Neuroscience Letters 486 (2010) 53–59

Contents lists available at ScienceDirect

Neuroscience Letters

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

Review

Electrophysiology and beyond: Multiple roles of Na^+ channel β subunits in development and disease

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ARTICLE INFO

Article history:
Received 29 April 2010
Received in revised form 2 June 2010
Accepted 16 June 2010

Keywords:
Voltage-gated Na+ channel
Beta subunit
Cell adhesion
Na+ current
Mouse model
Epilepsy

ABSTRACT

Voltage-gated Na^+ channel (VGSC) β Subunits are not "auxiliary." These multi-functional molecules not only modulate Na^+ current (I_{Na}), but also function as cell adhesion molecules (CAMs)—playing roles in aggregation, migration, invasion, neurite outgrowth, and axonal fasciculation. β subunits are integral members of VGSC signaling complexes at nodes of Ranvier, axon initial segments, and cardiac intercalated disks, regulating action potential propagation through critical intermolecular and cell–cell communication events. At least *in vitro*, many β subunit cell adhesive functions occur both in the presence and absence of pore-forming VGSC α subunits, and *in vivo* β subunits are expressed in excitable as well as non-excitable cells, thus β subunits may play important functional roles on their own, in the absence of α subunits. VGSC β1 subunits are essential for life and appear to be especially important during brain development. Mutations in β subunit genes result in a variety of human neurological and cardiovascular diseases. Moreover, some cancer cells exhibit alterations in β subunit expression during metastasis. In short, these proteins, originally thought of as merely accessory to α subunits, are critical players in their own right in human health and disease. Here we discuss the role of VGSC β subunits in the nervous system.

Contents

1. β Subunits are multi-functional CAMs

VGSCs in brain are heterotrimers, containing a single α subunit associated with one non-covalently (β1 or β3) and one covalently (β2 or β4) linked β subunit [9,85]. In mammals, *SCN1B–SCN4B* encode β1–β4, respectively. β1, β2, β3, and β4 are type I transmembrane proteins, containing an extracellular N-terminal signal peptide and Ig domain, one transmembrane domain, and an intracellular C-terminal domain (Fig. 1). *SCN1B* gives rise to two splice variants, β1 and β1B (also called β1A) [32,61]. β1B is formed through retention of intron 3, containing a stop codon and thus excluding the transmembrane domain in exon 4. The predicted amino acid sequence of the retained intronic region exhibits very low homology between species [61], however, hydrophobicity analysis of these sequences reveals no transmembrane domains in any species, predicting that β1B is a secreted protein that may func-

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doi:10.1016/j.neulet.2010.06.050

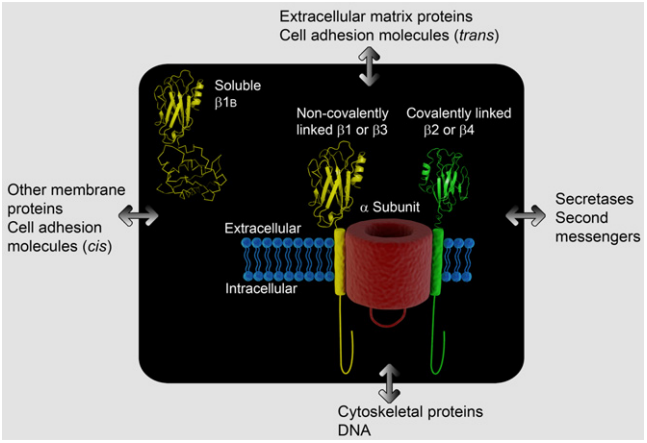

Fig. 1. Subunit structure of VGSCs. VGSCs in the CNS are multiprotein complexes composed of a single pore-forming α subunit, one non-covalently linked β subunit (β1 or β3), and one covalently linked β subunit (β2 or β4). β1B is a secreted, soluble subunit. α and β Subunits interact with multiple cell adhesion, ECM, cytoskeletal, and intracellular signal transduction proteins. Models of the β1 and β2 Ig loops and the C-terminus of β1B were obtained by analyzing amino acid sequences on the I-TASSER server [82,87,88]. We thank Mauricio Patino for his help in the design of this figure.

tion as a ligand for cell adhesion [58]. All of the β subunits, including β1B, belong to the Ig superfamily of CAMs [26,86]. Two *SCN1B* splice variants, including transmembrane and secreted forms, is consistent with other Ig superfamily CAMs [28,64].

VGSC β subunits are widely expressed in excitable and non-excitable cells in the nervous system. Table 1 provides a detailed description of nervous system expression of β1–β4 in mammals and zebrafish. In some tissues there is evidence that β subunits may be expressed in the absence of α, suggesting that they may play cell adhesive roles in the absence of ion conduction *in vivo*. β subunit expression in the CNS is developmentally regulated. Of the four genes, β1B and β3 mRNA predominate in fetal brain, with levels decreasing during late gestation and after birth. In contrast, levels of β1 and β2 mRNA increase progressively and become dominant after birth [26,32,65,69].

β Subunits associate with multiple VGSC α subunits [16,41,48,70]. In addition, β subunits interact both in *cis* and in *trans* with multiple CAMs, with components of the extracellular matrix (ECM), and with intracellular cytoskeletal and signaling molecules. A summary of interactions is presented in Table 2, with the majority of these studies focusing on β1. Similar studies have not been carried out for β1B, however, because β1 and β1B share the extracellular Ig domain, it is safe to assume that these two CAMs share many, if not all, extracellular binding partners.

β1 associates with multiple CAMs, including itself, contactin, neurofascin-186 and -155, NrCAM, N-cadherin, and VGSC β2 [30,41,43,63]. β2 does not associate with contactin, but does associate with β1 and the ECM proteins, tenascin-C and tenascin-R [43,71]. Fibroblasts expressing β1 or β2 are repelled by tenascin-R substrates, suggesting initial binding of this ECM molecule [83]. *Trans* homophilic β1 or β2 (but not β3) association results in recruitment of ankyrin to points of cell–cell contact [39,42]. Phosphorylation of a critical tyrosine residue (β1Y181) abolishes β1 association with $ankyrin_B$ or $ankyrin_G$ and this is postulated to be a mechanism regulating β1 subcellular localization [40,44]. Indirect evidence suggests that β1 may associate with the lipid raft tyrosine kinase fyn in response to extracellular *trans* β1–β1 adhesion [5]. Association of the β1 intracellular domain with receptor phosphotyrosine phosphatase β may provide a yin-yang mechanism of phosphorylation and dephosphorylation [62]. β1 and β2 also participate in heterophilic extracellular interactions and some of these interactions require the intracellular domain of at least one of the partners. For example, the intracellular domains of NrCAM and β2, respectively, are necessary for their extracellular association with β1, suggesting inside-out signaling mechanisms [43].

β Subunits are substrates for sequential cleavage by the β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) and γ-secretase [81]. β2 is also cleaved by the α-secretase ADAM10 [34]. Cleavage of β subunits by BACE1 or α-secretase at sites in the extracellular juxtamembrane region results in ectodomain shedding, leaving membrane-bound C-terminal fragments (CTFs) [34,81]. The shed ectodomain of β1 may function as a soluble ligand for cell adhesion to promote neurite outgrowth [14,39]. The CTFs are processed by γ-secretase, resulting in freed intracellular domains (ICDs) [34,81]. Inhibition of β2 cleavage by γ-secretase reduces cell–cell adhesion and migration [34] and β4 processing by BACE1 increases neurite outgrowth [49], predicting that proteolytic processing events are critical to the *in vivo* functioning of these subunits. The β2 ICD translocates to the nucleus and increases *Scn1a* expression, suggesting that this fragment may function as a transcriptional regulator of VGSC α subunits [33]. Although all four β subunits are BACE substrates *in vitro*, *in vivo* processing has only been confirmed for β2 and β4 [81], suggesting that this role may be specific to subunits that can be covalently linked to α.

Table 1
Tissue specific and subcellular domain specific expression of VGSC β subunits in the nervous system.

Tissue – subcellular domain	β1	β1B	β2	β3	β4
Central nervous system					
Hippocampal neurons	+ [12]		+ [86]	+ (RNA) [52,69]	+ [86]
Cortical neurons		+ [32,61]	+ [86]	+ (RNA) [52,69]	+ [86]
Basal ganglia	– (RNA) [52]		+ [86]	+ (RNA) [52,69]	+ [86]
Retinal ganglion cells	+ [18,29]		+ [29]		
Optic nerve nodes of Ranvier	+ [12,18]		+ [29]		
Optic nerve myelin	+ [19,29]				
Astrocytes	+ (RNA) [2,55]		+ [54]		
Dorsal root ganglia neurons		+ [61]	+ [60,86]	+ [8]	+ [86]
Ventral horn neurons		+ [32]	+ [86]		+ [86]
Radial glia	+ [19]				
Cerebellar Purkinje cells (soma)		+ [32,61]	+ [86]	+ (RNA, transitory) [69]	+ [86]
Cerebellar granule neurons (soma)	+ [4]			– (RNA) [52,69]	
Cerebellar granule neurons (AIS and growth cone)	+ [4]				
Deep cerebellar nuclei		+ [32]	+ [86]		+ [86]
Bergmann glia	+ [14]	– [32]	– [14]	– [14]	+ [14]
Peripheral nervous system					
Peripheral nerves		+ [61]	+ [60]	+ [8]	
Sciatic nerve nodes of Ranvier	+ [12]				
Schwann cells (zebrafish)	+ [19]				