Neuroscience Letters 464 (2009) 209–213

Contents lists available at ScienceDirect

Neuroscience Letters

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

The development of Kv4.2 expression in the retina

Juan Qu[a], Ira Mulo[a], Karen L. Myhr[a,b,*]

[a] Department of Biological Sciences, Wayne State University, 5047 Gullen Mall, Detroit, MI 48202, United States
[b] Department of Anatomy and Cell Biology, Wayne State University, 540 E. Canfield Ave., Detroit, MI 48201, United States

ARTICLE INFO

Article history:
Received 7 April 2009
Received in revised form 22 July 2009
Accepted 19 August 2009

Keywords:
Ganglion cell
Excitability
Potassium channel
Shal
Kcnd2

ABSTRACT

Throughout the brain, the potassium channel Kv4.2 regulates signal propagation in dendrites and action potential properties in subtypes of neurons. In adult rodents Kv4.2 is expressed predominantly in two bands in the inner plexiform layer (IPL) and in retinal ganglion cell (RGC) somas (Klumpp et al. [15]; Pinto and Klumpp [20]), suggesting a role regulating the activity of specific subtypes of RGCs. To understand the role of Kv4.2 in the regulation of the activity of RGCs during development we determined the developmental expression pattern of Kv4.2 immunoreactivity (Kv4.2-IR). At P4-6 Kv4.2-IR appeared diffusely throughout the IPL in cross-sectioned retinas. From postantal day 10 (P10) through adult there was an additional pair of brighter Kv4.2-IR bands between the ChAT bands that had a reticular pattern in flat-mounted retinas. Kv4.2-IR was not present in somas at P4–6, but appeared in ganglion cell layer (GCL) somas beginning at P10. The fraction of somas expressing Kv4.2 in the GCL was about 8% at P10–11, decreased to 5% at P20–21, then increased to 9% in adult retinas. The restriction of Kv4.2 expression to less than 10% of the GCL somas and the specificity of expression in the IPL suggest that Kv4.2 regulates activity in one or a few functional subtypes of RGCs. The pattern of Kv4.2-IR through postnatal development indicates that Kv4.2-mediated currents are important for development in a subset of RGCs, especially around P10 as the bipolar cells mature.

Throughout the brain, the potassium channel Kv4.2 alters the output of neurons by regulating signal propagation in dendrites and action potential patterns. Kv4.2 is one of the channels that mediates the A-current, which is a voltage-gated, fast, transient subthreshold potassium current [6]. Kv4.2 channels are unique from other channels mediating A-current in their rapid recovery from inactivation [6]. Kv4.2-mediated A-currents regulate neuronal output by attenuating signal propagation in dendrites [3,4,11,14,22], and by increasing the latency to firing, broadening action potentials and altering repetitive firing patterns [12,14,27,35]. These functions depend on subcellular expression, such as dendritic localization of Kv4.2, which underlies the role in regulating dendritic signal integration.

In developing retina, A-currents begin to be expressed when RGCs appear embryonically, but change expression through postnatal development and are not expressed in all RGCs [9,10,16,23–25,29,31]. During development, the pattern of action potentials, which are modified by A-currents, are critical for the normal refinement of the synaptic output of the retina [5,8,13,19,28]. Growth cone expression of Kv4.2 may also guide RGC growth cones directly during development [18,21]. Kv4.2 is expressed in adult mouse retina [15], but expression of Kv4.2 and channels generating A-current during retinal development are unknown. In this study, we determined the developmental expression pattern of Kv4.2 in mouse retinas comparing adult expression to the developmental pattern. The results lay the foundation for understanding the role of Kv4.2 in retinal development and adult function.

Retinal tissues were prepared as follows. Postnatal (P4–6, P10–11 and P20–21) and adult (P42–44) C57Bl/6 mice were anesthetized with halothane or carbon dioxide and decapitated to assure death according to National Institute of Health Guide for the Care and Use of Laboratory Animals. Eyes were removed and retinas were dissected from the rest of the eye and fixed for 2 h with 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Some retinas were sectioned into 50-μm thick retina cross-sections using a vibrating microtome (Vibratome, St. Louis, MO).

For immunohistochemistry, retinas were first incubated with blocking serum [0.5% Triton-X detergent, 5% normal goat serum (Chemicon, Temecula, CA) or normal donkey serum (Jackson Immuno Research, West Grove, PA) in 0.2 M pH 7.4 PB]. Flat-mounted retinas were incubated in goat blocking serum overnight, then in rabbit anti-Kv4.2 (Chemicon; 1:1000) then Alexa 647 goat-anti-rabbit-IgG (Chemicon; 1:1000) for a week each at 4 °C. Cross-sectioned retinas were incubated in donkey block-

* Corresponding author at: Department of Biological Sciences, Wayne State University, 5047 Gullen Mall, Detroit, MI 48202, Unites States.
Tel.: +1 313 577 1504; fax: +1 313 577 6891.
E-mail address: kmyhr@wayne.edu (K.L. Myhr).
URL: http://bio.wayne.edu/profhtml/myhr/myhr.html (K.L. Myhr).

0304-3940/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2009.08.048

ing serum for 4 h, mouse anti-Kv4.2 (NeuroMab, Davis, CA; 1:1000) and goat-anti-ChAT (Choline Acetyl transferase; Chemicon; 1:100) simultaneously overnight, and then Alexa 488 donkey anti-mouse-IgG (Invitrogen, Carlsbad, CA; 1:1000) and Alexa 633 donkey anti-goat-IgG (Invitrogen; 1:1000) simultaneously for 2.5 h. The NeuroMab mouse antibody has been validated in knockout mice [2]. Immunoblots of retinal extracts with rabbit anti-Kv4.2 antibody showed labeling at the appropriate molecular weight (Supplementary Fig. 1A). Controls with only the secondary antibodies did not show the specific labeling, and as expected, the donkey anti-mouse-IgG antibody labeled blood in vessels, which was ignored (Supplementary Fig. 1B). The goat-anti-rabbit-IgG antibody revealed no signal (Supplementary Fig. 1C). With rabbit anti-Kv4.2 antibodies there was some fluorescence in the outer retina with or without preabsorption with the antigen, which was not seen in a smaller sample with the NeuroMab antibody (data not shown). Preabsorption with the antigen eliminated all other fluorescence. Therefore, analysis focused on the inner retina, where there was no non-specific labeling.

Images were taken on a fluorescence microscope (Olympus, BX51WI fluorescence microscope; objectives X40, N.A. 0.8 or X20, N.A. 0.5) or confocal microscope (Leica, TCS SP2; objective X20, N.A. 0.7). For flat-mounts, Kv4.2-IR was imaged with Chroma filter set 41008 (excitation: 620/60, emission: 700/75). For cross-sections, images of Kv4.2-IR (filter set 41001, excitation: 480/40, emission: 535/50) were collected alone, or in combination with ChAT-IR (filter set 41008). The expression pattern of Kv4.2 in dendrites was quantified by determining the position of Kv4.2-IR in the inner plexiform layer (IPL) relative to ChAT-IR. To precisely align the Kv4.2-IR with the ChAT-IR, linescans were generated for each image at the same position on a cross-section. In Metamorph (version 6, Universal Imaging, Downingtown, PA), a line was drawn orthogonal to the layers of the retina in a flat region of the cross-section. The average pixel intensity from 50 μm left and right of the line was plotted as intensity at that depth of the retina for that fluorophore. Exposures and settings for contrast and brightness were constant across ages for accurate comparison of Kv4.2 labeling with Metamorph and Photoshop (version 7.0 or CS3).

Some flat-mounted retinas were further stained with TO-PRO-1 (Invitrogen; 1:500 in 0.2 M PB) for labeling nuclei and cell counting. Aligned images of TO-PRO-1-labeled nuclei and Kv4.2-IR were taken every 1 μm. The proportion of Kv4.2-IR positive cells was determined as the fraction of cells in the ganglion cell layer (GCL) that expressed Kv4.2. The numbers of TO-PRO-1 stained nuclei and Kv4.2-IR positive cells were counted in six regions in three flat-mounted retinas from three P10-11 animals, six regions in four retinas from two P20–21 animals, and ten regions in five retinas from five P42-44 animals. Spherical to oval shaped nuclei were counted throughout the depth of the GCL in a rectangular region of the TO-PRO-1 signal (filter set 41007a, excitation: 545/30, emission: 610/75). Nuclei that crossed edges were counted only if they crossed the upper and right edges. Cells with Kv4.2-IR were counted in the same region (filter set 41008). Criteria for counting an RGC as positive were clear labeling of the soma, corresponding nuclear staining, and in most cases labeling of primary dendrites. The percentage of positive cells was calculated as the number of cells with Kv4.2-IR divided by the number of nuclei.

Kv4.2-IR is described first for adult retinas, as a point of reference for developmental expression. In adult flat-mounted retinas there was discrete Kv4.2-IR in the IPL and somas in the GCL (Fig. 1). Kv4.2-IR appeared as a reticular pattern in the IPL. In the plane of the reticular labeling, discrete dendrites were labeled, and there was additional weaker Kv4.2-IR. The gaps in the Kv4.2-IR were irregularly shaped areas 2–5 μm across. There were occasional larger, rounder gaps without Kv4.2-IR that were about 10 μm in diameter. In cross-sections this reticular pattern appeared as two bright bands of Kv4.2-IR in the middle of the IPL with dimmer Kv4.2-IR throughout the IPL. The relative intensity of Kv4.2-IR can be seen in the linescan to the right of the image (Fig. 1B). Somas with Kv4.2-IR were seen in the GCL of cross-sections, consistent

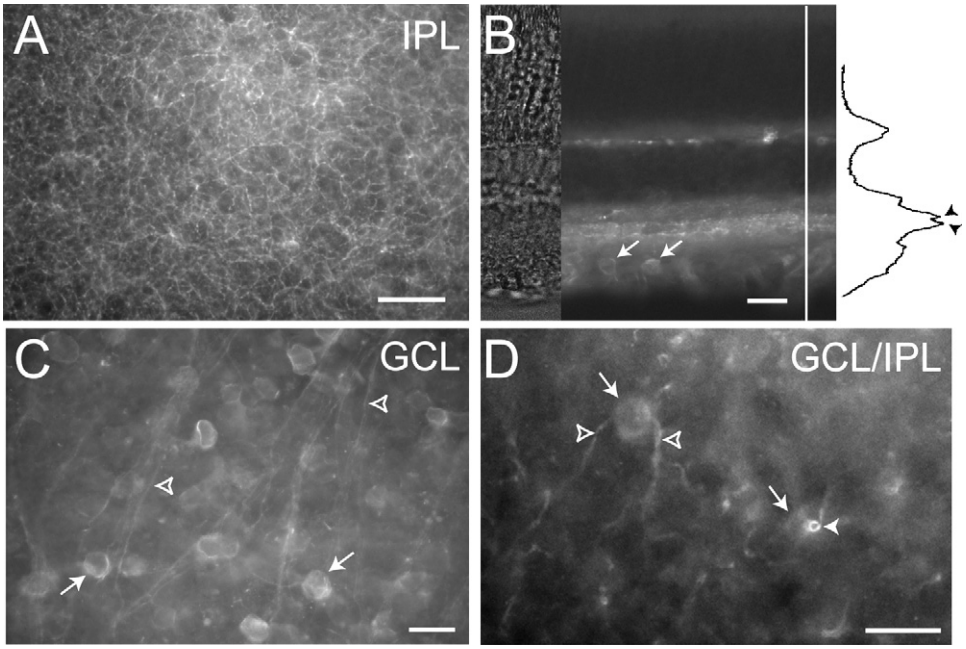

Fig. 1. *Kv4.2-IR in adult retinas.* (A) Flat-mounted retina focused in the IPL shows a network of Kv4.2-IR. (B) Cross-section of retina using the mouse anti-Kv4.2 antibody shows somas in GCL (arrows) and diffuse labeling in the IPL with two brighter bands of Kv4.2-IR in the central IPL (bright-field, left side; Kv4.2-IR, right side of image). Linescan to the right of image shows diffuse Kv4.2-IR through the IPL with brighter bands showing as peaks (arrowheads). Vertical white line is center of linescan. (C) Flat-mounted retina focused on GCL shows somas (arrows), and axons (open arrowheads). (D) Flat-mounted retina focused at interface between the GCL and IPL shows primary dendrites emerging from somas laterally (open arrowheads) and directly into the IPL (filled arrowhead). Kv4.2-IR appears white in each fluorescence image. All scale bars represent 20 μm.