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THE ROLE OF SODIUM CHANNELS IN CELL ADHESION

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TABLE OF CONTENTS

1. ABSTRACT

Voltage-gated sodium channels are unique in that they combine action potential conduction with cell adhesion. Mammalian sodium channels are heterotrimers, composed of a central, pore-forming α subunit and two auxiliary β subunits. The α subunits are members of a large gene family containing the voltage-gated sodium, potassium, and calcium channels. Sodium channel α subunits form a gene subfamily with at least eleven members. Mutations in sodium channel α subunit genes have been linked to paroxysmal disorders such as epilepsy, long QT syndrome (LQT), and hyperkalemic periodic paralysis in humans, and motor endplate disease and cerebellar ataxia in mice. Three genes encode the sodium channel β subunits with at least one alternative splice product. Unlike the pore-forming α subunits, the sodium channel β subunits are not structurally related to β subunits of calcium and potassium channels. Sodium channel β subunits are multifunctional. They modulate channel gating and regulate the level of channel expression at the plasma membrane. We have shown that β subunits also function as cell adhesion molecules (CAMs) in terms of interaction with extracellular matrix molecules, regulation of cell migration, cellular aggregation, and interaction with the cytoskeleton. A mutation in SCN1B has been shown to cause GEFS+1 epilepsy in human families. We propose that the sodium channel signaling complex at nodes of Ranvier involves β subunits as channel modulators as well as CAMs, other CAMs such as neurofascin and contactin, RPTPβ, and extracellular matrix molecules such as tenascin. Finally, we explore other subunits of voltage-gated ion channels as potential CAM candidates.

2. INTRODUCTION

Voltage-gated sodium channels are responsible for action potential initiation and conduction in excitable cells. In neurons and myocytes, sodium channels are not randomly distributed but instead are localized to specialized, subcellular domains such as nodes of Ranvier, axon initial segments, and cardiac z-lines and intercalated discs. What is the mechanism responsible for sodium channel localization and clustering? What proteins make up the sodium channel signaling complex? Finally, are the identities of these proteins the same in all excitable tissues, or are the members of the sodium channel signaling complex cell-specific or even subcellular domain-specific? We have shown that sodium channels are unique among voltage- and ligand-gated ion channels in that they contain auxiliary subunits that not only modulate channel kinetics, but also function as cell adhesion molecules that direct channel insertion into the plasma membrane and channel interaction with other signaling proteins. In this article we will present the novel idea that the cell adhesive functions of sodium channel β subunits may be as important or even more important than modulation of channel gating in mammalian excitable cells. We propose that, as cell adhesion molecules, sodium channel β subunits act as critical communication links between extra- and intracellular signaling molecules in excitable cells.

Voltage-gated sodium channels isolated from mammalian neurons are heterotrimeric protein complexes composed of α and β subunits (1). The mammalian pore-forming α subunits encode a gene family of at least eleven members (2, 3). The β subunit gene family consists of three members: β1 (SCN1B) (4), β2 (SCN2B) (5), and β3 (SCN3B) (6). β1A, a splice variant of the SCN1B gene expressed in embryonic brain, has also been described (7). α subunit cDNAs express functional sodium channels in heterologous expression systems such as *Xenopus* oocytes or mammalian fibroblasts. However, at least for brain and skeletal muscle α subunits, the currents characteristic of these channels expressed in isolation are quite different from native currents. Coexpression of the auxiliary β subunits with these channels results in hyperpolarizing shifts in the voltage-dependence of activation and inactivation, changes in channel modal gating behavior resulting in increases in the rates of inactivation and recovery from inactivation (8), and increases in channel density at the plasma membrane as assessed by ^{3}H-saxitoxin binding (9).

The behavior of sodium channel α and β subunits is critically dependent on the particular heterologous expression system chosen by the investigator. Functional modulation of α by β subunits is qualitatively different in *Xenopus* oocytes vs. mammalian cells. For example, brain and skeletal muscle α subunits gate slowly in *Xenopus* oocytes. Coexpression of β1 results in major changes in channel gating mode (8). In contrast, these same channels expressed in mammalian fibroblasts gate in a fast mode in the apparent absence of β subunits and β subunit coexpression has more subtle effects on channel gating than those observed in oocytes. These results suggested that a major function of β subunits in neurons may be something in addition to kinetic modulation of sodium channels (9).

A number of excellent reviews describing the effects of sodium channel β subunits on channel gating have been published (1, 8, 10-14). This review will focus instead on more recently described, novel functions of the sodium channel auxiliary subunits. Sodium channel β subunits and the β subunits of voltage-gated calcium and potassium channels are functionally homologous in terms of channel modulation (8, 12). However, calcium and potassium channel β subunits are not structurally homologous to sodium channel β subunits and only the sodium channel β subunits have been shown to function as cell adhesion molecules in addition to their roles in channel modulation. Thus, the sodium channel β subunits present a unique opportunity to study cell adhesion in terms of electrical signal transduction.

3.1. Sodium channel b subunits form a gene family

β1, β1A, β2, and β3 are transmembrane proteins with type I topology: containing an extracellular amino-terminus, a single transmembrane segment, and an intracellular carboxyl terminus. β1 and β1A are splice variants of SCN1B (7). β3 is homologous to β1 but is the product of a separate gene (6). β1 and β3 mRNA expression are developmentally and anatomically regulated. *In situ* hybridization studies comparing β1 and β3 mRNA localization in the adult rat central nervous system suggested that the expression of these two subunits is complementary (6). Interestingly, β3 mRNA is expressed in small-diameter c-fibers in rat dorsal root ganglia and its expression is significantly upregulated in a model of neuropathic pain (15). This expression pattern correlates with that of the peripheral nerve sodium channel PN3 (Nav1.8/SCN10A) under the same conditions. In contrast, β1 mRNA is expressed in large-diameter neurons but not in the small-diameter cells (15). The developmental expression patterns of β1 and β1A in total rat brain RNA showed that β1A is expressed early in embryonic brain development while β1 is not expressed until after postnatal day 2 (7, 16, 17). β1A expression in brain decreases after birth, concomitant with the emergence of β1 expression. In the adult rat, immunolocalization of β1A polypeptides showed expression in heart and small-diameter c-fiber and Aβ dorsal root ganglion neurons in addition to brain (7). In addition to brain, β1 is expressed in adult spinal cord (18, 19), dorsal root ganglion (20-22), sciatic nerve (18), skeletal muscle (18, 23), neuroendocrine cells (7, 18), glia (24-28), and heart (18, 29).

β2 is expressed at low levels during embryonic brain development and then at high levels in brain and spinal cord following postnatal day 7 (5, 30). While it was originally thought, based on Northern blot analysis of total RNA from various tissues (5) and Western blot analysis of sciatic nerve (30), that β2 expression was exclusive to the central nervous system, we now know this is not the case. More sensitive RT-PCR and *in situ* hybridization techniques have revealed β2 mRNA expression in peripheral sensory neurons and in heart muscle (22, 29). In addition, β2 polypeptides have been colocalized with sodium channel α and β1 subunits to z-lines of rat and mouse ventricular myocytes (29).

All four β subunit molecules contain a single extracellular immunoglobulin (Ig) domain that is structurally homologous to the V-set of the Ig superfamily and often found in CAMs (31). This unique property of the sodium channel auxiliary subunits was first discovered following sequence analysis of β2, revealing that its extracellular domain contained an Ig fold and an extended region with similarity to the CAM contactin (5). Two distinct regions of the extracellular domain of contactin have greater than 40% amino acid sequence identity with sodium channel β2 subunits. Subsequent analysis of the extracellular domains of β1/β1A, and β3 showed a similar homology to the CAM myelin Po, with β3 showing the highest degree of similarity (6, 32). Investigation of the amino acid sequences of β1, β1A, β2, and β3 showed that these proteins are closely related (Figure 1) (33). The extracellular Ig fold region (8) of all 4 proteins is highly conserved. While β3 is most closely related to β1 (Figure 1, amino acids indicated in green), there are a number of conserved residues between β3 and β2 outside of the Ig loop (Figure 1, outlined amino acids). The intracellular domain of β1A is very different from β1, due to alternative splicing (Figure 1, arrow). It will be interesting in the future to assign

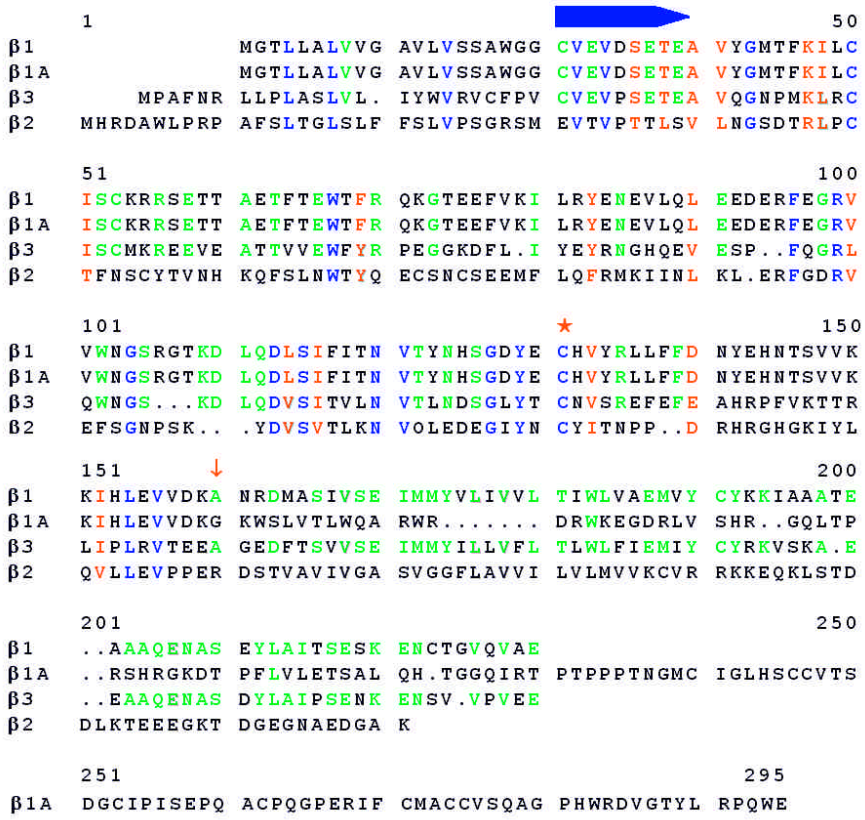

Figure 1. Comparison of β1, β1A, β3, and β2 amino acid sequences. Rat brain β1 (GenBank accession number P14860), β1A (accession number AF182949), β3 (accession number AJ243395), and β2 (accession number U37026) were aligned using the Pileup program in the Genetics Computer Group software package (GapWeight: 8. GapLengthWeight: 2). β1 and β1A are splice variants of the same gene and have identical sequence through …LLVVDK. Arrow indicates splice site. Blue: identical amino acid residues in all 4 sequences. Red: similar amino acid residues in all 4 sequences. Green: identical amino acid residues in β1/β1A and β3. Outlined type: identical amino acid residues in β2 and β3 sequences. ★: location of GEFS+1 mutation (C121W) in β1. Blue bar: A/A' face of β1 Ig loop involved in α subunit interactions (32).

specific functions to these regions using mutagenesis strategies.

3.2. Structure-function experiments define the Ig loop region of b1 and sites of a-b1 interaction

The extracellular domain of β1 is critical for channel gating and modulation of the voltage-dependence of brain and skeletal muscle sodium channels expressed in *Xenopus* oocytes (Fig. 2) (32, 34-36). Removal of the intracellular domain of β1, substitution of the intracellular β1 domain with the corresponding domains from either β2 or the structurally related protein myelin P_o, or attachment of the β1 extracellular domain to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor all resulted in retention of full β1-like modulation of Nav1.2 sodium channels expressed in *Xenopus* oocytes (32). Site-directed mutagenesis of the extracellular β1 Ig loop, based on comparison with the crystal structure of the Ig loop of myelin P_o, resulted in identification of key residues in the A/A' face of β1 required for interaction with Nav1.2 in *Xenopus* oocytes (Figure 1, blue bar) (32). In contrast, mutagenesis of hydrophobic residues at the juxtamembrane region of β1 or the sites of N-linked glycosylation had no effect on β1-mediated channel modulation in oocytes (32). A mutation in the putative transmembrane region of β1 did alter the voltage-dependence of sodium channel steady state inactivation, suggesting that this region may also play a role in α–β1 subunit interactions (32).

Where does β1 interact with the α subunit? The data suggest strongly that the extracellular Ig domain of β1 subunits interacts functionally with α subunits through a site formed by one or more extracellular loops of the α subunit. Experiments were designed to identify domains critical for α–β1 subunit interactions using brain, skeletal muscle, and cardiac sodium channel α subunit cDNAs expressed in *Xenopus* oocytes (36). The design of these experiments took advantage of the observation that Nav1.2 and Nav1.4 sodium channels are modulated from slow to fast gating modes by coexpression of β1 in *Xenopus* oocytes. In contrast, Nav1.5 channels gate in a fast mode in the absence of β1 subunits when expressed in oocytes. A series of Nav1.4/Nav1.5 chimeras expressed in oocytes showed that substitution of IS5-S6 and IVS5-S6 in Nav1.4

with the corresponding domains in Nav1.5 completely abolished the effects of β1 on channel inactivation (36). Substitution of just one of these domains resulted in channels with an attenuated response to β1, suggesting that β1 may interact with a discontinuous epitope formed by IS5-S6 and IVS5-S6 that are in close proximity to each other in the 3-dimensional structure of the sodium channel. Alternatively, β1 may interact elsewhere on the channel, but these domains are required for changes in gating behavior. Using a series of Nav1.2/Nav1.5 chimeras, it was shown that substitution of the IVSS2-S6 segment in Nav1.2 with the corresponding domain in Nav1.5 produced a channel that did not shift to a fast gating mode in the presence of β1 subunits. (37) (IVSS2-S6 is contained within the IVS5-S6 region used in the first study.) This reduced β1 effect could be partially overcome by the addition of higher levels of β1 subunits in the oocyte. It was suggested that the IVSS2-S6 substitution resulted in a reduction in the affinity of the α subunit for β1. It may also be possible that substitution of an additional domain in the α subunit, for example, IS5-S6 as shown in the first study, is required to completely abolish the effects of β1.

α and β1 also interact through intracellular domains. A mutation in the carboxyl-terminal domain of Nav1.5, D1790G, has been implicated in LQT-3 (39). According to topology predictions, D1790 is located intracellularly. This mutation has been shown to result in the disruption of α–β1 subunit interactions when expressed in transfected HEK cells. Removal of the carboxyl-terminal domain of β1 subunits (a mutant called $\beta1_{STOP}$) results in a protein that retains activity in *Xenopus* oocytes, but exhibits a significantly reduced affinity for α. Injection of approximately 10,000 times more mutant $\beta1_{STOP}$ mRNA was required to achieve the level of functional modulation observed with wild type β1. In transfected mammalian cells, $\beta1_{STOP}$ did not modulate sodium channel function, even when expressed at high levels under control of a CMV promoter. (Again, sodium channels behave differently in mammalian cells than in *Xenopus* oocytes.) Coimmunoprecipitation experiments suggested that the loss of functional modulation could be explained by inefficient association of the mutant β1 with α in the cell lines. These studies suggest that α and β1 likely have both intracellular and extracellular interacting domains and that both are required for high affinity association with Nav1.2 (38).

Interestingly, not all sodium channel α subunits are detectably modulated by β1 and β2 subunits, especially those channels primarily expressed in peripheral nerve (40, 41). Recent studies have shown that β3 subunits are expressed in peripheral, sensory neurons and β3 mRNA is upregulated in a chronic constriction injury model of neuropathic pain. Coexpression of PN3 (Nav1.8/SCN10A) sodium channels and β3 in *Xenopus* oocytes results in a hyperpolarizing shift in the voltage-dependence of channel activation as well as a 3-fold increase in peak current amplitude compared to the expression of PN3 alone (15). It has thus been suggested that β3 expression may be critical to action potential conduction in neuropathic pain and that development of small molecules targeting β3 subunits may yield therapeutic results.

3.3. b **subunits as cell adhesion molecules**

Do sodium channel β subunits have roles in addition to modulation of channel kinetics? Three key observations provided important clues: First, β2 subunit expression was found to be required to detect the translocation of newly synthesized sodium channels from intracellular stores to the plasma membrane of primary cultured embryonic neurons (42, 43). Second, expression of β2 subunits alone in *Xenopus* ooctyes resulted in dramatic changes in membrane capacitance through promotion of intracellular vesicle fusion with the microvillus membrane (5). Thus β2, in the absence of α, caused expansion of the oocyte surface membrane via intravesicular fusion. Third, coexpression of α and β1 subunits in mammalian cells resulted in a significant stabilization of the level of cell surface sodium channels (9). Thus, b subunits function to promote the expression of sodium channels on the cell surface.

Are β subunits merely homologous to CAMs or do they function as CAMs as well?Our laboratory as well as the Catterall laboratory, have asked whether β subunits function as cell adhesion molecules (44-46): Do they interact with extracellular matrix proteins? Do they interact with other CAMs leading to cellular aggregation and cytoskeletal changes? Do they participate in cell migration? If so, what are the implications of these activities on sodium channel localization and density at the node of Ranvier?

3.3.1. Sodium channel b subunits interact with extracellular matrix proteins and influence cell migration

Glial-derived extracellular matrix molecules, for example tenascin-C (TN-C) and tenascin-R (TN-R), play important roles in cellular interactions in developing or injured neurons, such as neuronal migration, neuritogenesis, and neuronal regeneration (47, 48). The tenascins are multi-functional molecules that can promote neurite outgrowth, inhibit growth cone advance, and induce axonal defasciculation *in vitro*. Sodium channel β subunits interact with TN-C (44) and TN-R (44, 45). The tenascins were chosen as candidate extracellular matrix molecules because they bind contactin, a CAM with homology to sodium channel β2 subunits (5). Incubation of purified sodium channels with TN-C showed saturable and specific binding (44). Glutathione *S*-transferase (GST) fusion proteins containing various domains of TN-C and TN-R were tested for their ability to bind purified sodium channels or the recombinant β2 subunit extracellular domain. Both sodium channels and β2 bound specifically to the fibronectin (FN) type III repeats 1-2, A, B, and 6-8 of TN-C and FN type III repeats 1-2 and 6-8 of TN-R. Transfected cells expressing β1 or β2 were repelled from TN-R spotted on a nitrocellulose substrate (45). The same TN-R GST fusion proteins used in the first study were used to determine which domains were responsible for the observed repulsion. Both the β1- and β2-expressing cell lines were strongly repelled by EGF-L (the cysteine-rich amino terminus of TN-R plus the EGF-like domains), but

adhered well to EGF-S (EGF-like domains only), FN 6-8, FG, and GST alone, suggesting that the amino-terminus of TN-R may be involved in repulsion of β1- or β2-expressing cells. In a modified assay to detect cell adhesion, cells expressing β1 subunits alone initially adhered to the TN-R recombinant domains FN 6-8 (as found in the first study for β2) and EGF-S, implying that β subunits recognize TN-R through a transient adhesive reaction prior to the repulsive response. A mixture of EGF-L, EGF-S, and FN6-8 fusion proteins added to the cell culture medium blocked the adhesion of β1-expressing cells to the EGF-like or FN-like domains of TN-R in a concentration-dependent manner. β subunit-mediated effects in response to TN-R occurred in the absence of α subunits, suggesting that β subunits may function as CAMs independently of the ion channel complex.

In two-electrode recordings in *Xenopus* oocytes, application of EGF-L fusion protein produced a rapid increase in the amplitude of sodium currents (45). Surprisingly, this EGF-L-mediated current potentiation was observed in oocytes expressing Nav1.2 alone as well as in oocytes coexpressing Nav1.2, β1, and β2 subunits, suggesting that the pore-forming α subunit can also recognize and respond to TN-R. Neither FN 6-8 fusion protein nor GST affected sodium currents in oocytes, suggesting that current potentiation is a specific effect of the EGF-L domain of TN-R. EGF-L-mediated potentiation was not accompanied by any detectable changes in the voltage-dependence of current activation or inactivation or in any obvious effects on current time course. The physiological significance of EGF-L-mediated current potentiation remains unclear. One intriguing possibility is that it is a means by which neurons or neuronal processes translate contact with TN-R into an intracellular signal. For example, we have proposed that TN-R mediated potentiation of sodium currents in growth cone membranes might lead to an increased influx of calcium through voltage-gated calcium channels and contribute to the mechanism of myelin-evoked growth cone collapse (45). However, further investigation is needed to correlate observations in heterologous expression systems with events in the developing brain.

3.3.2.Sodium channel b subunits interact homophilically to cause cellular aggregation and ankyrin recruitment

CAMs of the Ig superfamily interact homophilically and heterophilically to transduce signals between adjacent cells or adjacent axons where they participate in, for example, axonal fasciculation, growth cone guidance, and nodal formation. It has been shown that CAMs of the L1 subfamily interact homophilically in a *trans* mechanism to produce cellular aggregation (49, 50). Following homophilic binding, L1 family CAMs that have intracellular carboxyl-terminal domains transduce signals resulting in the recruitment of ankyrin and spectrin to points of cell-cell contact. *Drosophila* S2 cells are a classic model system in which potential CAMs have been tested for these properties (51). S2 cells do not express endogenous CAMs and grow as a suspension culture. cDNAs of interest are then cloned into the S2 cell expression vector under the control of an inducible *Drosophila* metallothionein promoter. Upon induction of protein expression, S2 cells transfected with CAMs aggregate. Immunocytochemical localization of endogenously expressed ankyrin can then be performed to determine whether *trans*-homophilic binding results in ankyrin recruitment to the plasma membrane. Using the S2 cell model system we tested whether sodium channel β1 and β2 subunits behave in a similar manner (Figure 2). S2 cells transfected with β1, β2, Nav1.2 + β1, or Nav1.2 + β2 displayed homophilic interactions while cells transfected with Nav1.2 alone did not (46). Immunocytochemical analysis of the cell aggregates revealed recruitment of ankyrin to sites of cell-cell contact (Figure 2B).

The intracellular domains of β1 and β2 are critical for ankyrin recruitment (46). cDNA constructs lacking the intracellular carboxyl-terminal domains of β1 and β2, respectively ($\beta1_{STOP}$ and $\beta2_{STOP}$, Figure 2A), were transfected into S2 cells and the cells were induced to aggregate. While both cell lines aggregated, ankyrin staining was diffuse and not concentrated to points of cell-cell contact as in cells expressing full-length β subunits (Figure 2C). It was concluded that the intracellular carboxyl-terminal domains of β1 and β2 are necessary for *trans*-homophilic mediated signal transduction to the cytoskeleton.

Cytoskeletal interactions through ankyrin and the β subunits may be critical to sodium channel placement in excitable cells. In *Xenopus* oocytes, Nav1.4 and Nav1.7 channels expressed in the absence of β1 subunits shift to a fast gating mode when sites of membrane attachment to the cytoskeleton are ruptured by patch excision, negative pressure, or agents that disrupt microtubule formation (52). Application of positive pressure prevented this shift in kinetics. The authors suggested that because β1 subunits cause similar changes in sodium channel gating in oocytes, β1 may also function through cytoskeletal interactions. In other studies, treatment of inside-out patches of ventricular cells with cytochalasin-D induced Nav1.5 channels to enter a mode characterized by lower peak open probability with a greater persistent activity, consistent with a decrease in the rate of inactivation (53). Cardiac sodium channels in $ankyrin_B$ knockout mice display reduced sodium current density and abnormal kinetics that contribute to prolonged action potential duration and abnormal QT-rate adaptation (54). We have recently colocalized β1 and β2 with sodium channel α subunits and α-actinin to z-lines of cardiac ventricular myocytes (29). Thus, cytoskeletal interactions are likely to be critical to sodium channel localization and gating in excitable tissues such as heart and brain. Interestingly, results from a more recent study show that Nav1.4 channels expressed in Chinese hamster ovary cells in the absence of added β subunits are not affected by treatment with Cytochalasin-D or Colchicine (55). The authors conclude that sodium channel α subunits do not interact with microfilaments or microtubules in transfected CHO cells and suggest that previous studies reporting cytoskeletal effects on channel gating may have been preparation-specific. Our data suggest that sodium channels require association with β subunits to interact with the cytoskeleton (46). It would be interesting to repeat the

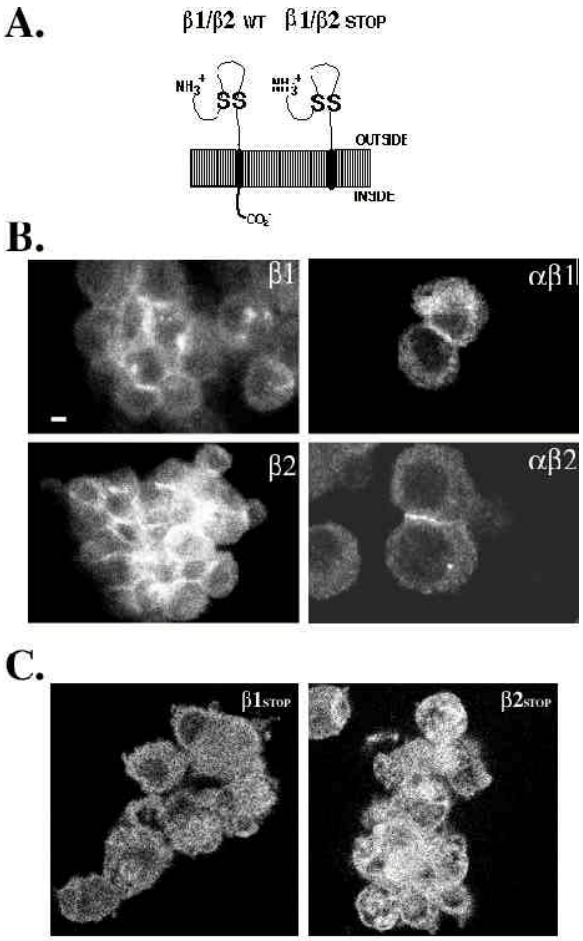

Figure 2. Sodium channel β1 and β2 subunits participate in homophilic cell adhesion. A. Comparison of the predicted membrane topologies of wildtype β1 or β2 (β1/β2 wt) with the β subunit truncation mutants that lack cytoplasmic domains ($\beta1_{STOP}$/$\beta2_{STOP}$).B. β subunit expression results in ankyrin recruitment to points of cell-cell contact. β1-, β2-, αβ1-, or αβ2-transfected S2 cells were induced in the presence of 0.7 mM $CuSO_4$ and aggregation was induced by rotary shaking. Cells were fixed with 2% paraformaldehyde, permeabilized with 0.5% Triton X-100, and stained with *Drosophila* anti-ankyrin antibody (1:200 dilution) followed by FITC-conjugated goat anti-mouse IgG antibody (1:100 dilution) and viewed with a Bio-Rad MRC-600 laser scanning confocal microscope attached to a Nikon Diaphot microscope**.** C. β subunit-mediated ankyrin recruitment requires β subunit cytoplasmic domains. $\beta1_{STOP}$ - and $\beta2_{STOP}$-transfected S2 cells were treated as described in Fig. 1B to visualize ankyrin immunofluorescence. Cells were viewed with a Bio-Rad MRC-600 laser scanning confocal microscope attached to a Nikon Diaphot microscope. All views were analyzed together on the confocal microscope using identical settings. Scale bar: 10 μm. Adapted from Malhotra *et al.*, 2000 with permission.

Moran *et al.* study in the presence and absence of added β subunits.

Sodium channel β subunits may be expressed in some cells as CAMs in the absence of α subunits. Immunolocalization of β1A subunit polypeptides showed expression in rat lung alveoli and in bronchus columnar epithelial and endothelial cells in pulmonary arterioles (7). β1 subunit polypeptides have been shown to be expressed at high levels in oligodendrocytes in the absence of known α subunits (28). Thus, sodium channel β subunits may be similar to AMOG/β2, a molecule that can participate in ion transport as a β2 subunit of the murine Na,K ATPase or can be expressed in the absence the transporter as a CAM (56).

3.4. Sodium channel b subunits interact with RPTPb, neurofascin, and contactin

Sodium channel β subunits are multifunctional and interact with other signaling molecules in complex with, or perhaps independently of, the pore-forming α subunits. In addition to ankyrin, recent studies have shown that β1 can interact with receptor tyrosine phosphatase β (RPTPβ) (57). Interestingly, the extracellular carbonic anhydrase domain of RPTPβ interacts with contactin, inducing neurite outgrowth (58). Phosphacan, a secreted catalytically inactive isoform of RPTPβ, is anchored at nodes of Ranvier through its interaction with TN-R (59), an extracellular matrix molecule that interacts with β1 and β2 resulting in cellular repulsion (44, 45). An intracellular site on β1, but not β2, binds the intracellular phosphatase domains of RPTPβ (57). Examination of the intracellular segments of β1 and β2 revealed that β1 contains a tyrosine residue while β2 does not. This may underlie the specificity of RPTPβ binding to β1 over β2. It has been shown previously that ankyrin binding to neurofascin is regulated by phosphorylation of a critical tyrosine residue in its intracellular domain (60). Perhaps the interaction of β1 and RPTPβ results in the regulation of ankyrin binding through modulation of this intracellular tyrosine residue in β1.

β1 subunits colocalize with the L1 family CAM neurofascin-186 at nodes of Ranvier and the two proteins associate in a heterologous expression system (61). Immunoprecipitation of sodium channel subunits and neurofascin in transfected ts-A201 cells showed that β1 and β3, but not α or β2, interact in *cis* with neurofascin-186. In similar immunoprecipitation experiments, a β1/β2 chimera containing the extracellular domain of β1 and the transmembrane and intracellular domains of β2 was able to interact with neurofascin-186, as did a GPI-linked β1 subunit. The first Ig-like domain and the second fibronectin type III-like domain of neurofascin were found to interact with β1. Interestingly, because both neurofascin-186 and neurofascin-155 contain these domains, it is possible that β1-neurofascin interactions occur in the node of Ranvier as well as in the paranode where they could be involved in formation of diffusion barriers in the axoglial apparatus (62).

β1 subunits also interact with the CAM contactin (63), a GPI anchored protein expressed by neurons and glia that is thought to play multiple roles in the nervous system (64-68) and that has significant homology with sodium channel β2. Tenascin-R, which accumulates at nodes of Ranvier in the CNS, binds to the Ig-like domains of contactin (69-72), as well as to β2 (44). Contactin also interacts with RPTPβ (58). Contactin is linked to the localization of axonal ion channels through its association with Caspr/paranodin, a neurexin-family protein that forms part of the axoglial junctions at paranodes (73-77), and whose expression precedes sodium channel clustering in the optic nerve (78). Contactin and sodium channels can be reciprocally coimmunoprecipitated from rat brain homogenates. Cells co-transfected with Nav1.2 α and β1 subunits and contactin have 3 to 4-fold higher peak sodium currents and correspondingly higher saxitoxin binding than cells with Nav1.2/β1, Nav1.2/contactin, Nav1.2/β2/contactin or Nav1.2/β1/β2, suggesting an increased channel surface membrane density. Coimmunoprecipitation of different subunits from cell lines showed that contactin interacts specifically with β1, and not β2 or α. In the CNS there is a high level of colocalization of sodium channels and contactin at nodes of Ranvier. Contactin may thus significantly influence the functional expression and distribution of sodium channels in neurons through heterophilic cell adhesive interactions with β1 subunits.

Sodium channel α subunits have also been shown to interact directly with RPTPβ and phosphacan (57), with members of the PDZ domain-containing syntrophin family (79), with the synaptic vesicle protein synaptotagmin (80), with GTP-binding proteins (81, 82), and with the extracellular matrix protein agrin (83), and indirectly with the cytoskeletal protein spectrin through ankyrin$_G$ (84). Thus, the sodium channel heterotrimeric complex is predicted to associate with many signaling and clustering molecules to form specialized membrane domains such as nodes of Ranvier, axon initial segments, or the neuromuscular junction.

3.5. Sodium channel b subunits are involved in human disease

Mutations in voltage-gated sodium channel α subunit genes have been shown to be linked to inherited human diseases causing paroxysmal events like LQT in the heart and hyperkalemic periodic paralysis in skeletal muscle (85). Mutations in SCN1A have been linked to idiopathic generalized epilepsy with febrile seizures plus type 2 (GEFS+2) (86-88). A mutation in SCN1B is linked to a large family with GEFS+1 (89). This mutation, C121W, changes a critical cysteine residue defining the carboxyl-terminal region of the Ig loop in β1 to a tryptophan, presumably destabilizing this critical cell adhesion and α subunit association domain (Figure 1, star). Coexpression of the mutant β1 subunit with Nav1.2 in *Xenopus* oocytes revealed a lack of the classic β1-mediated acceleration of the inactivation rate (89). The expressed current was virtually identical to that observed with α alone. It is not yet known whether C121W mutant β1 subunits are expressed at the plasma membrane or, if so, whether they are associated with α subunits. A similar mutation in the third Ig loop of L1CAM found in human patients (C264Y) produces a protein that is expressed at the cell surface, but has significantly impaired homophilic binding ability (90). Heterophilic binding of this mutant L1 protein to known L1CAM binding partners (chick F11, mouse F3, and chick axonin 1) was almost completely disrupted. The extracellular Ig loop of myelin P_o is stabilized by a disulfide bond between C21 and C98. A C98Y mutation in the Ig loop of myelin P_o causes Déjérine-Sottas syndrome, a hereditary demyelinating neuropathy (91). Mutation of C21 to A results in a protein that is expressed at the cell surface but cannot participate in homophilic cell adhesion (92). Interestingly, C21A myelin P_o has a dominant negative effect on adhesion of wild type protein (93). Thus, mutations that destabilize Ig loop domains of various CAMs result in debilitating human diseases and are dominant over the wild type allele. It will be interesting to screen for similar human disease mutations involving β2 and β3.

3.6. Other suspects

Is the idea that ion channel auxiliary subunits may play dual roles as channel modulators and CAMs exclusive to voltage-gated sodium channels, or are there other likely candidates? Calcium and potassium channel β subunits are intracellular proteins and thus cannot function as CAMs. However, there are other auxiliary subunits of voltage-gated ion channels that contain transmembrane segments and extracellular domains. Examples include calcium channel $\alpha_2\delta$ and γ subunits (94), the superfamily of small potassium channel MinK-related subunits (95), and the *Drosophila* sodium channel tipE subunit (96). Is it possible that these subunits interact with cell adhesive and/or extracellular matrix proteins in addition to channel modulation? Circumstantial evidence may suggest further investigation. A recent study has shown that a complex containing calcium channels, α4β2γ1 laminin, and spectrin can be immunoprecipitated from TritonX-100-solubilized synaptosomes isolated from the electric organ of *Torpedo californica* (97). Could calcium channel $\alpha_2\delta$ or γ subunits participate in laminin binding? Calcium channel $\alpha_2\delta$ subunits and N-CAM are colocalized during early myogenesis (98). The authors of this study suggested that $\alpha_2\delta$ may have functions other than calcium channel regulation and may be involved in cellular mechanisms related to molecular targeting to the plasma membrane, as we and our colleagues have proposed for sodium channel β2 (5, 42, 43). Is it possible that $\alpha_2\delta$ and N-CAM interact via heterophilic cell adhesion? The pore-forming α subunits of voltage-gated ion channels might also participate in cell adhesive interactions. For example, GIRK channels (GIRK1 and GIRK4) contain an extracellular RGD (arginine-glycine-aspartate) sequence that allows direct binding to integrin (99). Interaction with integrin appears to be required for proper GIRK channel membrane localization and function in the heart and brain. Opening of Kv1.3 channels in murine T lymphocytes results in activation of β1 integrin and subsequent integrin-mediated cell adhesion and migration (100). Kv1.3 and β1

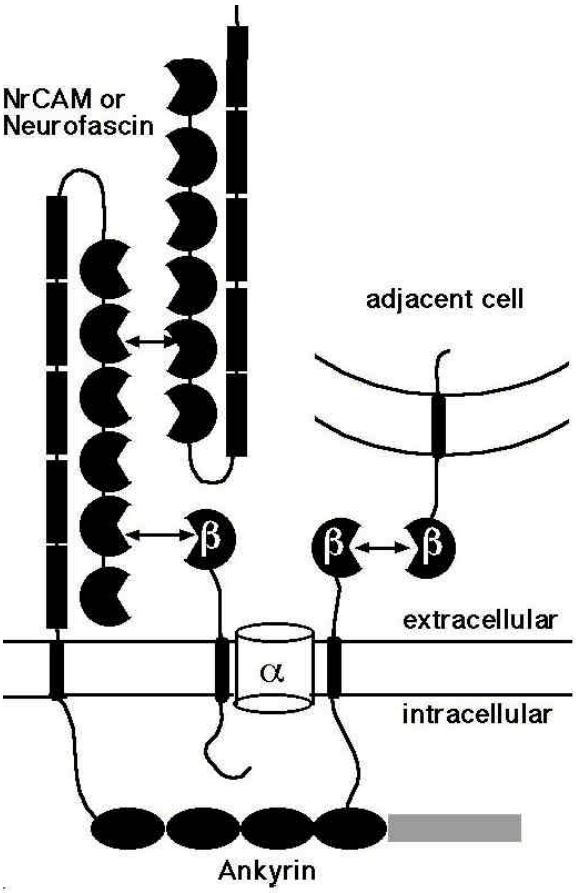

Figure 3. Model of β subunit-ankyrin interactions. Two possible mechanisms for sodium channel β subunit-ankyrin interactions are presented. As previously proposed (102, 107, 108), β subunits may interact via a cis-heterophilic mechanism with NrCAM or neurofascin. NrCAM and neurofascin bind ankyrin following trans-homophilic interactions with the same molecule on an adjacent neuron. Thus, interaction of β subunits with ankyrin by this mechanism is indirect. Alternatively, as suggested by the present study, β subunits may bind ankyrin directly following trans-homophilic interactions with β subunits on adjacent neurons. Reproduced with permission.

integrin can be co-immunoprecipitated from T cells even though Kv1.3 channels do not contain an RGD sequence (100). As reviewed above, we found that Nav1.2 subunits are functionally modulated by TN-R (45). Thus, it is possible that other ion channel subunits may have roles in cell adhesion. Analysis of the amino acid sequences of these subunits has not revealed homologies to known CAMs. However, further investigation into possible alternative functions may lead to unexpected and exciting results.

4. PERSPECTIVE

Sodium channel β subunits play important roles in channel modulation and regulation of channel density at the plasma membrane. We have shown that β subunits also function as cell adhesion molecules in heterologous expression systems. What are the potential implications of these findings? Ankyrin$_G$, the CAMs neurofascin and NrCAM, and voltage-gated sodium channels colocalize at the axonal membrane of the adult node of Ranvier in specialized membrane domains (Figure 3) (101, 102). Early clusters of neurofascin and NrCAM are joined later by ankyrin$_G$ and sodium channels during differentiation of myelinated axons. Formation of the node of Ranvier may then result from the fusion of two cluster intermediates. An ankyrin$_G$-mediated link between neurofascin, NrCAM, and ion channels may allow these CAMs to cluster sodium channels in the axonal membrane. It was proposed that sodium channel β2 subunits, because of their homology to contactin, may interact in a lateral or *cis* fashion with NrCAM or neurofascin and thus contribute to sodium channel localization (102). Recent experiments have shown that heterophilic *cis* β1- and β3-neurofascin interactions may occur (61, 103). Our data expand this model to propose a direct link between sodium channels and ankyrin$_G$ through *trans* homophilic β subunit interactions (46). The multivalent membrane binding domain of ankyrin$_G$ (104) may allow interaction with multiple CAMs, including neurofascin, NrCAM, as well as sodium channel β subunits, forming a sodium channel signaling complex at the node of Ranvier that may also include RPTPβ and contactin. β subunit mRNA expression has been described in sciatic nerve Schwann cells, astrocytes from spinal cord, optic nerve, and sciatic nerve, oligodendrocytes, and B104 oligodendrocyte precursor cells in culture (25, 28, 105, 106). *Trans*-homophilic cell adhesion may occur between axonal and glial cell sodium channel β subunits. β subunit *cis* homophilic adhesion may also take place in the plane of the axonal membrane. This putative adhesion may also contribute to sodium channel clustering in nodes of Ranvier during the process of myelination. Our challenge now is to relate these exciting observations in heterologous expression systems to physiological events.

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