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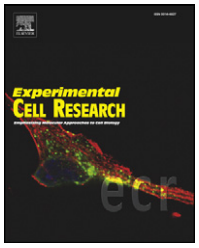

Research Article

A C-terminal PDZ binding domain modulates the function and localization of Kv1.3 channels

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ABSTRACT

The voltage-gated potassium channel, Kv1.3, plays an important role in regulating membrane excitability in diverse cell types ranging from T-lymphocytes to neurons. In the present study, we test the hypothesis that the C-terminal PDZ binding domain modulates the function and localization of Kv1.3. We created a mutant form of Kv1.3 that lacked the last three amino acids of the C-terminal PDZ-binding domain (Kv1.3ΔTDV). This form of Kv1.3 did not bind the PDZ domain containing protein, PSD95. We transfected wild type and mutant Kv1.3 into HEK293 cells and determined if the mutation affected current, Golgi localization, and surface expression of the channel. We found that cells transfected with Kv1.3ΔTDV had greater current and lower Golgi localization than those transfected with Kv1.3. Truncation of the C-terminal PDZ domain did not affect surface expression of Kv1.3. These findings suggest that PDZ-dependent interactions affect both Kv1.3 localization and function. The finding that current and Golgi localization changed without a corresponding change in surface expression suggests that PDZ interactions affect localization and function via independent mechanisms.

Introduction

The Kv1.3 voltage-gated potassium channel is a key determinant of function in many cell types. Kv1.3 plays a central role in immune function by regulating resting membrane potential [1], Ca^{2+} signaling cascades [2], and antigen-dependent activation of T-lymphocytes [3]. Kv1.3 also plays a role in energy homeostasis. Kv1.3-deficient mice have altered body weight [4,5] and Kv1.3 influences glucose transport in adipocytes and skeletal muscle [6,7]. In neurons, Kv1.3 influences resting membrane potential, action potential characteristics, and neurotransmission [4,8–10].

PDZ (postsynaptic density-95/disks-large/zona-occludens-1)-dependent interactions have emerged as important mechanisms of ion channel regulation. PDZ domain containing proteins interact primarily with the C-termini of channels to modulate their intracellular trafficking, localization and function [11–17]. For example, C-terminal PDZ-dependent interactions modulate endocytic recycling [16] and trafficking from the Golgi apparatus to the plasma membrane of the cystic fibrosis transmembrane conductance regulator (CFTR) [11]. PDZ-dependent interactions at the C-terminus also modulate Kv1 channels [12,13,17]. Kv1.3 contains a C-terminal PDZ binding domain, but the functional significance of PDZ domain-containing protein interactions with this channel are not well characterized.

In the present study we test the hypothesis that the C-terminal PDZ binding domain modulates the function and localization of

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Kv1.3. We demonstrate that truncation of the C-terminal PDZ domain has two effects on Kv1.3 channels expressed in HEK293 cells. It increased Kv1.3 current and decreased the amount of Kv1.3 localized in the Golgi apparatus. The truncation had no effect on surface expression. These findings suggest that PDZ-dependent interactions affect both Kv1.3 localization and function. The finding that current and Golgi localization were changed without a corresponding change in surface expression suggests that PDZ interactions affect localization and function via independent mechanisms.

Materials and methods

Animals

The use of animals in the present studies was in accordance with the National Institutes of Health guidelines for the humane care and use of animals in research, and was approved by the Institutional Animal Care and Use Committee of the University of Vermont.

Cell culture and transient transfection

Postganglionic sympathetic neurons were obtained from superior cervical ganglia of neonatal rats. The ganglia (SCG) were dissociated with collagenase/hyaluronidase digest followed by a trypsin digest. Cells were plated onto type I rat tail collagen coated dishes (RT-PCR and western analyses) or coverslips (immunohistochemistry). The day after plating, non-neuronal cells were growth arrested with mitomycin C (1 h, 10 μg/mL). All neuronal cultures were grown in neuronal growth medium (DMEM/F12 supplemented with 10% NuSerum, 5% FBS, 100 U/mL penicillin/streptomycin, and 50 ng/mL NGF). Vascular smooth muscle cells (VSM) were isolated from explants of adult postpartum Sprague Dawley rat tail arteries. VSM were grown in DMEM supplemented with 10% FBS, and 100 μ/mL penicillin/streptomycin. VSM were used at passage 2. Clonal human embryonic kidney 293 (HEK293) cells were maintained in DMEM/F-12 (Invitrogen) supplemented with 10% fetal bovine serum, 10 U/mL penicillin and streptomycin, and 2 mM L-glutamine. Cells were transiently transfected with 3.0 μg of GFP-Kv1.3/-Flag or GFP-Kv1.3-ΔTDV-Flag, using the Lipofectamine LTX transfection reagent (Invitrogen). Confluent transfected cultures were plated to a low density (25,000 cells/cm^2) onto poly-D-lysine-coated tissue culture plates (Corning Glass Works) and subsequently placed in serum-free medium overnight for experimentation the following day.

Mutagenesis

All mutagenesis reactions were performed using the Quickchange Lightning Site-Directed Mutagenesis Kit (Strategene), following the manufacturer's protocol. The GFP-Kv1.3-ΔTDV truncation was created by inserting two premature stop codons into the wild-type channel using the following primer sequence and its reverse complement: 5′-GTCAACATCATCAAAAAG ATATTCTGATGAACT-GATGTCTAATAGGGATCCACC-3′. Similar to previous reports [18,19], the Flag epitope was created by inserting the amino acid sequence, DYKDDDDK, into the first extracellular loop of GFP-Kv1.3/-ΔTDV and incorporating D222 as the first aspartic acid residue in the Flag epitope using the following primer sequence and its reverse complement: 5′-TCTCCGTCGCAGGACTACAAGGA CGACGACGACAAGGTGTTTGAGGCTGCC-3′.

Immunofluorescence

Cultures of postganglionic sympathetic neurons and vascular smooth muscle cells were each rinsed in PBS and fixed in 4% formaldehyde in PBS for 12 min. Cultures were permeabilized with 0.2% Triton X-100 in PBS for 5 min and subsequently rinsed in PBS. Cells were blocked for 1 h at room temperature in 3% goat serum, 0.1% fish skin gelatin in PBS. Cells were co-stained with anti-Kv1.3 mouse monoclonal antibody (0.84 μg/mL; NeuroMab) and anti-GM130 rabbit polyclonal antibody (0.7 μg/mL; Calbiochem) overnight at 4 °C, followed by three 5 min washes in PBS. Alexa Fluor Goat anti-mouse (488 nm) and goat anti-rabbit (647) secondary antibodies (4 μg/mL; Invitrogen) were applied for 1 h at room temperature.

To detect total Kv1.3, transiently transfected HEK293 cells were rinsed in PBS, fixed in 4% formaldehyde in PBS for 20 min, and subsequently permeabilized with acetone for 5 min and rinsed in PBS. Cells were then blocked for 20 min at 37 °C in 3% goat serum, 0.1% fish skin gelatin in PBS. Cells were incubated for 20 min at 37 °C in a rabbit polyclonal GM130 antibody, raised against recombinant protein containing amino acids 371–990 of human GM130 (Lot # D00004465), to identify the Golgi apparatus and subsequently rinsed 3× for 5 min in PBS. Alexa Fluor goat anti-rabbit (647 nm) secondary antibody (4.0 μg/mL; Invitrogen) was applied for 20 min at 37 °C.

Transiently transfected HEK293 cells were live labeled with anti-Flag M2 antibody (1 μg/mL; Sigma) at 37 °C for 15 min. Cells were then rinsed 3× for 5 min in PBS, and fixed, permeabilized and labeled as described above. Alexa Fluor goat anti-mouse (647 nm) and goat anti-rabbit (568 nm) secondary antibodies (4.0 μg/mL; Invitrogen) were used to detect Kv1.3 surface expression and GM130 localization, respectively. All cells were mounted using ProLong Gold antifade reagent (Invitrogen). All images were taken using the Olympus IX70 microscope and DeltaVision Restoration Imaging System (Applied Precision, LLC) and background subtracted with the appropriate IgG isotype controls (R&D Systems).

Golgi localization was quantified as follows: Golgi region of interest (ROI) was defined using the GM130 marker and the amount of GFP-Kv1.3-Flag signal intensity present in the Golgi ROI was expressed as a percent of the total Kv1.3 signal for the entire cell ((Kv1.3 in Golgi region/total Kv1.3) × 100). Surface expression was quantified as follows: the intensity of the live labeled anti-Flag M2 antibody was normalized to the total GFP-Kv1.3-Flag intensity of the cell (surface Kv1.3/total Kv1.3).

Immunoblot analysis

Cells were washed with ice-cold phosphate-buffered saline and lysed in radioimmune precipitation assay buffer (50 mM Tris, 150 mM NaCl, 11 mM EDTA, 0.25% deoxycholate, 1% Nonidet P-40, 10% glycerol, 1 mM NaF, 1 mM Na_3VO_4, 1 mM Na_4 BAPTA, 1 mM dithiothreitol, protease inhibitors (Sigma, catalog no. P8340), phosphatase inhibitors (Calbiochem, catalog nos. 524624 and 524625), pH 8.0). Lysates were centrifuged at 20,000 ×g for 5 min, and resulting supernatants were combined with sample buffer and separated by SDS-PAGE. Western blotting detection of Kv1.3 was