Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuroscience Letters 403 (2006) 315–317
Neuroscience Letters
www.elsevier.com/locate/neulet

Expression of sodium channels $Na_v1.2$ and $Na_v1.6$ during postnatal development of the retina

Audra Van Wart, Gary Matthews *

Graduate Program in Neuroscience, Department of Neurobiology and Behavior, State University of New York, Stony Brook, United States

Received 10 April 2006; accepted 4 May 2006

Abstract

During the second and third postnatal weeks, there is a developmental switch from sodium channel isoform $Na_v1.2$ to isoform $Na_v1.6$ at initial segments and nodes of Ranvier in rat retinal ganglion cells. We used quantitative, real-time PCR to determine if the developmental appearance of $Na_v1.6$ channels is accompanied by an increase in steady-state level of $Na_v1.6$ mRNA in the retina. Between postnatal day 2 (P2) and P10, $Na_v1.6$ levels did not change, but between P10 and P19, there was an approximately three-fold increase in $Na_v1.6$ transcript levels. This coincides with the appearance of $Na_v1.6$ channels in the retina and optic nerve. The steady-state level of $Na_v1.2$ mRNA also increased during this same period, which suggests that the rise in $Na_v1.6$ may be part of a general increase in sodium channel transcripts at about the time of eye opening at P14. The results are consistent with a developmental increase in steady-state transcripts giving rise to a corresponding increase in sodium channel protein expression.

Keywords: Retinal ganglion cell; Sodium channels; Quantitative PCR; Axon initial segment; Retina development

Voltage-gated sodium (Na_v) channels accumulate at high density at sites that are specialized for the initiation and conduction of action potentials, such as axon initial segments and nodes of Ranvier. In the adult nervous system, the high-density clusters of channels at both initial segments and nodes consist of a specific Na_v channel isoform, $Na_v1.6$ [1–4], but a different isoform, $Na_v1.2$, is typically found in unmyelinated axons. This distinction is especially clear in retinal ganglion cells, whose axons are unmyelinated within the eye and then become myelinated after exiting the eye and entering the optic nerve. $Na_v1.2$ channels are expressed uniformly along the unmyelinated intraocular axons of ganglion cells, but $Na_v1.6$ channels are sharply restricted to the axon initial segment and nodes of Ranvier [1,2].

During myelination, $Na_v1.2$ channels accumulate first at immature nodes of Ranvier but are replaced by $Na_v1.6$ channels as nodes mature, which occurs during the second and third postnatal weeks in the optic nerve [1]. A similar developmental switch from $Na_v1.2$ to $Na_v1.6$ channels also takes place concurrently at the axon initial segments of retinal ganglion cells [2]. Prior to approximately P10, $Na_v1.6$ immunoreactivity is undetectable in retinal ganglion cells, but by approximately P21, the adult pattern is established. Similarly, $Na_v1.6$ channel protein levels are low in Western blots of optic nerve extracts prior to about P10, and then rise progressively to adult levels [1]. It is interesting that the developmental increase in $Na_v1.6$ channel expression parallels the development of repetitive firing in retinal ganglion cells, which make the transition from single spikes to repetitive spikes during sustained depolarization between approximately P14 and P21 [10]. It is not yet clear, however, whether the developmental upregulation of $Na_v1.6$ channel protein is accompanied by an increase in steady-state levels of $Na_v1.6$ transcripts. To examine this question, we used real-time, quantitative PCR to determine relative amounts of $Na_v1.6$ and $Na_v1.2$ transcripts in mRNA isolated from rat retinas at various postnatal ages spanning the appearance of $Na_v1.6$ channel protein.

At each developmental age (P2, P5, P10, P19, P34, and >P60), poly-A RNA was extracted from retinas pooled from litters of rats using the Micro-Poly-A-Pure kit (Ambion), followed by DNase treatment (Ambion, RNase-free). Complementary

* Correspondence to: Center for Brain and Spinal Cord Research, Department of Neurobiology and Behavior, State University of New York, Stony Brook, NY 11794-5230, United States. Tel.: +1 631 632 8648; fax: +1 631 632 4858.
E-mail address: Gary.G.Matthews@sunysb.edu (G. Matthews).

0304-3940/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2006.05.019

DNA was synthesized using MultiScribe reverse transcriptase (Applied Biosystems). At each sampled age, four batches of mRNA were prepared from different litters and analyzed separately. All PCR analyses were performed in a total volume of 50 μl using the SYBR-Green PCR Master Mix and an ABI Prism 7700 Sequence Detector (Applied Biosystems). Primers selected for amplification of $Na_v1.6$, $Na_v1.2$, and β-actin were of equal efficiency across the range of template concentration used (1–10 ng cDNA). Amplification of β-actin served as an internal reference for each sample [8]. Reactions were performed in triplicate for each of the four samples of reverse transcribed mRNA at each developmental age, and steady-state expression levels for $Na_v1.2$ and $Na_v1.6$ transcripts relative to β-actin were calculated across development using the $2^{-\Delta\Delta CT}$ method [5]. In each experiment, control reactions were included in which reverse transcriptase was omitted (−RT control) and in which no template cDNA was added to the PCR mix (no DNA control). Forward and reverse PCR primers specific for $Na_v1.2$ and $Na_v1.6$ alpha subunits were designed within divergent regions near the 3′ end of rat sodium channel mRNA, corresponding to the C-terminus of the protein. As shown in Fig. 1, the specificity of the primers for $Na_v1.2$ and $Na_v1.6$ was verified by PCR from plasmids incorporating the corresponding regions of rat $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, and $Na_v1.6$. The primers were: $Na_v1.2$ forward, 5′-TCCGGTTTCGTCACGCTATC-3′; $Na_v1.2$ reverse, 5′-TCCAGAGAAGACTGATGTGACACC-3′; $Na_v1.6$ forward, 5′-CAAGCTGGAGAATGGAGGCA-3′; $Na_v1.6$ reverse, 5′-TAAGAGGGGAGGGAGGCTGT-3′; β-actin forward, 5′-GAGCGCAAGTACTCTGTGTGGA-3′; β-actin reverse, 5′-TCCACATCTGCTGGAAGGTG-3′.

Fig. 2 illustrates the developmental change in steady-state levels of $Na_v1.6$ and $Na_v1.2$ transcripts, relative to their levels at P2. Examples of single amplification curves for the three transcripts from an individual experiment at P2 are shown in Fig. 2A. For clarity, single curves are shown, although all reactions were actually carried out in triplicate. A threshold level

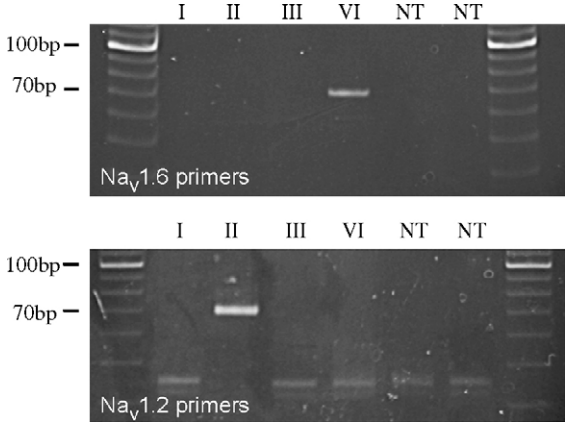

Fig. 1. Verification of specificity of primers used for amplification of $Na_v1.6$ and $Na_v1.2$ cDNAs in real-time quantitative RT-PCR. The primers were tested for specificity against plasmids containing the sequence corresponding to the C-terminus of $Na_v1.1$ (I), $Na_v1.2$ (II), $Na_v1.3$ (III), and $Na_v1.6$ (VI) (cloned in PGEM or PGEX-3X vector). Control lanes marked NT represent PCR with no added template, or with undigested template. Products were run on a 10% polyacrylamide gel.

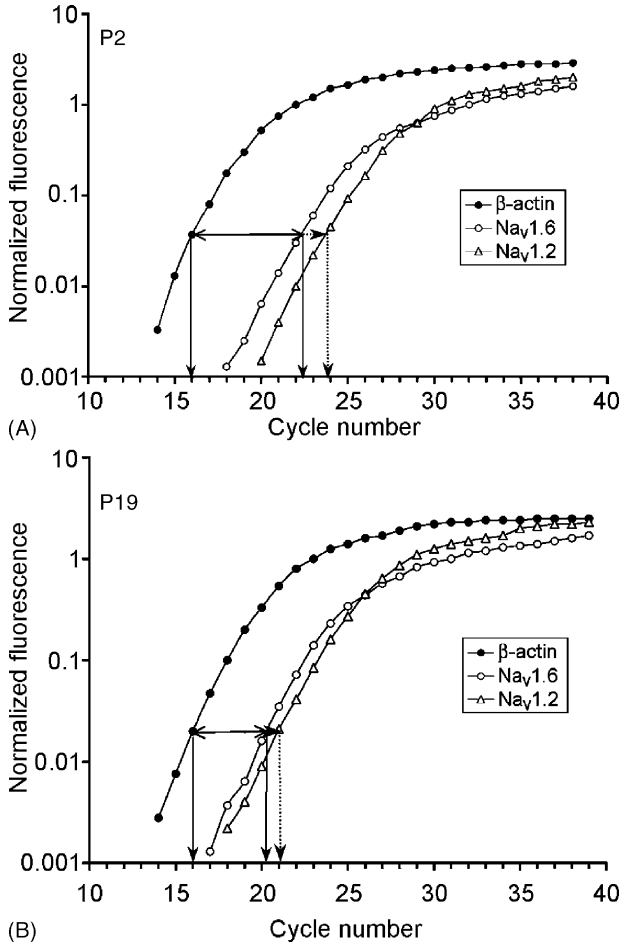

Fig. 2. Estimation of amounts of $Na_v1.2$ and $Na_v1.6$ transcripts by quantitative PCR. (A) Example fluorescence curves at P2, illustrating the shift in threshold cycle, ΔC_T, for $Na_v1.6$ (solid lines) and $Na_v1.2$ (dashed lines) compared with β-actin. (B) Example fluorescence curves at P19, demonstrating the developmental change in ΔC_T for $Na_v1.2$ and $Na_v1.6$.

was set within the range of PCR cycles over which the fluorescence signal doubled for each cycle, and the threshold cycle, C_T, necessary to reach threshold was determined for each reaction. After averaging the C_T values across the three reactions for each amplicon, the shift in average C_T (ΔC_T) relative to β-actin was measured for $Na_v1.6$ and $Na_v1.2$. As shown in Fig. 2B, ΔC_T was smaller for both $Na_v1.6$ and $Na_v1.2$ at P19 than at P2, which indicates an increase in steady-state levels of mRNA for both channel isoforms during developmental maturation of the retina. The change in ΔC_T from P2 to P19 (i.e., $\Delta\Delta C_T$; [5]) was then used to estimate the fold-change in $Na_v1.2$ and $Na_v1.6$ transcript levels between these two ages. Note, however, that the method does not allow conclusions regarding the amounts of $Na_v1.2$ and $Na_v1.6$ transcripts relative to each other.

Fig. 3 summarizes the developmental time course of the increase in mRNA levels for both $Na_v1.6$ and $Na_v1.2$. At P5 and P10, $Na_v1.6$ levels were the same as at P2, but by P19, $Na_v1.6$ mRNA increased approximately three-fold to a level that was maintained through adulthood. This rise in $Na_v1.6$ transcripts coincides with the appearance of $Na_v1.6$ protein at axon initial segments and nodes of Ranvier of retinal ganglion cells [1,2],

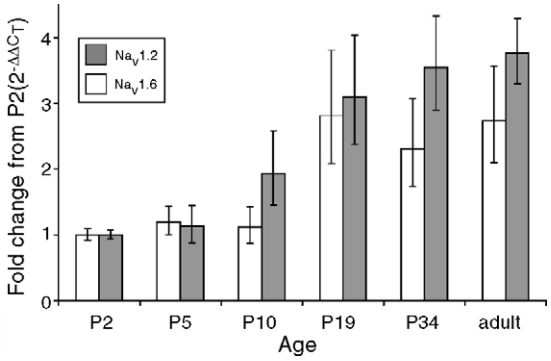

Fig. 3. Developmental increase in steady-state levels of mRNA for $Na_v1.2$ (white bars) and $Na_v1.6$ (gray bars), estimated from the shift in ΔC_T relative to P2. Each data point represents the mean of estimates from four RNA preparations. Error bars indicate ±1 standard deviation. The levels for both $Na_v1.2$ and $Na_v1.6$ at P19, P34, and adulthood are significantly elevated compared to P2 ($p < 0.03$).

which suggests that the increase in steady-state mRNA level gives rise to the increase in protein. The levels of $Na_v1.2$ transcript also increased significantly between P10 and P19, which might at first seem surprising given that $Na_v1.6$ channels replace $Na_v1.2$ channels at initial segments and nodes of Ranvier during this period. However, $Na_v1.2$ channels continue to be expressed in the unmyelinated axons of ganglion cells and in neurites of the inner plexiform layer throughout development, and so the steady-state level of $Na_v1.2$ mRNA would not necessarily be expected to decline. The fact that $Na_v1.2$ transcripts actually rose could indicate that $Na_v1.2$ channel expression increases in these other neuronal compartments at about the time of eye opening, at P14.

Although both $Na_v1.2$ and $Na_v1.6$ transcripts rise in parallel after P10, prior to this period only $Na_v1.2$ channels can be detected with immunocytochemistry or Western blots [1]. This implies that expression of $Na_v1.2$ channels before P10 is controlled by a selective mechanism that regulates $Na_v1.2$ only, whereas the later rise in $Na_v1.6$ may be governed by a nonselective mechanism that upregulates multiple sodium channel isoforms, including $Na_v1.2$. In this regard, it is interesting that $Na_v1.1$ channels have also been reported to appear in the retina after P10 [9], consistent with a generalized mechanism controlling expression of Na_v channels. In addition, the developmental period from P10 to P19 coincides with the development of the ability of rat retinal ganglion cells to fire repetitively during sustained depolarization [10], which in turn suggests that enhanced repetitive firing might result from increased expression of Na_v channels in ganglion cells. However, the density of sodium current in rat ganglion cells has already reached adult levels by P12 [6,7], and Wang et al. [10] attributed the developmental increase in repetitive firing to enhanced recovery of sodium channels from inactivation, not to an overall increase in sodium current density. Therefore, the appearance of repetitive firing after P10 seems more likely to be due to changes in isoform-specific targeting of Na_v channels at important sites like the axon initial segment than to an overall increase in Na_v channel expression.

Acknowledgements

Supported by NIH grant EY03821 (G.M.) and NRSA Fellowship F31 NS048762 (A.V.). We thank Diane Henry for useful discussions and technical advice, and Dr. Gail Mandel (HHMI, SUNY Stony Brook) for providing access to the quantitative, real-time PCR apparatus in her laboratory.

References

[1] T. Boiko, M.N. Rasband, S.R. Levinson, J.H. Caldwell, G. Mandel, J.S. Trimmer, G. Matthews, Compact myelin dictates the differential targeting of two sodium channel isoforms in the same axon, Neuron 30 (2001) 91–104.
[2] T. Boiko, A. Van Wart, J.H. Caldwell, S.R. Levinson, J.S. Trimmer, G. Matthews, Functional specialization of the axon initial segment by isoform-specific sodium channel targeting, J. Neurosci. 23 (2003) 2306–2313.
[3] J.H. Caldwell, K.L. Schaller, R.S. Lasher, E. Peles, S.R. Levinson, Sodium channel $Na_v1.6$ is localized at nodes of Ranvier, dendrites, and synapses, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 5616–5620.
[4] S.M. Jenkins, V. Bennett, Ankyrin-G coordinates assembly of the spectrin-based membrane skeleton, voltage-gated sodium channels, and L1 CAMs at Purkinje neuron initial segments, J. Cell Biol. 155 (2001) 739–746.
[5] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method, Methods 25 (2001) 402–408.
[6] S. Schmid, E. Guenther, Developmental regulation of voltage-activated Na+ and Ca2+ currents in rat retinal ganglion cells, NeuroReport 7 (1996) 677–681.
[7] S. Schmid, E. Guenther, Alterations in channel density and kinetic properties of the sodium current in retinal ganglion cells of the rat during in vivo differentiation, Neuroscience 85 (1998) 249–258.
[8] T. Suzuki, P.J. Higgins, D.R. Crawford, Control selection for RNA quantitation, BioTechniques 29 (2000) 332–337.
[9] A. Van Wart, T. Boiko, J.S. Trimmer, G. Matthews, Novel clustering of sodium channel $Na_v1.1$ with ankyrin-G and neurofascin at discrete sites in the inner plexiform layer of the retina, Mol. Cell. Neurosci. 28 (2005) 661–673.
[10] G.Y. Wang, G. Ratto, S. Bisti, L.M. Chalupa, Functional development of intrinsic properties in ganglion cells of the mammalian retina, J. Neurophysiol. 78 (1997) 2895–2903.