CHAPTER 3

Voltage-Gated Na⁺ Channels: Not Just for Conduction

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Voltage-gated sodium channels (VGSCs), composed of a pore-forming α subunit and up to two associated β subunits, are critical for the initiation of the action potential (AP) in excitable tissues. Building on the monumental discovery and description of sodium current in 1952, intrepid researchers described the voltage-dependent gating mechanism, selectivity of the channel, and general structure of the VGSC channel. Recently, crystal structures of bacterial VGSC α subunits have confirmed many of these studies and provided new insights into VGSC function. VGSC β subunits, first cloned in 1992, modulate sodium current but also have nonconducting roles as cell-adhesion molecules and function in neurite outgrowth and neuronal pathfinding. Mutations in VGSC α and β genes are associated with diseases caused by dysfunction of excitable tissues such as epilepsy. Because of the multigenic and drug-resistant nature of some of these diseases, induced pluripotent stem cells and other novel approaches are being used to screen for new drugs and further understand how mutations in VGSC genes contribute to pathophysiology.

Voltage-gated sodium channels (VGSCs) conduct inward current that depolarizes the plasma membrane and initiates the action potential (AP) in excitable cells, including neurons, cardiomyocytes, and skeletal muscle cells. Because of the intrinsic link between VGSCs and cellular excitability, it is not surprising that mutations in VGSC genes are linked with epilepsy, cardiac arrhythmia, neuropathic pain, migraine, and neuromuscular disorders. The goal of this review is to provide an overview of critical discoveries in VGSC physiology and discuss the challenges of studying VGSCs in disease, including some of the exciting techniques to address these challenges.

VGSC DISCOVERY AND STRUCTURE

In 1952, the Nobel Laureates Alan Lloyd Hodgkin and Andrew Fielding Huxley first recorded sodium current ($I_{Na}$) using their voltage-clamp technique on the squid giant axon. Their experiments showed three key features of $I_{Na}$—selective Na⁺ conductance, voltage-dependent activation, and rapid inactivation (Hodgkin and Huxley 1952). The Hodgkin–Huxley model mathematically described voltage-dependent initiation of the AP by inward $I_{Na}$, followed by fast inactivation of $I_{Na}$, and simultaneous activation of outward $I_{K}$. Outward $I_{K}$ was postulated to reestablish the charge balance across the plasma membrane (Hodgkin and Huxley 1952). These results were supported by studies of peripheral motor neurons by Sir John Carew Eccles, who shared the Nobel Prize with Hodgkin and Huxley. Among other contributions, he identified deviations in membrane potential and APs because of injections of sodium into motor neurons (Coombs et al. 1955). In addition to this monumental discovery, refinement of the voltage-clamp technique opened the door for a plethora of research on membrane potential physiology.

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In the 1960s, Bertil Hille and Clay Armstrong proposed the idea that \( I_{\text{Na}} \) and \( I_K \) are conducted through specific ion channels. At that time, many studies centered on performing voltage-clamp recordings of squid axon with sodium-free solutions containing organic cations (e.g., ammonium) to determine the size of the ion-conducting pore. The axon membrane became transiently permeable to these ions following depolarization, and this permeability was abolished by tetrodotoxin (TTX) (Larramendi et al. 1956; Lorente De No et al. 1957; Tasaki et al. 1965, 1966; Tasaki and Singer 1966; Binstock and Lecar 1969). Hille added to this body of work by using additional metal and many organic cations to develop a model of the narrowest region of the ion-conducting pore of the channel (Hille 1971, 1972). Hille’s model proposed a partial dehydration of \( \text{Na}^+ \) through interaction with a high-field-strength site at the extracellular end of the pore followed by rehydration in the lumen of the pore. This model is astonishingly close to what we now know to be true about VGSCs from crystal structure information (Payandeh et al. 2011; McCusker et al. 2012; Zhang et al. 2012). Armstrong and Bezanilla developed signal-averaging techniques to detect the movement of “gating charges” (Armstrong and Bezanilla 1973, 1974) corresponding to what we now understand to be the movement of voltage sensors, which respond to changes in membrane potential. Remarkably, without having any of the knowledge that we possess today about nucleotide/amino acid sequence and crystal structures, these early studies made many accurate predictions about VGSC structure and the functions of various channel domains.

Although early physiological studies provided evidence for the existence of a VGSC, the biochemical proof that tetrodotoxin/saxitoxin (STX) receptors were also ion-conducting did not yet exist. Membrane protein purification techniques were being established, but they required an extraordinarily large amount of time and biological starting material compared to today’s techniques. Photo-affinity labeling of STX receptors with a scorpion toxin derivative by William Catterall’s group identified a family of VGSC proteins that were designated \( \alpha \) and \( \beta \) subunits (Beneski and Catterall 1980). Purification of VGSC protein to theoretical homogeneity was challenging because of the difficulty of solubilizing a high molecular weight, highly lipophilic membrane protein and the large amount of biological starting material required. Again, the Catterall laboratory was the first to overcome these challenges and purify VGSCs from rat brain (Hartshorne and Catterall 1981, 1984; Hartshorne et al. 1982). This work was closely followed by purification of VGSCs from rat and rabbit skeletal muscle (Barchi 1983; Kraen et al. 1985), and from chicken heart (Lombet and Lazdunski 1984). Reconstitution of purified VGSCs in a lipid bilayer membrane allowed observation of \( \text{Na}^+ \) flux and confirmed that a functional VGSC protein had been purified (Hartshorne et al. 1985). The evolution of VGSC purification methods is discussed in detail in Catterall (1992). Purification from rat brain revealed that VGSCs are heterotrimers, composed of a single \( \alpha \) subunit and two non-pore-forming \( \beta \) subunits, \( \beta 1 \) and \( \beta 2 \) (Hartshorne and Catterall 1981). We now know that there are more than two VGSC \( \beta \) subunits. Subsequent homology cloning and heterologous expression studies showed that each VGSC \( \alpha \) is associated at the plasma membrane with a noncovalently linked \( \beta 1 \) or \( \beta 3 \) subunit and a covalently linked \( \beta 2 \) or \( \beta 4 \) subunit (Morgan et al. 2000; Yu et al. 2003).

To clone the first VGSC \( \alpha \) subunit complementary DNA (cDNA), researchers from Shosaku Numa’s group took on the arduous task of purifying brain VGSCs and obtaining partial amino acid sequence by amino-terminal Edman degradation as well as through cleaved peptides from the \( \alpha \) subunit. They then generated and screened multiple cDNA libraries based first on predicted degenerate cDNA sequences and then on cloned sequences. Assembly of the cloned VGSC cDNA fragments revealed what we now know to be \( \text{Scn1a} \) (Noda et al. 1984). Heterologous expression of the cloned cDNAs gave a functional channel, which we now recognize as the VGSC \( \alpha \) subunit \( \text{Na}_1.1 \).

**VGSC \( \beta \) Subunits Are Required to Recapitulate Physiological Expression of \( I_{\text{Na}} \)**

Early cloning of \( \text{Na}_1.2 \) cDNA and its expression in oocytes resulted in \( I_{\text{Na}} \) that inactivated more slowly than \( I_{\text{Na}} \) recorded from neurons. Co-injection of low-molecular-weight rat brain messenger RNA (mRNA) was required for recapitulation of physiological \( I_{\text{Na}} \) in terms of rates of activation and
Voltage-Gated Na⁺ Channels

MECHANISTIC INSIGHTS ON VGSC STRUCTURE AND FUNCTION

VGSC α subunits are highly evolutionarily conserved. Each α subunit is composed of four homologous domains, each containing six transmembrane helices (S1–S6), which come together in pseudotetradal symmetry to form the ion-conducting pore (Fig. 1). As discussed earlier, long before the emergence of crystal structures, much of VGSC structure and function was inferred through studies modeling gating and using site-specific antibodies and toxins during electrophysiological recordings.

The sliding-helix model of VGSC voltage-dependent activation was proposed in the 1980s by Catterall (1986a,b). The S4 segments of each domain that serve as voltage sensors contain arginine residues at every third position, which pair with negatively charged residues in nearby transmembrane segments. On membrane depolarization, these residues change ion-pair partners, causing each S4 helix to rotate or slide outward, pushing a single arginine residue in each of the four segments out, and generating gating current that activates, or opens, the channel pore (Payandeh et al. 2011). Data from potassium channel structures prompted the paddle model as an alternate mechanism of voltage-dependent activation (Jiang et al. 2003); however, the solving of multiple prokaryotic α subunit crystal structures showed the validity of the sliding-helix model for VGSCs (Payandeh et al. 2011; Vargas et al. 2012).

Another critical property of VGSCs is voltage-dependent inactivation, which occurs despite an ongoing depolarizing pulse. Early studies identified the intracellular loop between domains III and IV as the inactivation gate (Fig. 1). This was proposed to “swing” into the pore in a voltage-dependent manner, physically blocking ion conduction, and effectively inactivating the pore (Vassilev et al. 1988, 1989). A short amino acid sequence in the inactivation gate, isoleucine, phenylalanine, and methionine (IFM), was identified as critical to the process of inactivation. Mutation of these three residues to glutamine resulted in a noninactivating channel (West et al. 1992; Kellenberger 1997). These studies, combined with a three-dimensional nuclear magnetic resonance (NMR) structure of the inactivation gate (Rohl et al. 1999), helped form the current understanding of the molecular basis for fast inactivation of VGSCs.
FIGURE 1. Voltage-gated sodium channel (VGSC) α and β subunit topology. The pore-forming α subunit is composed of four homologous domains (I–IV), each with six transmembrane segments. The fourth segment in each domain contains the voltage-sensing domain of the channel. The inactivation loop between domains III and IV contains the isoleucine, phenylalanine, and methionine (IFM) domain required for channel inactivation. Up to two β subunits may be associated with VGSCs. β1 and β3 subunits associate noncovalently, whereas β2 and β4 subunits associate via a disulfide link. (From Brackenbury and Isom 2011; reprinted, with permission, © Frontiers Media SD.)
Recent crystal structures of bacterial VGSCs have led to a more detailed understanding of the pore and selectivity filter. The membrane reentrant P-loop, located between transmembrane helices S5 and S6 of each domain that line the pore, was identified as the target for the pore-blocking TTX, suggesting its close location to the pore (Noda et al. 1989; Terlau et al. 1991). The crystal structure of a bacterial VGSC showed that the four P-loops together form a ring of glutamates, located near the extracellular end of the pore (Payandeh et al. 2011). As predicted in 1992, these highly conserved glutamates form a high-field-strength site critical in determining ion selectivity of the channel (Heinemann et al. 1992). These residues stabilize multiple ionic occupancy states, preferentially conducting hydrated Na\(^+\) through the pore (Payandeh et al. 2011). In contrast, potassium channels conduct dehydrated K\(^+\) (Doyle et al. 1998).

Capturing crystal structures of bacterial VGSCs in different activation states has provided new insights and biochemical modeling tools. A closed-pore conformation of the Arcobacter channel (Na\(_a\),Ab), crystallized within a lipid-based bicelle, offered the first VGSC 3D structure (Payandeh et al. 2011). A putatively inactive Na\(_a\),Rh channel from the marine bacterium Rickettsiales showed significant differences from Na\(_a\),Ab and supplied new information about conformational rearrangements required for inactivation (Zhang et al. 2012). Finally, the apparently open conformation of the Na\(_a\),M structure from the marine bacterium Magnetococcus presented further insights into channel gating and selectivity (McCusker et al. 2012).

Despite the wealth of information provided by these prokaryotic VGSC structures, crystallization of a eukaryotic VGSC is the essential next step. Although prokaryotic VGSCs are homotetramers, mammalian VGSC \(\alpha\) subunits are single polypeptides containing four nonidentical domains. Further, the extensive extracellular and intracellular loops of mammalian channels are not present in their prokaryotic orthologs. Thus, major gaps in the field include the crystal structure of a mammalian \(\alpha\) subunit and the co-crystal structure of an \(\alpha\) subunit associated with \(\beta\) subunits. This information will be critical in fully understanding mammalian VGSC structure.

**VGSC Diversity**

Originally, VGSC \(\alpha\) subunit proteins were named according to the tissue from which they were purified. For example, brain type II, rat II, and R-II are all outdated terms for Na\(_{1.2}\) (Catterall et al. 2005). To date, nine VGSC \(\alpha\) proteins (Na\(_{1.1–1.9}\)) and five \(\beta\) proteins (\(\beta_1–4\) and \(\beta_1B\)), encoded by SCN(X)A and SCN1B-SCN4B genes, respectively, have been identified in mammalian genomes.

A BLAST search of the cDNAs encoding \(\beta_1\) and \(\beta_2\) in 1995 revealed important, and unexpected, new information. Both proteins contained areas of homology with known CAMs of the immunoglobulin (Ig) superfamily, for example, contactin and myelin P0. This led to the hypothesis that \(\beta_1\) and \(\beta_2\) function as CAMs in addition to channel modulation (Isom et al. 1995b). We now know that all five \(\beta\) subunit proteins contain an extracellular Ig domain and have CAM function, as discussed later in this review. \(\beta_1–4\) (encoded by SCN1B-SCN4B) are type 1 transmembrane proteins with an extracellular amino terminus (containing the Ig domain) and an intracellular carboxyl terminus (Isom et al. 1992, 1995b; Morgan et al. 2000). In contrast, \(\beta_1B\), a splice variant of SCN1B, is soluble and, thus, a portion is secreted. Retention of, intron 3 results in generation of an alternate carboxy-terminal domain that does not contain a transmembrane region (Patino et al. 2011). Recently, the three-dimensional crystal structures of the human Ig domains of \(\beta_3\) and \(\beta_4\) were solved. \(\beta_3\) formed a trimer when crystalized (Namadurai et al. 2014). In contrast, the \(\beta_4\) Ig domain was monomeric in crystal form (Gilchrist et al. 2013). Although this could represent a physiological difference in homophilic interactions, it could also be because of the removal of 31 amino acids at the \(\beta_4\) amino terminus to facilitate crystallization (Gilchrist et al. 2013). Comparisons of these structures will give new insights into the function of \(\beta\) subunits, but the co-crystal structure of \(\alpha\) and \(\beta\) subunits remains the next frontier.

VGSC \(\alpha\) and \(\beta\) subunits are highly expressed in central and peripheral neurons, cardiac myocytes, skeletal muscle, and some nonexcitable cells (Maier et al. 2004; Patino and Isom 2010; Brunklaus et al. 2011). A putatively inactive NavRh channel from the marine bacterium Magnetococcus presented further insights into channel gating and selectivity (McCusker et al. 2012).


Table 1. VGSC genes, expression patterns, and disease associations

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Type</th>
<th>Tissue distribution</th>
<th>Consequence of mutations</th>
<th>TTX sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN1A</td>
<td>Na,1.1</td>
<td>CNS, PNS, heart, DRG</td>
<td>DS, familial autism, FHIM3, FS', GEFS', SUDEP</td>
<td>+</td>
</tr>
<tr>
<td>SCN2A</td>
<td>Na,1.2</td>
<td>CNS, PNS, DRG</td>
<td>BFNIS, DS, EOEE, familial autism, GEFS, OS</td>
<td>+</td>
</tr>
<tr>
<td>SCN3A</td>
<td>Na,1.3</td>
<td>CNS, PNS, heart, DRG</td>
<td>Unclear</td>
<td>+</td>
</tr>
<tr>
<td>SCN4A</td>
<td>Na,1.4</td>
<td>Skeletal muscle, heart, DRG</td>
<td>PAM, PMC, HyperPP, HypoPP, SNEL</td>
<td>+</td>
</tr>
<tr>
<td>SCN5A</td>
<td>Na,1.5</td>
<td>Skeletal muscle, heart, CNS, DRG</td>
<td>AF, AS, BS, DCM, LQTS, PCCD, SIDS,</td>
<td>-</td>
</tr>
<tr>
<td>SCN8A</td>
<td>Na,1.6</td>
<td>CNS, PNS, heart, DRG</td>
<td>EOEE; cognitive impairment, paralysis, ataxia, dystonia</td>
<td>+</td>
</tr>
<tr>
<td>SCN9A</td>
<td>Na,1.7</td>
<td>DRG</td>
<td>CIP, IEM, PEPP, PPN</td>
<td>+</td>
</tr>
<tr>
<td>SCN10A</td>
<td>Na,1.8</td>
<td>DRG</td>
<td>PPN</td>
<td>-</td>
</tr>
<tr>
<td>SCN11A</td>
<td>Na,1.9</td>
<td>DRG</td>
<td>PPN</td>
<td>-</td>
</tr>
<tr>
<td>SCN11B</td>
<td>β1</td>
<td>CNS, PNS, heart, DRG, (oligodendrocytes, Schwann cells, astrocytes, radial glia)</td>
<td>AF, BS, DS, GEFS', LQTS, PCCD, TLE</td>
<td>N/A</td>
</tr>
<tr>
<td>SCN2B</td>
<td>β2</td>
<td>CNS, PNS, heart, DRG</td>
<td>AF, BS</td>
<td>N/A</td>
</tr>
<tr>
<td>SCN3B</td>
<td>β3</td>
<td>CNS, PNS, heart, DRG</td>
<td>AF, BS, PCCD, SIDS, ventricular fibrillation</td>
<td>N/A</td>
</tr>
<tr>
<td>SCN4B</td>
<td>β4</td>
<td>CNS, PNS, heart, DRG</td>
<td>LQTS, SIDS</td>
<td>N/A</td>
</tr>
<tr>
<td>SCN11B</td>
<td>β1B</td>
<td>Fetal CNS, PNS, heart, DRG</td>
<td>BS, PCCD, epilepsy</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Modified, with permission, from Patino and Isom 2010; Elsevier Ireland; Catterall 2012, © Wiley; Brunklaus et al. 2014; BMJ.
AF, Atrial fibrillation; AS, atrial standstill; BFNIS, benign familial neonatal-infantile seizures; BS, Brugada syndrome; CIP, channelopathy-associated insensitivity to pain; CNS, central nervous system; DCM, dilated cardiomyopathy; DRG, dorsal root ganglia; DS, Dravet syndrome; EOEE, early-onset epileptic encephalopathy; FHIM3, familial hemiplegic migraine type 3; FS', febrile seizures plus; GEFS', genetic epilepsy with febrile seizures plus; HyperPP, hyperkalemic periodic paralysis, HypoPP, hypokalemic periodic paralysis; IEM, inherited erythromelalgia; LQTS, long QT syndrome; OI, Ohtahara syndrome; PAM, potassium-aggravated myotonia; PCCD, progressive cardiac conduction disease; PEPP, paroxysmal extreme pain disorder formally known as familial rectal pain syndrome; PMC, paramyotonia congenital; PNS, peripheral nervous system; PPNN, painful peripheral neuropathies; SIDS, sudden infant death syndrome; SNEL, severe neonatal episodic laryngospasm; SSS, sick sinus syndrome; SUDEP, sudden unexplained death in epilepsy; TLE, temporal lobe epilepsy.

2014), including breast cancer cells (Fraser et al. 2005), astrocytes, and oligodendrocytes. Table 1 shows the gene name, known tissue expression, and diseases associated with mutations for each VGSC subunit (adapted from Patino and Isom 2010; Catterall 2012; Brunklaus et al. 2014). VGSC expression is developmentally regulated. For example, Na,1.3, β3, and β1B are most prevalent in embryonic and neonatal rodent brain. This expression profile then changes such that Na,1.1, Na,1.2, Na,1.6, β1, β2, and β4 are predominant in the adult brain, albeit distribution is not equivalent across brain regions (Kazen-Gillespie et al. 2000; Catterall et al. 2005; Patino et al. 2011).

VGSC subcellular localization is critical for normal physiological functions. In neurons, VGSCs are concentrated at the axon initial segment (AIS), where the AP is initiated, and nodes of Ranvier in myelinated axons, which are critical for saltatory conduction. In cardiomyocytes, VGSCs are differentially localized at intercalated discs and transverse tubules (see Bao and Isom 2014; Calhoun and Isom 2014 for reviews that discuss this in more detail).

Despite their high sequence similarity, each VGSC α subunit has subtle differences in biological properties and pharmacological sensitivities (as summarized in Catterall et al. 2005; Kwong and Carr 2015). Many of the agents that target α subunits are toxins, including TTX, STX, μ-conotoxins, and their derivatives, such as 4,9-anhydro-TTX, which preferentially blocks Na,1.6 (Rosker et al. 2007; Kwong and Carr 2015). VGSCs are canonically separated into TTX-sensitive (TTX-S) and TTX-resistant categories (TTX-R), which are blocked by nanomolar or micromolar concentrations of TTX, respectively (Catterall et al. 2005). Interestingly, modulation of a single residue changes TTX sensitivity (Noda et al. 1989). Modulation of INa by these toxins (reviewed in Gilchrist et al. 2014) can be affected by β subunit expression. For example, expression of β1 and β3, but not β2 or β4, tend to increase the rate of association and binding affinity of μ-conotoxins for VGSCs. However, modulation of INa by STX or TTX was unaffected by β subunit expression (Isom et al. 1995a; Zhang et al. 2013). In vivo, a combination of electrophysiology and toxicology are used to infer which VGSCs contribute to
Aspects of endogenous $I_{Na}$. Although challenging, in vivo studies are crucial, as heterologous results can be difficult to translate to in vivo physiological and pathophysiological mechanisms.

**Posttranslational Modifications of α and β Subunits**

VGSC α subunits are heavily glycosylated, although the extent of glycosylation varies among subunits (Bennett 2002). 40%–50% of these added carbohydrate residues are sialic acid moieties such that each channel contains ~100 sialic acid residues (Miller et al. 1983; Roberts and Barchi 1987). Differential glycosylation of VGSC subunits can affect channel gating, putatively because of the local negative charge of sialic acid residues. In general, when α subunits are less glycosylated, channel gating occurs at more depolarized voltages (Recio-Pinto et al. 1990; Bennett et al. 1997; Zhang et al. 1999; Tyrrell et al. 2001). For example, Na$_{1.9}$ expressed in neonatal DRG neurons is more glycosylated than in adult DRG, suggesting developmental regulation of glycosylation. This may account for developmental differences in gating of persistent $I_{Na}$ attributed to Na$_{1.9}$ (Tyrrell et al. 2001). Voltage-dependent activation and inactivation of Na$_{1.4}$, but not Na$_{1.5}$, shifts when expressed in cells incapable of sialylation, indicating that not all VGSCs are affected equally (Bennett 2002). Glycosylation accounts for about one-third of the molecular weights of β subunits. Mature β subunits contain three to four N-linked glycosylation sites in the Ig domain (Isom et al. 1992; McCormick et al. 1998) all of which are exposed in the β3 crystal structure (Namadurai et al. 2014). Sialic acid residues in these sites affect β1- and β2-mediated modulation of $I_{Na}$ and channel surface expression (Johnson et al. 2004; Johnson and Bennett 2006). Critically, VGSC glycosylation varies developmentally and among cell types; for example, neonatal and adult myocytes have distinct differences in glycogen expression, as do atrial and ventricular myocytes (Stocker and Bennett 2006). Therefore, tissue-specific variations in β subunit processing likely include altered sialylation/glycosylation and may contribute to differential modulation of $I_{Na}$ by β subunits among cell types (Zhang et al. 1999; Bennett 2002).

β1 can be phosphorylated on intracellular tyrosine residue Y-181 (Malhotra et al. 2002, 2004). Tyrosine phosphorylation regulates β1-mediated recruitment of ankyrin to points of cell–cell contact (Malhotra et al. 2002) and likely occurs via fyn kinase, an src family kinase (Brackenbury et al. 2008; Nelson et al. 2014). Receptor tyrosine phosphatase β interacts with the intracellular domain of β1 and may modulate β1 phosphorylation (Ratcliffe et al. 2000). In cardiac myocytes, nonphosphorylated β1 localizes at the T-tubules with ankyrinB, whereas phosphorylated β1 localizes at intercalated discs with connexin-43, N-cadherin, and Na$_{1.5}$ (Malhotra et al. 2004). Therefore, the phosphorylation state Y-181 regulates β1 association with cytoskeletal proteins, as well as its subcellular localization.

β subunits are substrates for sequential proteolytic cleavage by BACE1 (β-secretase) and γ-secretase, similar to amyloid precursor protein. BACE1 cleavage releases the extracellular domain (ECD), followed by a γ-secretase-mediated cleavage event to release the carboxy-terminal fragment (CTF) (Wong et al. 2005). These cleaved peptides may have important physiological functions. For example, the β2-CTF translocates to the nucleus and promotes Scn1a mRNA and protein expression when heterologously expressed in cultured neurons (Kim et al. 2005, 2007). Consistent with this proposed mechanism, total and surface expression of Na$_{1.1}$ in acutely dissociated hippocampal slices is significantly reduced in BACE1 null mice compared to wild-type (Kim et al. 2011). The physiological importance of BACE1 and γ-secretase cleavage of the other β subunits is not understood. As β1B can act as a CAM, it is possible that the β1-ECD, which contains the identical Ig loop domain, functions as a ligand for cell adhesion (Patino et al. 2011). γ-Secretase activity and, thus, formation of the β1-CTF is critical for β1-mediated neurite outgrowth in vitro (Brackenbury and Isom 2011). Determining whether these functions occur in vivo represents a critical next step in our understanding of posttranslational processing of VGSCs.

**β Subunits Are Multifunctional**

**Effects on Surface Expression**

Coexpression of β subunits with α results in increased $I_{Na}$ density, in part, as a result of promotion of α cell-surface expression. Following posttranslational processing, α subunits are stored in an intracel-
Cell Adhesion and Neurite Outgrowth

β Subunits are multifunctional. They play critical roles in neurite outgrowth, migration, and maintenance of nodes of Ranvier. β Subunits are CAMs and, as such, recruit other signaling proteins to the VGSC complex. Most is known about the CAM interactions of β1 and β2, which participate in trans-homophilic adhesion, via the Ig loop, with β1 or β2 molecules on adjacent cells (Malhotra et al. 2000). β1 or β2 trans-homophilic adhesion recruits the cytoskeletal protein ankyrin to points of cell–cell contact (Malhotra et al. 2000). Ankyrin recruitment is abolished by phosphorylation of β1-Y181 (Malhotra et al. 2002). β1–β1 trans-homophilic adhesion promotes neurite outgrowth via a pathway requiring fyn kinase, the CAM contactin, γ-secretase activity, and localized Ina (Brackenbury et al. 2008, 2010; Brackenbury and Isom 2011). Because β1B contains the identical Ig loop, it similarly promotes neurite outgrowth of neurons expressing β1 (Patino et al. 2011). Together, these properties may contribute to neuronal development in vivo; Scn1b null mice have neuronal pathfinding dysfunction and differences in subcellular localization of α subunits at AIS (Chen et al. 2004; Brackenbury et al. 2008).

β1, and likely β1B, interacts heterophilically with other CAMs and extracellular matrix proteins, including contactin, neurofascin-186, NrCAM, N-cadherin, and tenascin-R (Kazarinova-Noyes et al. 2001; Ratcliffe et al. 2001; Malhotra et al. 2004; McEwen and Isom 2004; Patino et al. 2011). Some of these interactions affect β1-modulation of α surface expression. For instance, coexpression of NF186 or contactin with Na1.2 and β1 increases Na1.2 surface expression compared with Na1.2 and β1 alone, although NF186 and contactin have no impact on Na1.2 expression in the absence of β1 (Kazarinova-Noyes et al. 2001; McEwen and Isom 2004). Heterophilic interactions with tenascin-R, an extracellular matrix protein secreted by oligodendrocytes during myelination, repels cells expressing β1 or β2 and, therefore, may play a role in restricting VGSCs to nodes of Ranvier in myelinated axons (Xiao et al. 1999). The cell-adhesive properties of β1 and β1B have likely clinical implications.
relevance, as most known SCNIB epilepsy mutations are located in or near the Ig loop domain (reviewed in O’Malley and Isom 2015).

Much less is known about the CAM functions of β3 and β4. Studies of β3 homophilic adhesion give conflicting results: the β3 Ig domain appears to interact with full-length β3 when expressed heterologously (Yereddi et al. 2013), yet another group reported that β3 expression could not induce aggregation of Drosophila S2 cells, unlike β1 and β2 (McEwen et al. 2009). β3 and β1 likely do not associate, as β3 does not interact with a peptide containing the β1-ECD (McEwen and Isom 2004). β4 did not promote neurite outgrowth of cerebellar granule neurons, but this observation does not preclude other CAM interactions (Davis et al. 2004).

**PATHOPHYSIOLOGICAL ROLES OF VGSCs**

Mutations in VGSC genes are associated with many types of genetic epilepsy, peripheral neuropathy, long QT syndrome, neuromuscular disorders, and other diseases associated with dysfunction of excitable tissues. Recent reviews discuss the complex pathophysiological mechanisms of sodium channelopathies associated with pain and cardiac disease (Dib-Hajj et al. 2010; Adsit et al. 2013; Bao and Isom 2014; O’Malley and Isom 2015). Here, we will focus on the role of VGSCs in the genetic epilepsies and cancer as examples of disease resulting from aberrant or loss of VGSC function in mammals.

**Epilepsy**

Epilepsy results from an imbalance between excitation and inhibition in the brain. More than two million Americans have epilepsy, which can be caused by genetic mutations or brain injury (Hirtz et al. 2007). Epilepsy significantly impacts the quality of life for both patients and caregivers because of the unpredictability of seizures and the risk of comorbidities including cognitive decline, intellectual disability, developmental delay, and sudden unexpected death in epilepsy (SUDEP).

Mutations in genes encoding VGSC α and β subunits are associated with epilepsies with a wide range of phenotypic severities, including genetic epilepsy with febrile seizures plus (GEFS+), an inherited epilepsy with a wide range of phenotypes, and Dravet syndrome (DS), one of the most devastating pediatric epileptic encephalopathies. An intriguing discussion of the complex mechanisms underlying DS was published recently (Chopra and Isom 2014). In 1998, the first VGSC mutation associated with epilepsy was identified in SCN1B in a patient with GEFS+. However, GEFS+ is incompletely penetrant and family members with the same SCN1B mutation present with a wide range of epilepsy severities (Wallace et al. 1998), a now familiar characteristic of the genetic epilepsies. Since this initial discovery, a large number of mutations in SCN1A, SCN2A, SCN8A, and SCN1B (Escayg et al. 2000; O’Malley and Isom 2015) have been implicated in multiple types of epilepsy (see Table 1) (reviewed in part by Shi et al. 2012; Steinlein 2014; Wagnon and Meisler 2015). More than 1200 mutations have been identified in SCN1A alone (Meng et al. 2015). Identifying the pathophysiological mechanisms underlying DS is of particular importance, as traditional antiepileptic drugs often aggravate seizures in DS patients and development of new therapeutics to help these patients is especially critical. Although 70%–80% of DS patients have an identified heterozygous de novo SCN1A mutation (Marini et al. 2011), homozygous mutations in SCN1B have also been reported (Patino et al. 2009; Ogiwara et al. 2012).

Studies of DS SCN1A mutations have shown that a majority result in loss-of-function (Meng et al. 2015). Studies of Scn1a<sup>−/−</sup> null mice and knockin mice expressing human mutations have led to the “interneuron hypothesis,” suggesting that selective INa loss in GABAergic interneurons causes epilepsy via loss of inhibitory tone (Yu et al. 2006). However, this has become controversial (Chopra and Isom 2014; Isom 2014). More recent studies of DS patient-derived induced pluripotent stem cell (iPSC) neurons showed either that both inhibitory and excitatory (Liu et al. 2013) or excitatory neurons (Jiao et al. 2013) are hyperexcitable compared to nonepileptic controls (Mistry et al. 2014). These studies...
suggest that SCN1A haploinsufficiency may result in compensatory up-regulation of other VGSC α subunits. Hyperexcitability of both inhibitory and excitatory neurons may lead to seizures in DS via increased network excitability or synchronization of firing.

Scn1b null mice also model DS; they experience spontaneous seizures and early lethality. At least one DS SCN1B mutation causes β1 to be retained intracellularly, preventing surface expression and, thus, function (Patino et al. 2009). Scn1b null mice have differences in neuronal pathfinding that are observed before seizure onset (Brackenbury et al. 2013). Thus, SCN1B-linked DS mutations may alter neuronal excitability by affecting α subunit surface expression/function as well as by altering neuronal development via changes in cell adhesion.

A major concern for epilepsy patients and their families is the risk of SUDEP. Research suggests that a “perfect storm” of seizures, cardiac arrhythmias, respiratory dysfunction, and autonomic and/or parasympathetic dysfunction contributes to SUDEP (Auerbach et al. 2013; Kalume 2013; Massey et al. 2014). Heterozygous Scn1a-R1407X knockin GEFS+/DS mice, as well as Scn1b null DS mice, have increased transient and persistent INa in cardiac myocytes, which may provide substrates for cardiac arrhythmias and contribute to SUDEP (Lopez-Santiago et al. 2007; Auerbach et al. 2013). Fully understanding the mechanisms underlying SUDEP will be essential to epilepsy treatment.

One of the greatest challenges in epilepsy research is the need for novel antiepileptic drugs. Even with optimal treatment, 20%–30% of all epilepsy patients are pharmacoresistant, defined as being unresponsive to at least two different, tolerated antiepileptic drugs (Kwan et al. 2010, 2011). Even in nonrefractory patients, antiepileptic drugs treat disease symptoms rather than preventing disease progression and often have intolerable side effects. A major goal of studying epilepsy-associated VGSC mutations is to further understand epileptogenesis (how a brain becomes epileptic) to inform new antiepileptic drug discovery.

Emerging Roles for VGSCs in Cancer

An exciting area of research is the role of VGSCs in nonexcitable cells, including multiple cancer cell types. Almost two decades ago, researchers observed that cancer cell lines with higher VGSC expression have increased cell motility and metastatic potential. Furthermore, the invasive capacity of these cells could be reduced by incubation with TTX (Grimes et al. 1995; Laniado et al. 1997). Since then, both α and β subunits have been detected in many cancer cell lines and in some patient biopsies. VGSC α subunits are expressed in some cervical, ovarian, breast, and colon tumors in vivo (Fraser et al. 2005; Gao et al. 2010; House et al. 2010; Hernandez-Plata et al. 2012), although some prostate, breast, cervical, and lung cancers express β subunits (reviewed in Patel and Brackenbury 2015). Studies have identified both positive and negative associations between VGSC expression and metastatic potential. Na+,1.5 expression in breast cancer cells correlates positively with increased risk of recurrence and metastasis (Fraser et al. 2005; Yang et al. 2012), and a similar trend has been described for colon, prostate, and ovarian cancers. However, there is an inverse correlation between α expression and clinical grade in glioma and no correlation in lung cancer cell lines (Schrey et al. 2002; Onganer and Djamgoz 2005; Roger et al. 2007). Expression of α subunits can potentiate lateral motility, adhesion, process extension, and other cellular behaviors associated with metastasis (Brackenbury 2012). It is not clear how increased expression of the pore-forming α subunits potentiates invasive changes in the tumor cells. Three potential models have been proposed: increased Na+ influx enhances H+ efflux, thus activating pH-dependent extracellular matrix degradation and invasion; regulation of an “invasion gene network,” particularly by SCN5A, and increased intracellular Ca2+, which enhances formation of invasive projections (reviewed in Brackenbury 2012).

SCN1B and SCN2B expression are associated with metastatic potential in prostate cancer cells (Diss et al. 2008; Jansson et al. 2012). However, studies of the invasive potential of SCN1B-expressing breast cancer in vitro have been contradicted by in vivo studies. β1 expression is associated with less invasive breast cancer cell lines (Chioni et al. 2009); however, in a mouse model of breast cancer, overexpression of β1 increased tumor growth, metastasis, and angiogenesis (Nelson et al. 2014). β1 trans-homophilic adhesion mediates process outgrowth from breast cancer cells (Nelson et al. 2014),
Thus it is proposed that \( \beta_1 \) promotes metastasis by a mechanism similar to \( \beta_1 \)-mediated neurite outgrowth.

NEW FRONTIERS AND NOVEL TECHNIQUES

The list of pathogenic VGSC gene mutations is growing rapidly. The explosion of whole-genome sequencing has led to multi-institution efforts, such as the NINDS-funded Epi4K Project (www.ninds.nih.gov/news_and_events/news_articles/pressrelease_childhood_epilepsy_genes_08112013.htm), with the goal of sequencing the genomes of 4000 patients with epilepsy, and the England Department of Health funded 100,000 Genomes Project (genomicsengland.co.uk) (Kearney 2014; Siva 2015). However, even with familial genetic information in hand, determining whether mutations are causative and understanding their pathophysiology are not simple tasks. A given patient can have mutations in several genes, which may be causative, benign, or modify another mutation. A single causative mutation may result in a wide range of phenotypes among individuals, for example, DS and GEFS\(^+\) patients may express the same \( SCN1A \) or \( SCN1B \) mutation. Genetic background is critical. \( Scn1a^{+/−} \) mouse models of DS have phenotypes of varying severity depending on genetic background (Yu et al. 2006; Miller et al. 2014). This issue can now be addressed directly with the induced-pluripotent stem cell (iPSC) technique. Patient iPSCs, usually derived from a skin cell biopsy, can theoretically be differentiated into any cell type, including cardiomyocytes, and specific neuronal subtypes (Parent and Anderson 2015). With this remarkable technique, we now have the opportunity to study disease-causing mutations in human cells within the context of the patient’s unique genomic background (Takahashi et al. 2007). Furthermore, endogenous genes that may contribute to pathology remain present. Brain organoids made from human iPSC-derived cells with a mutation in a gene involved in pluripotent cell maintenance were used to model microcephaly, providing proof-of-concept for use of organoids to model other neurological diseases (Lancaster et al. 2013). Another advantage of iPSCs is the opportunity to perform drug screening on differentiated human cells, alongside animal models, thus providing insight into toxicity and species-specific effects before clinical trials (reviewed in Ko and Gelb 2014). Patient-derived iPSC cells are critical for precision medicine approaches. For instance, antiepileptic drug effectiveness or toxicity may be optimized on patient-derived iPSC-neurons before patient treatment. As the iPSC technique is further refined and developed, it will provide additional, critical tools in therapeutic development.

Another challenge to the field is the non-specificity of clinically available VGSC-targeted drugs. There are six different potential sites for small molecule targeting on VGSCs, as shown by the wide variety of toxin binding sites. However, most of the current antiarrhythmic, antiepileptic, and analgesic VGSC drugs on the market, for example, lamotrigine, phentoin, and lidocaine, target the “local anesthetic” site located in domain IVS6 (Kwong and Carr 2015). This site is highly conserved among \( \alpha \) subunits; thus, these drugs have little selectivity for specific VGSCs and often cause adverse effects. Development of VGSC agents that target other binding sites remains an important task. Although, in most cases, toxins cannot be used directly for therapeutics, they can greatly inform drug design; small molecules can be developed that preserve beneficial effects while reducing harm. Drugs targeting specific toxin binding sites may allow more selective blockade of specific \( \alpha \) subunits to treat disease, such as neuropathic pain, cardiac arrhythmias, epilepsies, and perhaps even cancer (Stevens et al. 2011; Xiao et al. 2014).

Transgenic zebrafish are an economical and rapid vertebrate system that can be used to screen small molecule libraries and FDA-approved drugs for novel applications. Zebrafish with mutations in \( scn1Lab \), the gene orthologous to \( SCN1A \), have spontaneous seizures that are resistant to many antiepileptic drugs and appear to model DS. Peter de Witte’s group showed that DS zebrafish responded to fenfluramine (Zhang et al. 2015). Scott Baraban used locomotion tracking in mutant zebrafish to monitor behavioral seizures in a drug screen, which was validated using diazepam, valproate, and stiripentol, antiepileptic drugs effective in some DS patients. Out of 320 compounds

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tested, clemizole, an FDA-approved antihistamine, suppressed spontaneous seizures in vivo (Baraban et al. 2013). This approach can be adapted for screening potential therapeutics for any monogenic epilepsy, especially if combined with the CRISPR/Cas9 technique. Discovered in 2010 as a bacterial defense mechanism against phages (Deveau et al. 2010), the CRISPR/Cas9 system has since been adapted to easily and quickly introduce heritable genetic mutations in mice, rats, zebrafish, and rabbits (Chang et al. 2013; Li et al. 2013; Yang et al. 2014a). Compared to zinc-finger nucleases and transcription activator-like effector nucleases (TALENs), CRISPR/Cas creates double-stranded DNA breaks in a sequence-specific manner at sites complementary to a “single-guide RNA.” Because these are easy to design, the CRISPR/Cas9 system vastly improves the precision, speed, and accuracy of creating transgenic cell lines (including iPSCs) and animal models for single gene mutations (Yang et al. 2014b). These and other cutting edge techniques will allow us to discover novel disease mechanisms and therapeutics for diseases linked to VGSC mutations for another six decades (Catterall 2012).

REFERENCES


