currents in COS-7 cells coexpressing Kv4.2 with either NIL-16/GFP or a control GFP vector. Kv4.2-dependent current densities of cells cotransfected with the control vector were significantly greater than of cells cotransfected with NIL-16/GFP at clamp potentials of −40 to +30 mV (Figs. 1A and B). Time courses of inactivation were similar regardless of NIL-16 transfection.

It is possible that the reduction of Kv4.2 currents in the presence of NIL-16, rather than being due to a direct interaction between NIL-16 and Kv4.2, results from reduced Kv4.2 transfection or expression efficiency with NIL-16 coexpression. We addressed this possibility by comparing Kv4.2 protein expression levels using Western blot analysis of whole-cell lysates. Kv4.2 protein expression levels were similar in cells expressing either the Kv4.2 and the control vector or Kv4.2 with NIL-16/GFP (Fig. 1D, lanes 1 and 2). No Kv4.2 was detected in lysates of cells that were transfected with either GFP or NIL-16/GFP alone (Fig. 1D, lanes 3 and 4).