Posttranslational Modification of Sodium Channels

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Abstract

Voltage-gated sodium channels (VGSCs) are critical determinants of excitability. The properties of VGSCs are thought to be tightly controlled. However, VGSCs are also subjected to extensive modifications. Multiple posttranslational

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modifications that covalently modify VGSCs in neurons and muscle have been identified. These include, but are not limited to, phosphorylation, ubiquitination, palmitoylation, nitrosylation, glycosylation, and SUMOylation. Posttranslational modifications of VGSCs can have profound impact on cellular excitability, contributing to normal and abnormal physiology. Despite four decades of research, the complexity of VGSC modulation is still being determined. While some modifications have similar effects on the various VGSC isoforms, others have isoform-specific interactions. In addition, while much has been learned about how individual modifications can impact VGSC function, there is still more to be learned about how different modifications can interact. Here we review what is known about VGSC posttranslational modifications with a focus on the breadth and complexity of the regulatory mechanisms that impact VGSC properties.

Keywords
Nav · Nitrosylation · Palmitoylation · Phosphorylation

1 Brief Overview of VGSCs

Cell membrane forms the boundary of the cell and separates cells from their exterior environment. The lipid bilayer of biological membranes has selective permeability to different molecules (ions and polar molecules), controlling the flow of substance across the membrane. There are different types of membrane transport proteins that determine the membrane permeability. Among them, active transporters facilitate an "active" transport of molecules (usually against their concentration gradient) using cellular energy. Passive transporters (e.g., ion channels), on the other hand, allow the "passive" diffusion of molecules (usually in the same direction as the concentration or electrochemical gradient) without energy consumption. Ion channels are pore-forming integral membrane proteins that gate the flow of ions across the cell membrane and therefore contribute to setting the resting membrane potential and determining cellular excitability. Landmark experiments by Hodgkin and Huxley (1952) demonstrated that the transmembrane sodium and potassium currents were responsible for the action potential generation. Moreover, they were able to identify the influence of membrane potential on sodium current kinetics and quantitatively describe the sodium conductance and gating mechanism. This work provided the foundation for understanding action potential generation and propagation and paved the way for later ion channel studies. Our knowledge of voltage-gated sodium channel (VGSC) proteins and mechanisms underlying membrane excitability has progressed significantly over the last six-plus decades. As the result of the development of patch clamp and other advanced techniques (Hamill et al. 1981; Payandeh et al. 2011; Schmidt and Catterall 1986; Messner and Catterall 1985; Costa and Catterall 1984), we now have a clearer understanding of sodium channel structure, kinetics, and function. We also know that VGSCs are subject to extensive modulation which can have significant impact on cell excitability. This review focuses on VGSC posttranslational modifications, with specific attention to more recent discoveries and studies.

VGSCs are integral membrane protein complexes that allow sodium ion flow across the membrane and conduct transmembrane sodium currents in response to changes in membrane voltage (Goldin 2001). VGSCs consist of the pore-forming α subunit and one or more β auxiliary subunits that regulate functions of channels (Catterall et al. 2005). Although β auxiliary subunits can be posttranslationally modified (Malhotra et al. 2004), here we focus on posttranslational modification (PTM) of α subunits. The VGSC α subunit consists of four transmembrane domains (DI–DIV), with each containing six transmembrane segments (S1–S6, Fig. 1). S1–S4 serve as voltage sensor and change confirmations in response to membrane potential change. S5 and S6 form the pore of the channel, which allows ion conduction through the channel (Corry and Thomas 2012). The N-terminus, C-terminus, and large cytoplasmic linkers are frequent targets of PTMs. However, smaller intracellular linkers can also be subject to modification. Extracellular regions such as the linker between specific S5 segments and the pore loops that form the selectivity filter can also be subject to important modifications. Indeed, α subunits can be covalently linked to β2 and β4 subunits via disulfide bonds between extracellular residues (Chen et al. 2012).

The VGSC family consists of nine members, and they share more than 50% common amino acid sequence in transmembrane segments and extracellular domains (Yu and Catterall 2003). The α subunit has a molecular weight of about 220–260 kDa (Payandeh et al. 2011; Catterall et al. 2005; Levinson and Ellory 1973). Some of the difference in molecular weight between isoforms and preparations can arise from extensive PTMs. The other major difference in molecular weight results from substantial differences in the length of the linker between domains I and II, with Nav1.4 and Nav1.9 having significantly shorter linkers than other mammalian isoforms. Numerous phosphorylation sites have been reported in the DI–DII linkers from the longer isoforms (Berendt et al. 2010) (Fig. 1).

In general, studies of PTM consequences have focused on modulation of VGSC gating properties and surface expression. In terms of gating, VGSCs have three basic configurations: resting (closed), activated (open), and inactivated. At resting membrane potential, VGSCs are closed. They activate (open) in response to the membrane potential depolarization. When membrane depolarization begins, several positively charged residues on the S4 segments are able to sense the membrane potential change, which leads to outward movement of S4 segments and the conformational change of VGSCs (Yarov-Yarovoy et al. 2012). The channel is then in transition to activated (open) configuration, providing a conduction pathway for the sodium currents through the channel pore (Stuhmer et al. 1989). Very soon after channel activation (usually within milliseconds), the inactivation gate that tethered to the sodium channel protein will block the channel and prevent sodium conductance (Goldin 2003). VGSCs transition to a nonconducting, inactivated conformation. This process is often referred to as "fast inactivation." Structure function studies revealed that the IFM motif (located in the intracellular linker of DIII and DIV) with three hydrophobic amino acids isoleucine, phenylalanine, and methionine is largely responsible for the channel fast inactivation (West et al. 1992). Modifications to fast inactivation can lead to altered persistent currents,

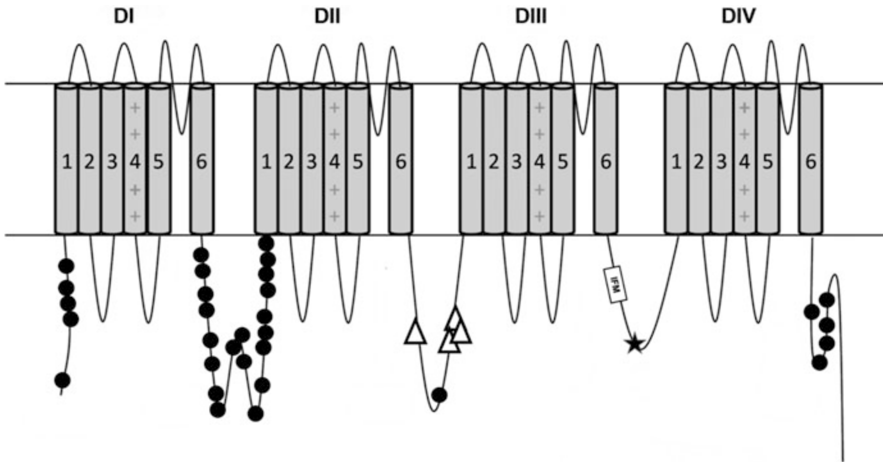

Fig. 1 Schematic representation of the linear structure of α subunit for voltage-gated sodium channels (VGSCs) with long I–II linkers. The α subunit consists of four homologous transmembrane domains (DI–DIV). Each domain contains six transmembrane segments (S1–S6). Among all, S1–S4 serve as the voltage sensor of the channel and S5–S6 form the pore. The IFM inactivation particle is located in DIII–DIV linker. The *black circles* represent likely phosphorylation sites in Nav1.2 and the *black star* is S1506. The *white triangles* represent putative palmitoylation sites in Nav1.5

also referred to as late or non-inactivating currents (Fig. 2a, b). There is also another type of inactivation that develops in response to longer depolarization time periods (usually hundreds of milliseconds) called "slow inactivation" (Silva 2014). Slow inactivation will sustain for a few seconds or even tens of seconds and is associated with altered excitability in neurons and myocytes, contributing to various disease phenotypes such as hyperkalemic periodic paralysis and long QT syndrome (Vilin and Ruben 2001). However, the detailed molecular mechanism of slow inactivation is still unknown (Ulbricht 2005). Once VGSCs enter an inactivated configuration, the channels are refractory to future stimuli and will not be able to open again until the membrane potential has repolarized to negative potentials. This process is called "recovery from inactivation," which prevents the cells from premature re-excitation and is critical in regulating action potential firing in excitable cells. Alteration in recovery rate may contribute to a disrupted action potential generation pattern. Previous study on skeletal muscle sodium channel reveals that defective recovery from fast inactivation may lead to disease phenotype producing myotonic discharges (Richmond et al. 1997). The voltage dependence of inactivation, and to a lesser extent activation, can be modulated by posttranslational modifications. This can change the degree of overlap between activation and steady-state inactivation and impact window currents that typically are active near cell resting membrane

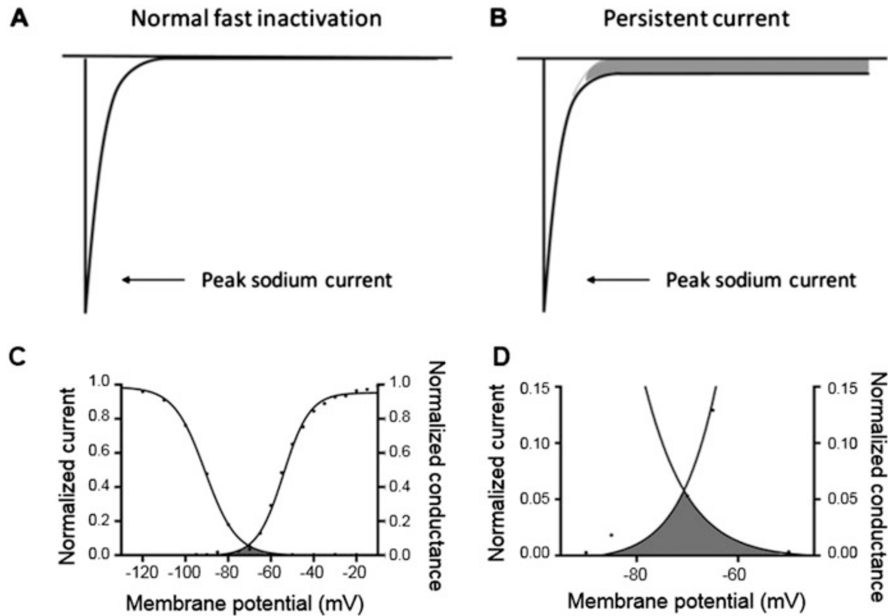

Fig. 2 Impaired fast inactivation leads to the generation of persistent (late) sodium current. (**a**) Representative current trace of normal fast inactivation. Sodium channels open and inactivate quickly after opening. (**b**) Persistent current generation when sodium channel fast inactivation is impaired, with the *gray* color indicating the non-inactivating persistent current. (**c**) Representative curves of cardiac sodium channel voltage dependence of activation and steady-state inactivation. (**d**) Representative window current (*gray* area). Window currents can be observed at voltages that are depolarized enough to activate a fraction of sodium channels but not sufficient to fully inactivate all sodium channels

potential (Fig. 2c, d). Window currents are related to classic persistent currents, but result from distinct gating alterations.

2 Posttranslational Modifications of VGSCs

Sodium channels can be modulated by a number of substances. Calcium and hydrogen ions can alter sodium channel gating. G proteins, ATP, cAMP, glycolytic metabolites, and local anesthetics have all been reported to have modulatory effects on sodium channels. VGSC function is determined by the intrinsic biophysical properties (i.e., rapid activation and inactivation), but it can also be regulated by posttranslational modifications.

Posttranslational VGSC modulation appears to be critically important in neurons and cardiac tissue. It is less clear what the physiological role of sodium channel modulation is in skeletal muscle, although it is likely to be involved in disease states such as critical illness myopathy (Teener and Rich 2006). Neuronal sodium current

can be downregulated by anoxia and cyanide (Cummins et al. 1991, 1993; O'Reilly et al. 1997), and it is thought that this can be important in the anoxic response. CaMKII phosphorylation of brain VGSCs has been implicated as a major regulator of VGSC persistent currents and is likely to contribute to pathophysiologies such as epilepsy. Cardiac sodium channel modulation is believed to be important in rhythmogenesis (Schubert et al. 1990; Wagner et al. 2015). As a result, Nav1.5 modulation has been extensively studied, and Nav1.5 is the target of a multitude of posttranslational modifications including glycosylation, phosphorylation, methylation, acetylation, redox modifications, palmitoylation, and ubiquitination (Ashpole et al. 2012; Pei et al. 2016; Marionneau and Abriel 2015). Because of modern protein analysis technologies such as mass spectrometry, researchers are able to better determine the precise location of post-translational modification sites and consensus sequence among different VGSC subtypes, leading to a better understanding of the mechanistic details of these regulations.

2.1 Phosphorylation

Phosphorylation is the most extensively studied posttranslational modification of VGSCs. Various protein kinases have been identified to modulate different aspects of VGSC function through diverse pathways including Ca2+/calmodulin-dependent serine/threonine protein kinase (CaMK) (Ashpole et al. 2012; Koval et al. 2012; Aiba et al. 2010), protein kinase A and C (PKA and PKC) (Shin and Murray 2001; Murray et al. 1997; Murray et al. 1994; Hallaq et al. 2012), phosphatidylinositol 3-kinase (PI3K) (Lu et al. 2013; Lu et al. 2012), and adenosine monophosphate-activated protein kinase (APMK) (Wallace et al. 2003). These kinases can add a negatively charged phosphate group to select serine, threonine, or tyrosine residues. It is not possible to give a comprehensive review of all the findings relating to this powerful form of VGSC modulation, so we will cover only a few specific areas to provide highlights of what can be learned from the study of VGSC phosphorylation. Many of the phosphorylation sites that have been identified are located in the first intracellular linker loop (DI–DII linker, Fig. 1), and a number of these are conserved among different species and subtypes (Marionneau et al. 2012). Early estimates indicated that the α subunit could be phosphorylated at somewhere between 2 and 20 sites by protein kinase A (PKA), protein kinase C (PKC), and possibly other protein kinases. Numann et al. (1991) studied the effect of PKC on rat brain channels (Nav1.2) expressed in Chinese hamster ovary cells. They reported that activation of PKC with a membrane-permeant agent, l-oleoyl-2-acetyl-sn-glycerol (OAG), both inhibited the peak current and slowed macroscopic fast inactivation of rat neuronal sodium channels. West et al. (1991) reported that phosphorylation of serine 1506 in the III–IV linker (shown by the black star in Fig. 1) by PKC was responsible for the slowing of RIIA macroscopic inactivation. After this potential PKC site, located in the III–IV linker, was removed by mutating serine 1506 to an alanine, neither the slowing nor the inhibition by OAG was observed. Additional experiments seemingly confirmed that phosphorylation of

S1506 was necessary and sufficient for the observed slowing of inactivation. However, while phosphorylation of S1506 was also necessary for the decrease in current, it was not sufficient: phosphorylation of another site, possibly in the I–II linker, also seemed to be needed for the inhibition (Li et al. 1993). However, subsequent studies on PKC modulation of neuronal sodium channels in hippocampal neurons did not observe pronounced slowing of inactivation (Chen et al. 2005). This could be due to complex interactions between posttranslational modifications and/or accessory subunits. The putative PKC site in the III–IV linker is conserved in most VGSCs. The site is conserved in the cardiac sodium channel (Nav1.5), and PKC phosphorylation of the corresponding residue in the III–IV linker causes a major negative shift in the voltage dependence of inactivation (Qu et al. 1996). Interestingly, this site is also conserved in Nav1.4, but while PKC also induces a negative shift in the voltage dependence of inactivation for Nav1.4, this effect is not dependent on the corresponding serine in the III–IV linker (Bendahhou et al. 1995). Data from *Xenopus* oocyte experiments indicate that both Nav1.7 and Nav1.8 currents are inhibited by PKC activation and that this also shifts the voltage dependence of activation in the depolarizing direction (Vijayaragavan et al. 2004). By contrast PKA activation enhanced Nav1.8 but inhibited Nav1.7 currents in this experimental system. Recently it was shown that Nav1.7 resurgent sodium currents are enhanced by PKC, and this effect is modulated by the state of the corresponding III–IV linker residue in Nav1.7 (Tan et al. 2014). This illustrates some of the complexities of determining the functional consequences of VGSC phosphorylation and comparing the effects on various isoforms in various tissues.

Mass spectrometry is providing enhanced estimates of VGSC phosphorylation. Obtaining full coverage can be difficult with complex transmembrane proteins. In one study of Nav1.2 (Berendt et al. 2010), 66% coverage of the cytoplasmic linkers was obtained. Fifteen sites were identified, 1 in the N-terminus, 11 in the I–II linker, and 3 in the C-terminus. Unfortunately there was insufficient coverage in the III–IV linker to determine if the conserved serine residue discussed above was phosphorylated in the brain tissue. In a follow-up study on Nav1.2 (Baek et al. 2014), it was found that acute kainate-induced seizures induced a significant reduction in phosphorylation of nine sites in Nav1.2. Not surprisingly, these are primarily sites located in the I–II linker. However, this study also revealed that this downregulation was due, at least in part, to an increase in methylated arginines at three sites. Thus distinct regulation of phosphorylation and methylated arginines in Nav1.2 is likely to contribute to functional changes in Nav1.2, modulation of neuronal excitability, and perhaps seizure activity.

Calcium/calmodulin protein kinase II (CaMKII) is believed to be an important regulator of excitability in neurons and muscle. CaMKII modulation of VGSCs has also been implicated in physiological and pathophysiological control of excitability. Increased CaMKII activity has been implicated in animal models of heart failure as well as in studies of failing human hearts (Zhang et al. 2003; Hoch et al. 1999). In one study of CaMKII and Nav1.5 (Ashpole et al. 2012), it was found that a negative shift in Nav1.5 steady-state inactivation resulted from CaMKII-dependent phosphorylation of Nav1.5 at two specific phosphor sites. However a mass spectroscopy analysis of

human Nav1.5 purified from HEK293 cells with >80% coverage identified 23 sites that could be phosphorylated by CaMKII in vitro (Herren et al. 2015). This suggests that CaMKII can extensively modify VGSCs by phosphorylation. A study of sodium currents in neurons from a SCN2a (Nav1.2) mutant mouse with epilepsy found that CaMKII phosphorylation of Nav1.2 increases persistent sodium currents and excitability. Maltsev et al. (2008) reported that CaMKII can also increase persistent sodium currents in cardiac myocytes. Burel et al. (2017) identified two distinct CaMKII phosphor sites in the C-terminus of Nav1.5 that contribute in part to increased Nav1.5 persistent currents; however this modulation involved altered binding of FGF13 to Nav1.5. Interestingly, these two sites were not among the 23 sites identified by Herren et al. Although several hundred studies have investigated how a multitude of kinases modulate VGSC isoforms and a substantial amount of insight has been gained, our knowledge of the interplay between different phosphor sites and how this impacts interactions with accessory proteins is still incomplete.

2.2 Arginine Methylation

As mentioned above, VGSCs can also be modified at lysine residues by arginine methylation. Much less is known about this compared to VGSC phosphorylation. Arginine methylation of Nav1.5 in stable cell lines and human ventricles has been examined using mass spectrometry (Beltran-Alvarez et al. 2015; Beltran-Alvarez et al. 2014; Beltran-Alvarez et al. 2013). Studies revealed that arginine R513, R526, and R680, located in the DI and DII intracellular linker of cardiac sodium channel, are likely subject to arginine methylation. Nav1.5 methylation also enhances cell surface expression and sodium current density. It is likely that other isoforms in addition to Nav1.2 and Nav1.5 are regulated by arginine methylation. Differential expression of protein arginine methyl transferases may play a role in how VGSCs are modulated in different tissue and cell compartments.

2.3 Glycosylation

Glycosylation, another common post-translational modification of ion channels, is an enzymatic process that attaches glycans to ion channel proteins. These sugar groups can be quite complex and extensive, making analysis complicated. Early reports (Waechter et al. 1983) indicated that glycosylation of neuronal VGSCs played a crucial role in biosynthesis, trafficking, and degradation of VGSCs. N-linked glycans (attached to a nitrogen of asparagine) and O-linked glycans (attached to the hydroxyl oxygen of serine, threonine) are often terminated by sialic acids which can, at least in theory, alter VGSC function through their negative charges. Glycosylation of Nav1.5 was determined 20 years ago by Cohen and Levitt (1993). This study indicated that Nav1.5 mass was only increased by about 5% due to glycosylation, compared to 25–30% increases observed with some other VGSC isoforms. Despite the lesser degree of modification, there is still compelling evidence

that glycosylation can be an important determinant of Nav1.5 membrane trafficking (Mercier et al. 2015). Pathophysiologically, reduced cardiac sodium channel sialylation has been shown to shorten the cardiomyocyte refractory time and enhance susceptibility to ventricular arrhythmias by slowing fast inactivation and increasing the rate of recovery from inactivation (Ednie et al. 2013). Glycosylation can also impact functional properties for multiple VGSCs. It has been shown that sialylation shifts the voltage dependence of activation and inactivation toward hyperpolarized potentials, enhances the rate of fast inactivation, and reduces the rate of recovery from fast inactivation (Johnson et al. 2004; Bennett et al. 1997). The impact on Nav1.4 voltage dependence of activation and inactivation can be quite large (Ednie et al. 2015), with sialylation, N-linked glycans, and O-linked glycans likely all playing a role in this. The sensory neuronal channel Nav1.9 is also not extensively glycosylated, yet changes in glycosylation can still impact voltage dependence of steady-state inactivation, and this appears to be developmentally regulated (Tyrrell et al. 2001). In addition to α subunits, it is well established that β-subunits can also be glycosylated (Laedermann et al. 2013a). However the extent to which glycosylation is involved in control of excitability is not fully understood, and improved strategies for determining how VGSC glycosylation is controlled will undoubtedly provide invaluable insight in the near future.

2.4 Ubiquitination

Ubiquitination, another well-studied posttranslational modification of VGSCs, refers to the enzymatic process in which an ubiquitin protein is attached to the sodium channel protein. Ubiquitin is a small protein, only about 8.5 kDa, but is a fairly large addition to a protein compared to most posttranslational modifications. The addition can alter protein function in several ways. Ubiquitination can target a protein to proteasomes and induce degradation. It can also alter localization of proteins and/or their functional properties. Modifications can involve addition of a single ubiquitin subunit or a chain of ubiquitin molecules. Addition of an ubiquitin molecule involves three proteins with distinct functions. Abriel et al. (2000) investigated the functional consequences of a PY (PPXY) motif in the C-terminus region of Nav1.5. This motif can bind Nedd4, an ubiquitin-protein ligase. Interestingly Nav1.4 lacks a PY motif. Mutation of the PY motif in Nav1.5 increased current density. Overexpression of Nedd4 could decrease Nav1.5 but not Nav1.4 current. This indicated that ubiquitination can be closely associated with Nav1.5 VGSC internalization. Ubiquitination of Nav1.5 has been demonstrated from both in vitro and in vivo studies (van Bemmelen et al. 2004; Laedermann et al. 2014a). Rougier et al. (2005) noted that most VGSC isoforms contain a PY motif (the exceptions being Nav1.4 and Nav1.9). They found that Nedd4-2 could downregulate Nav1.2, Nav1.3, and Nav1.5 currents in HEK293 cells with a corresponding reduction in surface expression. Fotia et al. (2004) demonstrated that Nedd4-2 could downregulate Nav1.7 and Nav1.8 currents.

While many studies have suggested that regulation of ubiquitination of VGSCs could lead to an altered surface expression level of channels (Laedermann et al. 2014b; Rougier et al. 2013), the detailed mechanism involved in this process and the study of its clinical relevance are still lacking. Interestingly, several studies were able to demonstrate that reduced Nedd4-2 levels led to DRG hyperexcitability, and conditions that increase pain in rodents downregulate Nedd4-2 expression in DRG, indicating that reduced ubiquitination of VGSCs might be involved in the development of neuropathic pain (Laedermann et al. 2013b; Cachemaille et al. 2012). Although Nav1.6 also has a PY motif in its C-terminus, it also appears to have a potential Nedd4 binding site in the I–II linker. In an elegant study, Gasser et al. (2010) demonstrated that downregulation of Nav1.6 can be enhanced by p38 kinase phosphorylation of the region involved in the I–II linker, indicating that Nedd4-induced ubiquitination and subsequent internalization of Nav1.6 involve at least two distinct Nedd4 binding sites and may be a stress response that limits cell excitability under pathophysiological conditions.

2.5 SUMOylation

The small ubiquitin-like modifier (SUMO) protein can also be conjugated to proteins, including ion channels. SUMO proteins are roughly 12 kDa in size and, as with ubiquitin, are added to other proteins by enzymes. Plant et al. (2016) found that adding SUMO protein to the pipet solution can increase the amplitude of Nav1.2-mediated currents in HEK293 cells. They also showed that SUMO modulated Nav1.2 currents in cerebellar neurons. SUMOylation occurs on lysine residues. Plant et al. reported that mutating K38 in the N-terminus of Nav1.2 eliminated the effect of SUMO. Thus Nav1.2 seems to be directly regulated by SUMOylation. This increase in current due to SUMOylation occurred rapidly in response to hypoxic conditions and may underlie some of the initial toxicity associated with hypoxia. SUMOylation also shifted the voltage dependence of activation and steady-state inactivation in the negative direction. While a negative shift in activation can increase excitability, a negative shift in inactivation can decrease excitability. SUMOylation has also been implicated in control of excitability in sensory neurons (Dustrude et al. 2016). Reduced SUMOylation of collapsin response mediator protein 2 (CRMP2) can alter CRMP2 binding to Nav1.7. As a consequence, Nav1.7 membrane localization and current density is reduced. CRMP2 enhances ubiquitination and endocytosis of Nav1.7. This illustrates the sometimes complex web of posttranslational modifications that can influence VGSCs.

2.6 Palmitoylation

Palmitoylation has been recognized as an important post-translational mechanism for the regulation of various membrane proteins, but our knowledge of how

palmitoylation regulates ion channels has been limited (Shipston 2011). Protein S-palmitoylation involves the addition of a 16-carbon palmitic acid chain to an intracellular cysteine residue through a thioester linkage (Fig. 3a). Recent studies have shown that palmitoylation can have profound impact on VGSC properties.

S-palmitoylation is a reversible process (Fig. 3b) that can dynamically regulate protein life cycle and function (Shipston 2011). Multiple enzymes that facilitate the palmitoylation process have been identified in recent years. Palmitoyltransferases (PATs), the catalytic enzymes for protein palmitoylation, form a diverse family of proteins (23 members in mammals) (Fukata et al. 2004; Korycka et al. 2012). PATs are characterized by the presence of an aspartate-histidine-histidine-cysteine (DHHC) motif within a cysteine-rich domain. The reverse process, depalmitoylation, is mediated by acyl protein thioesterases (APTs) (Zeidman et al. 2009). According to previous studies, nonenzymatic palmitoylation could be possible but is very rare and has only been reported in vitro.

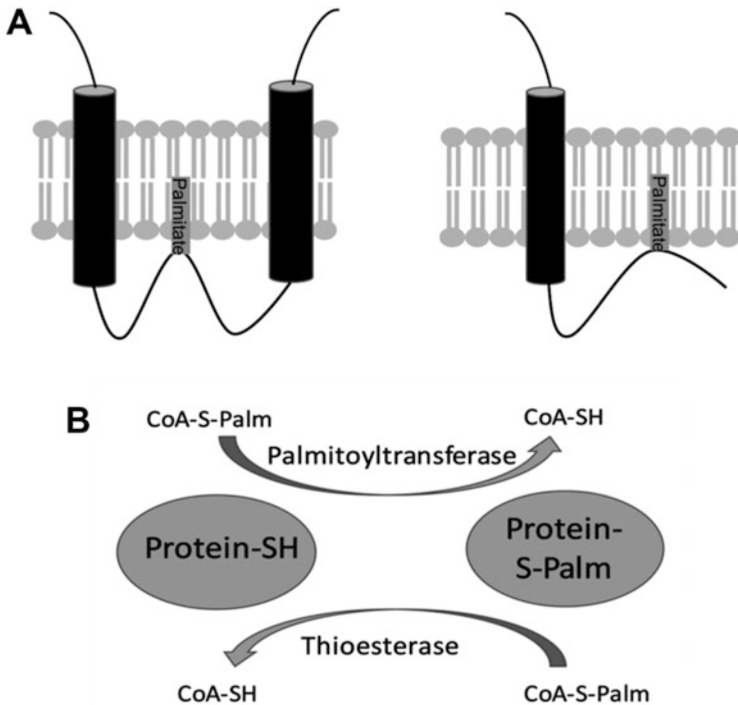

Fig. 3 (**a**) Palmitoylation can provide additional anchors for intracellular linkers or termini of channel proteins, depending on the location of the cysteine that is palmitoylated. (**b**) A cartoon illustration of the dynamic regulation of proteins by palmitoylation. Palmitoylation is a reversible process due to the labile nature of the thioester bond. Palmitoyltransferases mediate the palmitoylation process with the presence of palmitoyl-CoA. Depalmitoylation is mediated by thioesterases

Palmitoylation is involved in various phases of ion channel life cycle, including synthesis, maturation, trafficking, membrane targeting, internalization, and recycling. The formation of ligand binding sites in nicotinic acetylcholine receptors is regulated by palmitoylation (Drisdel et al. 2004; Alexander et al. 2010). Palmitoylation also regulates trafficking of AMPA receptors (Thomas et al. 2012; Hayashi et al. 2005) and the spatial organization of aquaporin channels (Suzuki et al. 2008). The addition of palmitic acid also regulates protein hydrophobicity and facilitates association to the membrane. However, few studies have focused on how palmitoylation directly alters channel biophysical activity at the membrane. Palmitoylation of the intracellular linker between S2 and S3 in Kv1.1 has been shown to increase the intrinsic voltage sensitivity of the channel (Gubitosi-Klug et al. 2005).

In 1987, brain sodium channel palmitoylation was identified to occur in the early stages of biosynthesis (Schmidt and Catterall 1987). This biochemical study indicated that brain sodium channels were palmitoylated after N-glycosylation of the channels. The addition of palmitate had a negligible impact on channel mass. It was not until over 30 years later that insight was gained into the functional consequences of palmitoylation (Bosmans et al. 2011). Interestingly, the initial functional work was on a recombinant channel that contained an early cloning artifact. An artificial cysteine in Nav1.2 that introduced a palmitoylation site had significant impact on the pharmacology of the channels. This indicated that lipid modification, at least in theory, is capable of altering the pharmacological properties of Nav1.2. Bosmans et al. also presented evidence that an endogenous cysteine might impact channel functional properties based on whether or not it was palmitoylated. However biochemical analysis of palmitoylation was not part of this short report.

The functional effect of VGSC palmitoylation is likely dependent on both the channel type and the location of the palmitoylation sites. Palmitate lipid may attach to different locations of channels, including C-terminus, N-terminus, juxta-transmembrane region, or intracellular loops (Fig. 3a), leading to the distinct alteration in various aspects of ion channel protein function (Shipston 2014). It is still unclear how palmitoylation alters the functional activity of VGSC protein. One hypothesized mechanism is that the palmitate molecules interact with the membrane lipids, changing the lipid membrane environment surrounding the targeted channel as well as impacting protein configuration, thus potentially modulating channel activity.

In recent years, the progress of ion channel palmitoylation research has been facilitated by the development of new biochemical and proteomics tools and the identification of palmitoyltransferases (Korycka et al. 2012). The traditional method of detecting protein palmitoylation involves metabolic labeling of cells with radioactive palmitate followed by immunoprecipitation and further identification of target proteins by autoradiography (Schmidt et al. 1988). This can provide direct evidence of protein palmitoylation. The development of the nonradioactive acyl-biotin exchange (ABE) assay allows more rapid detection of protein palmitoylation (Brigidi and Bamji 2013). This method provides higher sensitivity

compared to traditional metabolic labeling and allows for quantitative estimates of palmitoylation. However, one caveat of this methodology is the detection of all existing S-acylated proteins, instead of more specific identification of protein palmitoylation. Another commonly used nonradioactive assay for palmitoylation detection is the click chemistry method. This method uses the alkyne fatty acid analog 17-octadecynoic acid (17-ODYA), which can be metabolically incorporated into endogenous cellular machinery at palmitoylation sites (Martin and Cravatt 2009). 17-ODYA-labeled proteins can be further linked to azide reporter tags by copper-catalyzed click chemistry and visualized by in-gel fluorescence analysis. One important advantage of this method is that it largely reduces the chance of false positive results compared with ABE assay. The most convincing demonstration of palmitoylation sites will be the mass spectrometry results. However, it is often lacking in most studies due to the complexity of this lipid modification and difficulties of resolving the palmitoylated peptides in mass spectrometry. Pei et al. (2016) demonstrated palmitoylation of Nav1.5 using three lines of biochemistry evidence. Nav1.5 palmitoylation was detected with tritiated palmitate, ABE experiments, and 17-ODYA labeling. Although most biochemical work was done in HEK293 cells with human Nav1.5, they also confirmed that rodent cardiac sodium channels were palmitoylated in myocytes (Fig. 4).

They also determined that Nav1.5 gating properties were modulated by palmitoylation (Pei et al. 2016). Surprisingly, palmitoylation did not induce a significant change in current density, indicating that palmitoylation has negligible impact on

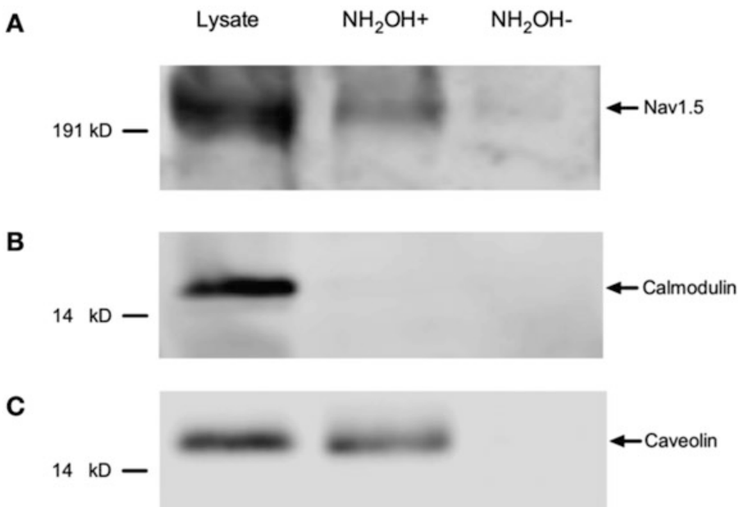

Fig. 4 Identification of Nav1.5 palmitoylation using an ABE assay. The *left lane* indicates total input lysate. The *middle lane* (NH2OH+) indicates palmitoylated protein. The *right lane* (NH2OH−) indicates the negative control group treated with tris solution. (**a**) Nav1.5 is palmitoylated in cardiac tissues. (**b**) Calmodulin is present but not palmitoylated in cardiac tissues. (**c**) Caveolin is palmitoylated in cardiac tissues. Reprinted with permission from Pei et al. (2016)

cell surface expression of Nav1.5. Instead, the data demonstrated that Nav1.5 palmitoylation has profound impact on channel availability by regulating the voltage dependence of steady-state inactivation in both HEK293 cells and cardiomyocytes. Nav1.5 inactivation was shifted in the negative direction by approximately 15–20 mV with the palmitoylation inhibitor 2-Br-palmitate (Fig. 5b). In contrast, Nav1.5 availability was significantly enhanced by palmitic acid treatment, increasing the voltage range of where window currents could be generated (Fig. 5c). Consistent with this, an elevated persistent sodium current was observed with palmitic acid treatment (Fig. 5a). As palmitic acid is the substrate for palmitoylation, this indicated that palmitoylation of Nav1.5 can enhance late sodium currents in myocytes. Together with the alteration in Nav1.5 inactivation, this could potentially lead to the reactivation of Nav1.5 and enhancement of sodium conductance during

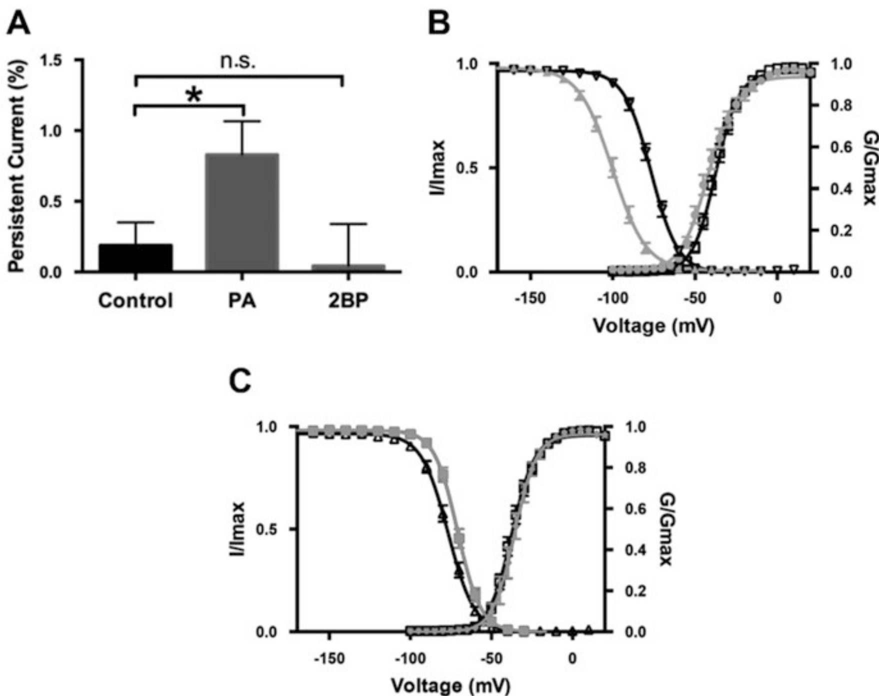

Fig. 5 (**a**) Statistical analysis comparing the relative amplitude of persistent currents from the control condition and treatment groups in neonatal cardiomyocytes. The persistent current was measured as the percentage of peak current. The persistent current is 0.2 ± 0.2% (control, *left bar*); 0.8 ± 0.2% (palmitic acid, *middle bar*); 0.0 ± 0.3% (2-Br-palmitate, *right bar*); $n = 10$. The difference between control group and palmitic acid treatment group is statistically significant ($p = 0.037$). (**b**, **c**) Comparison of sodium channel voltage dependence of activation and steady-state inactivation in neonatal cardiomyocytes. The *black* curves and symbols indicate the non-treatment group. In (**b**) the *gray* curves and symbols indicate the 2-Br-palmitate treatment group. In (**c**) the *gray* curves and symbols indicate the palmitic acid treatment group. Adapted with permission from Pei et al. (2016)

action potential phase 3 in cardiac myocytes. The opposite direction of changes in sodium channel gating properties between these two treatment groups suggests that palmitoylation can dynamically regulate Nav1.5 functions by altering channel inactivation gating. In addition, a mutant Nav1.5 with four internal cysteines mutated to alanine had a similar voltage dependence of activation to that obtained with wild-type 2-Br-palmitate treatment, indicating that specific cysteine residues on Nav1.5 were involved in the modulation of inactivation.

Pei et al. also reported that palmitoylation of Nav1.5 has a pronounced impact on cardiomyocyte excitability. Specifically, inhibiting palmitoylation greatly reduced myocyte excitability. The treatment of myocytes with 2-Br-palmitate abolished beating cells, and no action potential (spontaneously or stimulated) was observed. In contrast, enhancing palmitoylation with excess substrate increased action potential duration (Fig. 6a, b). Computer simulations indicated that changes in Nav1.5 gating due to palmitoylation are sufficient to cause the excitability changes that were observed in myocytes (Fig. 6c). Bankston and colleagues reported that the F1473C Nav1.5 mutation, a long QT mutation associated with a severe clinical phenotype, had similar biophysical consequences to those of Pei et al. observed with palmitic acid treatment (Bankston et al. 2007). The F1473C mutation shifted the midpoint of Nav1.5 inactivation in the depolarizing direction by 9 mV and increased persistent (late) currents to ~0.6% of the peak current. All together these data suggest that while depalmitoylation of Nav1.5 cysteine(s) can result in reduced cardiac excitability similar to that observed with Brugada syndrome, excessive palmitoylation of Nav1.5 can lead to enhanced cardiac activity similar to that observed with long QT syndrome mutations.

2.7 S-nitrosylation

Nitric oxide (NO) is known to be an important signaling molecule in many physiological processes, including host defense, neuronal communication, and vascular regulation. Renganathan et al. (2002) showed that an NO donor (papa-NONOate) significantly inhibited fast TTX-sensitive, slow TTX-resistant, and persistent TTX-resistant Na^+ currents in small diameter DRG neurons (Fig. 7). The NO scavenger hemoglobin blocked this inhibition, indicating that NO or a related molecule was responsible for the inhibition of multiple VGSCs (most likely Nav1.7, Nav1.8, and Nav1.9). The inhibition was independent of guanylyl cyclase and cGMP signaling pathways. Posttranslational modification of sulfhydryl groups, S-nitrosylation, can mediate the actions of NO and NO-related molecules (Stamler 1994). Renganathan et al. (2002) presented evidence indicating that S-nitrosylation was likely to mediate the inhibition of three different types of Na^+ channels, fast TTX-sensitive, slow TTX-resistant, and persistent TTX-resistant Na^+ channels in C-type DRG neurons via modification of sulfhydryl groups on the VGSCs.

Thiol sites have been considered as selective bioregulatory targets for NO and NO-related molecules. It has been proposed that protein targets of S-nitrosylation

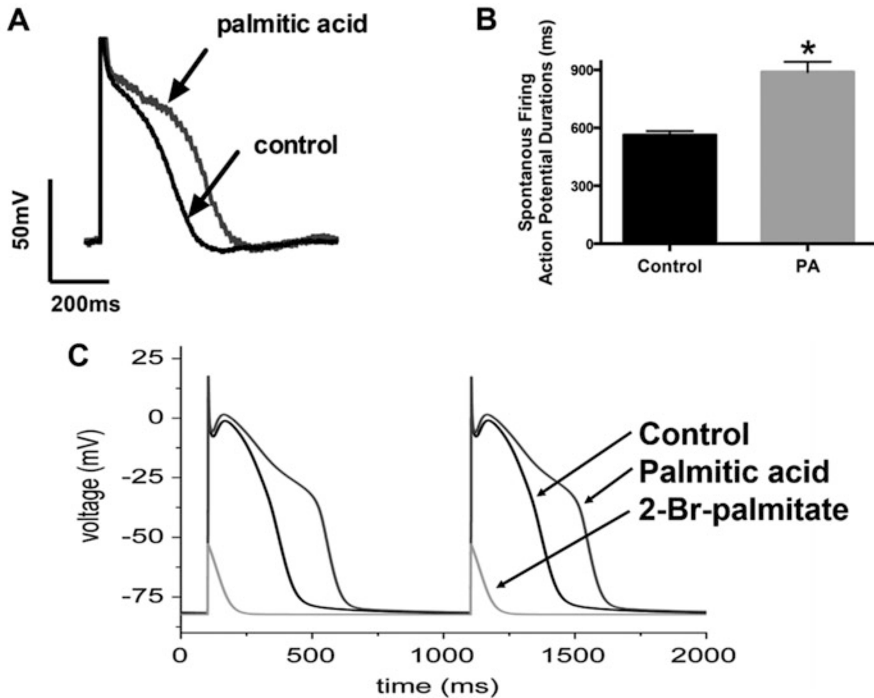

Fig. 6 Palmitoylation regulates cardiac action potential duration and firing frequency in myocytes. (**a**) Representative action potential traces of cardiomyocytes with (*blue*) and without (*black*) palmitic acid treatment. Palmitic acid (PA) treatment increases the action potential duration. (**b**) Averaged APD measurements (from the beginning of depolarization phase to the end of repolarization phase) from the spontaneous firing myocytes. Averaged APD is 563 ms in the control group and 890 ms in the palmitic acid treatment group. (**c**) Simulated action potentials from a modeled cardiac myocyte are shown. Action potentials were paced at 1 Hz in these simulations, and the second and third action potentials are shown. The action potentials from control Nav1.5 channels, Nav1.5 channels modeled to reflect the changes induced by enhanced palmitoylation (palmitic acid) and Nav1.5 channels modeled to reflect the changes induced by depalmitoylation (2-Br-palmitate). Adapted with permission from Pei et al. (2016)

often contain a consensus nitrosylation motif consisting of a cysteine (C) flanked by charged amino acids (Stamler et al. 1997). However, some proteins which do not have a consensus sequence can also be modulated by NO and NO-related products. Analysis of DRG sodium channel α subunits indicated the presence of cysteines flanked by charged amino acids at −1,2 and +1,2 positions. While both Nav1.7 and Nav1.8 are reported to have at least one consensus sequence for nitrosylation, Nav1.9 does not have a classic consensus sequence. The block of persistent Nav1.9 currents by a NO donor indicates that partial consensus sequences may be sufficient for nitrosylation of a resident cysteine and Na^+ channel block. The precise cysteine sites involved have not yet been identified, but it was speculated that

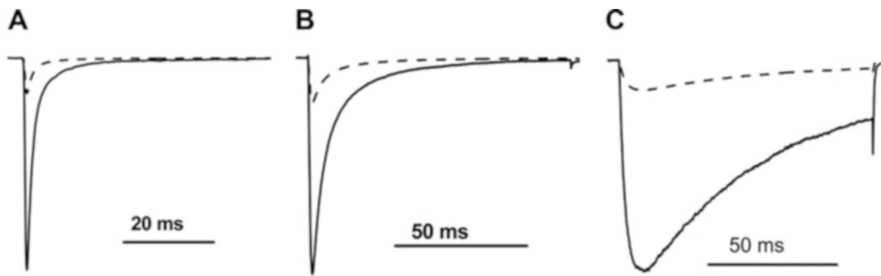

Fig. 7 Schematic illustration of relative inhibition of sensory neuron (**a**) TTX-sensitive, (**b**) slow TTX-resistant, and (**c**) persistent TTX-resistant sodium currents after putative S-nitrosylation. All three types of currents were reduced by 79–85% by nitric oxide donors in Renganathan et al. (2002)

current inhibition could play a role in regulating the excitability of the DRG neurons and might contribute to impaired impulse conduction in disease states.

As with phosphorylation, there is evidence that S-nitrosylation might differentially impact distinct VGSC isoforms. Hammarström and Gage (1999) reported that NO donors could increase persistent TTX-sensitive sodium currents in rat hippocampal neurons without impacting peak sodium current density. A two- to threefold increase in persistent current was observed, and an increase of this magnitude would undoubtedly contribute to increased excitability and thus could contribute to pathophysiological activity associated with stroke or epilepsy.

Because the increase in persistent current was relatively stable, it was proposed that a conformational change in the VGSC protein might have occurred as the result of disulfide bond formation between two closely located *S*-nitrosylated thiols in the VGSC.

S-nitrosylation has also been implicated in modulation of Nav1.5 cardiac sodium channels. Ahern et al. (2000) reported that NO donors could increase persistent currents by fivefold in both nerve terminals and myocytes. This effect was independent of cGMP, was blocked by *N*-ethylmaleimide, and was proposed to also involve S-nitrosylation. Interestingly, caveolin-3 activity seems to suppress S-nitrosylation of Nav1.5 channels and thus attenuates Nav1.5 persistent currents. However, LQT-9 mutations in caveolin-3 seem to disrupt this suppression, leading to larger Nav1.5 persistent currents and cardiac abnormalities (Cheng et al. 2013). It is not clear if S-nitrosylation and palmitoylation can both impact specific cysteine residues in VGSCs.

2.8 ROS Modifications

Reactive oxygen species (ROS) has also been implicated in regulation of VGSC activity. It has long been known that mild oxidizing agents such as chloramine-T can substantially impair sodium channel inactivation (Wang et al. 1985). Chloramine-T can impair inactivation of Nav1.2, Nav1.4, Nav1.5, and Nav1.7 channels (Kassmann

et al. 2008). Kassmann et al. found that chloramine-T removed more than 50% of Nav1.4 inactivation but had negligible impact on current activation properties. The effect on inactivation was not readily reversible. ROS can oxidize the sulfur-containing amino acids cysteine and methionine. Chloramine-T is thought to preferentially target methionines. By mutating intracellular methionines, Kassmann et al. found that three specific methionines were responsible for the chloramine-T effect on inactivation. One of these was the methionine in the IFM motif. The two other methionines are located next to each other in the S4–S5 linker of DIV. Interestingly, several disease mutations that impair inactivation occur at these positions. However, it should be noted that there is indication that VGSCs may be differentially regulated by ROS. Schluter and Leffler (2016) reported that chloramine-T enhances activation in addition to impairing inactivation of sensory neuronal Nav1.7 and Nav1.8 VGSCs. Normally the cytosol is a reducing environment, but it is speculated that increased persistent sodium currents in response to ROS allow neurons and muscle to regulate firing frequency when under conditions of oxidative stress. This could be adaptive in some scenarios, but could also be problematic in other situations.

3 Conclusions

During over 40 years of research on VGSCs, we have learned that these crucial regulators of cellular excitability are themselves subject to extensive modulation. This review focused on a number of different covalent modifications of VGSCs. In addition to the modifications discussed here, it is likely that VGSCs are subject to other posttranslational modifications. Sulfation, lipoxidation, acetylation, and amidation are possibilities. Protease modification of channel proteins is also an important way to modulate their properties. The array of accessory proteins that can modulate VGSC properties continues to expand. Much has been learned about VGSCs using heterologous expression systems. However, it is clear that modulation can depend on cellular background. Differences in protein partners and enzyme activity can greatly impact the extent to which VGSCs are posttranslational modified. Furthermore, posttranslational modifications can interact, and therefore it is difficult to predict what impact a particular ensemble of modification will have on VGSC properties. Regardless, it is clear that VGSCs can be extensively modified and that this can alter trafficking, localization, gating, and pharmacology, among other properties. Active research will continue to help us understand how VGSC posttranslational modifications impact normal and abnormal excitability.

References

Abriel H, Kamynina E, Horisberger JD, Staub O (2000) Regulation of the cardiac voltage-gated Na+ channel (H1) by the ubiquitin-protein ligase Nedd4. FEBS Lett 466(2–3):377–380

Ahern GP, Hsu SF, Klyachko VA, Jackson MB (2000) Induction of persistent sodium current by exogenous and endogenous nitric oxide. J Biol Chem 275(37):28810–28815. https://doi.org/10.1074/jbc.M003090200

Aiba T, Hesketh GG, Liu T, Carlisle R, Villa-Abrille MC, O'Rourke B, Akar FG, Tomaselli GF (2010) Na+ channel regulation by Ca2+/calmodulin and Ca2+/calmodulin-dependent protein kinase II in guinea-pig ventricular myocytes. Cardiovasc Res 85(3):454–463. https://doi.org/10.1093/cvr/cvp324

Alexander JK, Govind AP, Drisdel RC, Blanton MP, Vallejo Y, Lam TT, Green WN (2010) Palmitoylation of nicotinic acetylcholine receptors. J Mol Neurosci 40(1–2):12–20. https://doi.org/10.1007/s12031-009-9246-z

Ashpole NM, Herren AW, Ginsburg KS, Brogan JD, Johnson DE, Cummins TR, Bers DM, Hudmon A (2012) Ca2+/calmodulin-dependent protein kinase II (CaMKII) regulates cardiac sodium channel NaV1.5 gating by multiple phosphorylation sites. J Biol Chem 287(24):19856–19869. https://doi.org/10.1074/jbc.M111.322537

Baek JH, Rubinstein M, Scheuer T, Trimmer JS (2014) Reciprocal changes in phosphorylation and methylation of mammalian brain sodium channels in response to seizures. J Biol Chem 289(22):15363–15373. https://doi.org/10.1074/jbc.M114.562785

Bankston JR, Yue M, Chung W, Spyres M, Pass RH, Silver E, Sampson KJ, Kass RS (2007) A novel and lethal de novo LQT-3 mutation in a newborn with distinct molecular pharmacology and therapeutic response. PLoS One 2(12):e1258. https://doi.org/10.1371/journal.pone.0001258

Beltran-Alvarez P, Espejo A, Schmauder R, Beltran C, Mrowka R, Linke T, Batlle M, Perez-Villa F, Perez GJ, Scornik FS, Benndorf K, Pagans S, Zimmer T, Brugada R (2013) Protein arginine methyl transferases-3 and -5 increase cell surface expression of cardiac sodium channel. FEBS Lett 587(19):3159–3165. https://doi.org/10.1016/j.febslet.2013.07.043

Beltran-Alvarez P, Tarradas A, Chiva C, Perez-Serra A, Batlle M, Perez-Villa F, Schulte U, Sabido E, Brugada R, Pagans S (2014) Identification of N-terminal protein acetylation and arginine methylation of the voltage-gated sodium channel in end-stage heart failure human heart. J Mol Cell Cardiol 76:126–129. https://doi.org/10.1016/j.yjmcc.2014.08.014

Beltran-Alvarez P, Feixas F, Osuna S, Diaz-Hernandez R, Brugada R, Pagans S (2015) Interplay between R513 methylation and S516 phosphorylation of the cardiac voltage-gated sodium channel. Amino Acids 47(2):429–434. https://doi.org/10.1007/s00726-014-1890-0

van Bemmelen MX, Rougier JS, Gavillet B, Apotheloz F, Daidie D, Tateyama M, Rivolta I, Thomas MA, Kass RS, Staub O, Abriel H (2004) Cardiac voltage-gated sodium channel Nav1.5 is regulated by Nedd4-2 mediated ubiquitination. Circ Res 95(3):284–291. https://doi.org/10.1161/01.RES.0000136816.05109.89

Bendahhou S, Cummins TR, Potts JF, Tong J, Agnew WS (1995) Serine-1321-independent regulation of the mu 1 adult skeletal muscle Na+ channel by protein kinase C. Proc Natl Acad Sci U S A 92(26):12003–12007

Bennett E, Urcan MS, Tinkle SS, Koszowski AG, Levinson SR (1997) Contribution of sialic acid to the voltage dependence of sodium channel gating. A possible electrostatic mechanism. J Gen Physiol 109(3):327–343

Berendt FJ, Park KS, Trimmer JS (2010) Multisite phosphorylation of voltage-gated sodium channel alpha subunits from rat brain. J Proteome Res 9(4):1976–1984. https://doi.org/10.1021/pr901171q

Bosmans F, Milescu M, Swartz KJ (2011) Palmitoylation influences the function and pharmacology of sodium channels. Proc Natl Acad Sci U S A 108(50):20213–20218. https://doi.org/10.1073/pnas.1108497108

Brigidi GS, Bamji SX (2013) Detection of protein palmitoylation in cultured hippocampal neurons by immunoprecipitation and acyl-biotin exchange (ABE). J Vis Exp (72). https://doi.org/10.3791/50031
Burel S, Coyan FC, Lorenzini M, Meyer MR, Lichti CF, Brown JH, Loussouarn G, Charpentier F, Nerbonne JM, Townsend RR, Maier LS, Marionneau C (2017) C-terminal phosphorylation of NaV1.5 impairs FGF13-dependent regulation of channel inactivation. J Biol Chem. https://doi.org/10.1074/jbc.M117.787788
Cachemaille M, Laedermann CJ, Pertin M, Abriel H, Gosselin RD, Decosterd I (2012) Neuronal expression of the ubiquitin ligase Nedd4-2 in rat dorsal root ganglia: modulation in the spared nerve injury model of neuropathic pain. Neuroscience 227:370–380. https://doi.org/10.1016/j.neuroscience.2012.09.044
Catterall WA, Goldin AL, Waxman SG (2005) International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. Pharmacol Rev 57(4):397–409
Chen Y, Cantrell AR, Messing RO, Scheuer T, Catterall WA (2005) Specific modulation of Na+ channels in hippocampal neurons by protein kinase C epsilon. J Neurosci 25(2):507–513. https://doi.org/10.1523/JNEUROSCI.4089-04.2005
Chen C, Calhoun JD, Zhang Y, Lopez-Santiago L, Zhou N, Davis TH, Salzer JL, Isom LL (2012) Identification of the cysteine residue responsible for disulfide linkage of Na+ channel alpha and beta2 subunits. J Biol Chem 287(46):39061–39069. https://doi.org/10.1074/jbc.M112.397646
Cheng J, Valdivia CR, Vaidyanathan R, Balijepalli RC, Ackerman MJ, Makielski JC (2013) Caveolin-3 suppresses late sodium current by inhibiting nNOS-dependent S-nitrosylation of SCN5A. J Mol Cell Cardiol 61:102–110. https://doi.org/10.1016/j.yjmcc.2013.03.013
Cohen SA, Levitt LK (1993) Partial characterization of the rH1 sodium channel protein from rat heart using subtype-specific antibodies. Circ Res 73(4):735–742
Corry B, Thomas M (2012) Mechanism of ion permeation and selectivity in a voltage gated sodium channel. J Am Chem Soc 134(3):1840–1846. https://doi.org/10.1021/ja210020h
Costa MR, Catterall WA (1984) Phosphorylation of the alpha subunit of the sodium channel by protein kinase C. Cell Mol Neurobiol 4(3):291–297
Cummins TR, Donnelly DF, Haddad GG (1991) Effect of metabolic inhibition on the excitability of isolated hippocampal CA1 neurons: developmental aspects. J Neurophysiol 66(5):1471–1482
Cummins TR, Jiang C, Haddad GG (1993) Human neocortical excitability is decreased during anoxia via sodium channel modulation. J Clin Invest 91(2):608–615. https://doi.org/10.1172/JCI116241
Drisdel RC, Manzana E, Green WN (2004) The role of palmitoylation in functional expression of nicotinic alpha7 receptors. J Neurosci 24(46):10502–10510. https://doi.org/10.1523/JNEUROSCI.3315-04.2004
Dustrude ET, Moutal A, Yang X, Wang Y, Khanna M, Khanna R (2016) Hierarchical CRMP2 posttranslational modifications control NaV1.7 function. Proc Natl Acad Sci U S A 113(52): E8443–E8452. doi: https://doi.org/10.1073/pnas.1610531113
Ednie AR, Horton KK, Wu J, Bennett ES (2013) Expression of the sialyltransferase, ST3Gal4, impacts cardiac voltage-gated sodium channel activity, refractory period and ventricular conduction. J Mol Cell Cardiol 59:117–127. https://doi.org/10.1016/j.yjmcc.2013.02.013
Ednie AR, Harper JM, Bennett ES (2015) Sialic acids attached to N- and O-glycans within the Nav1.4 D1S5-S6 linker contribute to channel gating. Biochim Biophys Acta 1850(2):307–317. https://doi.org/10.1016/j.bbagen.2014.10.027
Fotia AB, Ekberg J, Adams DJ, Cook DI, Poronnik P, Kumar S (2004) Regulation of neuronal voltage-gated sodium channels by the ubiquitin-protein ligases Nedd4 and Nedd4-2. J Biol Chem 279(28):28930–28935. https://doi.org/10.1074/jbc.M402820200
Fukata M, Fukata Y, Adesnik H, Nicoll RA, Bredt DS (2004) Identification of PSD-95 palmitoylating enzymes. Neuron 44(6):987–996. https://doi.org/10.1016/j.neuron.2004.12.005
Gasser A, Cheng X, Gilmore ES, Tyrrell L, Waxman SG, Dib-Hajj SD (2010) Two Nedd4-binding motifs underlie modulation of sodium channel Nav1.6 by p38 MAPK. J Biol Chem 285(34): 26149–26161. https://doi.org/10.1074/jbc.M109.098681

Goldin AL (2001) Resurgence of sodium channel research. Annu Rev Physiol 63:871–894. https://doi.org/10.1146/annurev.physiol.63.1.871

Goldin AL (2003) Mechanisms of sodium channel inactivation. Curr Opin Neurobiol 13(3): 284–290. doi: S0959438803000655 [pii]

Gubitosi-Klug RA, Mancuso DJ, Gross RW (2005) The human Kv1.1 channel is palmitoylated, modulating voltage sensing: identification of a palmitoylation consensus sequence. Proc Natl Acad Sci U S A 102(17):5964–5968. https://doi.org/10.1073/pnas.0501999102

Hallaq H, Wang DW, Kunic JD, George AL Jr, Wells KS, Murray KT (2012) Activation of protein kinase C alters the intracellular distribution and mobility of cardiac Na+ channels. Am J Physiol Heart Circ Physiol 302(3):H782–H789. https://doi.org/10.1152/ajpheart.00817.2010

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 391(2):85–100

Hammarstrom AK, Gage PW (1999) Nitric oxide increases persistent sodium current in rat hippocampal neurons. J Physiol 520(Pt 2):451–461

Hayashi T, Rumbaugh G, Huganir RL (2005) Differential regulation of AMPA receptor subunit trafficking by palmitoylation of two distinct sites. Neuron 47(5):709–723. https://doi.org/10.1016/j.neuron.2005.06.035

Herren AW, Weber DM, Rigor RR, Margulies KB, Phinney BS, Bers DM (2015) CaMKII phosphorylation of Na(V)1.5: novel in vitro sites identified by mass spectrometry and reduced S516 phosphorylation in human heart failure. J Proteome Res 14(5):2298–2311. https://doi.org/10.1021/acs.jproteome.5b00107

Hoch B, Meyer R, Hetzer R, Krause EG, Karczewski P (1999) Identification and expression of delta-isoforms of the multifunctional Ca2+/calmodulin-dependent protein kinase in failing and nonfailing human myocardium. Circ Res 84(6):713–721

Hodgkin AL, Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol 117(4):500–544

Johnson D, Montpetit ML, Stocker PJ, Bennett ES (2004) The sialic acid component of the beta1 subunit modulates voltage-gated sodium channel function. J Biol Chem 279(43):44303–44310. https://doi.org/10.1074/jbc.M408900200

Kassmann M, Hansel A, Leipold E, Birkenbeil J, Lu SQ, Hoshi T, Heinemann SH (2008) Oxidation of multiple methionine residues impairs rapid sodium channel inactivation. Pflugers Arch 456(6):1085–1095. https://doi.org/10.1007/s00424-008-0477-6

Korycka J, Lach A, Heger E, Boguslawska DM, Wolny M, Toporkiewicz M, Augoff K, Korzeniewski J, Sikorski AF (2012) Human DHHC proteins: a spotlight on the hidden player of palmitoylation. Eur J Cell Biol 91(2):107–117. https://doi.org/10.1016/j.ejcb.2011.09.013

Koval OM, Snyder JS, Wolf RM, Pavlovicz RE, Glynn P, Curran J, Leymaster ND, Dun W, Wright PJ, Cardona N, Qian L, Mitchell CC, Boyden PA, Binkley PF, Li C, Anderson ME, Mohler PJ, Hund TJ (2012) Ca2+/calmodulin-dependent protein kinase II-based regulation of voltage-gated Na+ channel in cardiac disease. Circulation 126(17):2084–2094. https://doi.org/10.1161/CIRCULATIONAHA.112.105320

Laedermann CJ, Syam N, Pertin M, Decosterd I, Abriel H (2013a) beta1- and beta3-voltage-gated sodium channel subunits modulate cell surface expression and glycosylation of Nav1.7 in HEK293 cells. Front Cell Neurosci 7:137. https://doi.org/10.3389/fncel.2013.00137

Laedermann CJ, Cachemaille M, Kirschmann G, Pertin M, Gosselin RD, Chang I, Albesa M, Towne C, Schneider BL, Kellenberger S, Abriel H, Decosterd I (2013b) Dysregulation of voltage-gated sodium channels by ubiquitin ligase NEDD4-2 in neuropathic pain. J Clin Invest 123(7):3002–3013. https://doi.org/10.1172/JCI68996

Laedermann CJ, Decosterd I, Abriel H (2014a) Ubiquitylation of voltage-gated sodium channels. Handb Exp Pharmacol 221:231–250. https://doi.org/10.1007/978-3-642-41588-3_11

Laedermann CJ, Pertin M, Suter MR, Decosterd I (2014b) Voltage-gated sodium channel expression in mouse DRG after SNI leads to re-evaluation of projections of injured fibers. Mol Pain 10:19. https://doi.org/10.1186/1744-8069-10-19

Levinson SR, Ellory JC (1973) Molecular size of the tetrodotoxin binding site estimated by irradiation inactivation. Nat New Biol 245(143):122–123

Li M, West JW, Numann R, Murphy BJ, Scheuer T, Catterall WA (1993) Convergent regulation of sodium channels by protein kinase C and cAMP-dependent protein kinase. Science 261(5127): 1439–1442

Lu Z, Wu CY, Jiang YP, Ballou LM, Clausen C, Cohen IS, Lin RZ (2012) Suppression of phosphoinositide 3-kinase signaling and alteration of multiple ion currents in drug-induced long QT syndrome. Sci Transl Med 4(131):131ra150. https://doi.org/10.1126/scitranslmed.3003623

Lu Z, Jiang YP, Wu CY, Ballou LM, Liu S, Carpenter ES, Rosen MR, Cohen IS, Lin RZ (2013) Increased persistent sodium current due to decreased PI3K signaling contributes to QT prolongation in the diabetic heart. Diabetes 62(12):4257–4265. https://doi.org/10.2337/db13-0420

Malhotra JD, Thyagarajan V, Chen C, Isom LL (2004) Tyrosine-phosphorylated and nonphosphorylated sodium channel beta1 subunits are differentially localized in cardiac myocytes. J Biol Chem 279(39):40748–40754. https://doi.org/10.1074/jbc.M407243200

Maltsev VA, Reznikov V, Undrovinas NA, Sabbah HN, Undrovinas A (2008) Modulation of late sodium current by Ca2+, calmodulin, and CaMKII in normal and failing dog cardiomyocytes: similarities and differences. Am J Physiol Heart Circ Physiol 294(4):H1597–H1608. https://doi.org/10.1152/ajpheart.00484.2007

Marionneau C, Abriel H (2015) Regulation of the cardiac Na+ channel NaV1.5 by post-translational modifications. J Mol Cell Cardiol 82:36–47. https://doi.org/10.1016/j.yjmcc.2015.02.013

Marionneau C, Lichti CF, Lindenbaum P, Charpentier F, Nerbonne JM, Townsend RR, Merot J (2012) Mass spectrometry-based identification of native cardiac Nav1.5 channel alpha subunit phosphorylation sites. J Proteome Res 11(12):5994–6007. https://doi.org/10.1021/pr300702c

Martin BR, Cravatt BF (2009) Large-scale profiling of protein palmitoylation in mammalian cells. Nat Methods 6(2):135–138. https://doi.org/10.1038/nmeth.1293

Mercier A, Clement R, Harnois T, Bourmeyster N, Bois P, Chatelier A (2015) Nav1.5 channels can reach the plasma membrane through distinct N-glycosylation states. Biochim Biophys Acta 1850(6):1215–1223. https://doi.org/10.1016/j.bbagen.2015.02.009

Messner DJ, Catterall WA (1985) The sodium channel from rat brain. Separation and characterization of subunits. J Biol Chem 260(19):10597–10604

Murray KT, Fahrig SA, Deal KK, Po SS, Hu NN, Snyders DJ, Tamkun MM, Bennett PB (1994) Modulation of an inactivating human cardiac K+ channel by protein kinase C. Circ Res 75(6): 999–1005

Murray KT, Hu NN, Daw JR, Shin HG, Watson MT, Mashburn AB, George AL Jr (1997) Functional effects of protein kinase C activation on the human cardiac Na+ channel. Circ Res 80(3):370–376

Numann R, Catterall WA, Scheuer T (1991) Functional modulation of brain sodium channels by protein kinase C phosphorylation. Science 254(5028):115–118

O'Reilly JP, Cummins TR, Haddad GG (1997) Oxygen deprivation inhibits Na+ current in rat hippocampal neurons via protein kinase C. J Physiol 503(Pt 3):479–488

Payandeh J, Scheuer T, Zheng N, Catterall WA (2011) The crystal structure of a voltage-gated sodium channel. Nature 475(7356):353–358. https://doi.org/10.1038/nature10238

Pei Z, Xiao Y, Meng J, Hudmon A, Cummins TR (2016) Cardiac sodium channel palmitoylation regulates channel availability and myocyte excitability with implications for arrhythmia generation. Nat Commun 7:12035. https://doi.org/10.1038/ncomms12035

Plant LD, Marks JD, Goldstein SA (2016) SUMOylation of NaV1.2 channels mediates the early response to acute hypoxia in central neurons. Elife 5. https://doi.org/10.7554/eLife.20054

Qu Y, Rogers JC, Tanada TN, Catterall WA, Scheuer T (1996) Phosphorylation of S1505 in the cardiac Na+ channel inactivation gate is required for modulation by protein kinase C. J Gen Physiol 108(5):375–379

Renganathan M, Cummins TR, Waxman SG (2002) Nitric oxide blocks fast, slow, and persistent Na+ channels in C-type DRG neurons by S-nitrosylation. J Neurophysiol 87(2):761–775
Richmond JE, VanDeCarr D, Featherstone DE, George AL Jr, Ruben PC (1997) Defective fast inactivation recovery and deactivation account for sodium channel myotonia in the I1160V mutant. Biophys J 73(4):1896–1903. https://doi.org/10.1016/S0006-3495(97)78220-1
Rougier JS, van Bemmelen MX, Bruce MC, Jespersen T, Gavillet B, Apotheloz F, Cordonier S, Staub O, Rotin D, Abriel H (2005) Molecular determinants of voltage-gated sodium channel regulation by the Nedd4/Nedd4-like proteins. Am J Physiol Cell Physiol 288(3):C692–C701. https://doi.org/10.1152/ajpcell.00460.2004
Rougier JS, Gavillet B, Abriel H (2013) Proteasome inhibitor (MG132) rescues Nav1.5 protein content and the cardiac sodium current in dystrophin-deficient mdx (5cv) mice. Front Physiol 4:51. https://doi.org/10.3389/fphys.2013.00051
Schluter F, Leffler A (2016) Oxidation differentially modulates the recombinant voltage-gated Na (+) channel alpha-subunits Nav1.7 and Nav1.8. Brain Res 1648(Pt A):127–135. https://doi.org/10.1016/j.brainres.2016.07.031
Schmidt JW, Catterall WA (1986) Biosynthesis and processing of the alpha subunit of the voltage-sensitive sodium channel in rat brain neurons. Cell 46(3):437–444
Schmidt JW, Catterall WA (1987) Palmitoylation, sulfation, and glycosylation of the alpha subunit of the sodium channel. Role of post-translational modifications in channel assembly. J Biol Chem 262(28):13713–13723
Schmidt M, Schmidt MF, Rott R (1988) Chemical identification of cysteine as palmitoylation site in a transmembrane protein (Semliki Forest virus E1). J Biol Chem 263(35):18635–18639
Schubert B, Vandongen AM, Kirsch GE, Brown AM (1990) Inhibition of cardiac Na+ currents by isoproterenol. Am J Physiol 258(4 Pt 2):H977–H982
Shin HG, Murray KT (2001) Conventional protein kinase C isoforms and cross-activation of protein kinase A regulate cardiac Na+ current. FEBS Lett 495(3):154–158
Shipston MJ (2011) Ion channel regulation by protein palmitoylation. J Biol Chem 286(11): 8709–8716. https://doi.org/10.1074/jbc.R110.210005
Shipston MJ (2014) Ion channel regulation by protein S-acylation. J Gen Physiol 143(6):659–678. https://doi.org/10.1085/jgp.201411176
Silva J (2014) Slow inactivation of Na(+) channels. Handb Exp Pharmacol 221:33–49. https://doi.org/10.1007/978-3-642-41588-3_3
Stamler JS (1994) Redox signaling: nitrosylation and related target interactions of nitric oxide. Cell 78(6):931–936
Stamler JS, Toone EJ, Lipton SA, Sucher NJ (1997) (S)NO signals: translocation, regulation, and a consensus motif. Neuron 18(5):691–696
Stuhmer W, Conti F, Suzuki H, Wang XD, Noda M, Yahagi N, Kubo H, Numa S (1989) Structural parts involved in activation and inactivation of the sodium channel. Nature 339(6226): 597–603. https://doi.org/10.1038/339597a0
Suzuki H, Nishikawa K, Hiroaki Y, Fujiyoshi Y (2008) Formation of aquaporin-4 arrays is inhibited by palmitoylation of N-terminal cysteine residues. Biochim Biophys Acta 1778(4): 1181–1189. https://doi.org/10.1016/j.bbamem.2007.12.007
Tan ZY, Priest BT, Krajewski JL, Knopp KL, Nisenbaum ES, Cummins TR (2014) Protein kinase C enhances human sodium channel hNav1.7 resurgent currents via a serine residue in the domain III-IV linker. FEBS Lett 588(21):3964–3969. doi: 10.1016/j.febslet.2014.09.011 S0014-5793(14)00686-3 [pii]
Teener JW, Rich MM (2006) Dysregulation of sodium channel gating in critical illness myopathy. J Muscle Res Cell Motil 27(5–7):291–296. https://doi.org/10.1007/s10974-006-9074-5
Thomas GM, Hayashi T, Chiu SL, Chen CM, Huganir RL (2012) Palmitoylation by DHHC5/8 targets GRIP1 to dendritic endosomes to regulate AMPA-R trafficking. Neuron 73(3): 482–496. https://doi.org/10.1016/j.neuron.2011.11.021

Tyrrell L, Renganathan M, Dib-Hajj SD, Waxman SG (2001) Glycosylation alters steady-state inactivation of sodium channel Nav1.9/NaN in dorsal root ganglion neurons and is developmentally regulated. J Neurosci 21(24):9629–9637

Ulbricht W (2005) Sodium channel inactivation: molecular determinants and modulation. Physiol Rev 85(4):1271–1301. https://doi.org/10.1152/physrev.00024.2004

Vijayaragavan K, Boutjdir M, Chahine M (2004) Modulation of Nav1.7 and Nav1.8 peripheral nerve sodium channels by protein kinase A and protein kinase C. J Neurophysiol 91(4): 1556–1569. https://doi.org/10.1152/jn.00676.2003

Vilin YY, Ruben PC (2001) Slow inactivation in voltage-gated sodium channels: molecular substrates and contributions to channelopathies. Cell Biochem Biophys 35(2):171–190. https://doi.org/10.1385/CBB:35:2:171

Waechter CJ, Schmidt JW, Catterall WA (1983) Glycosylation is required for maintenance of functional sodium channels in neuroblastoma cells. J Biol Chem 258(8):5117–5123

Wagner S, Maier LS, Bers DM (2015) Role of sodium and calcium dysregulation in tachyarrhythmias in sudden cardiac death. Circ Res 116(12):1956–1970. https://doi.org/10.1161/CIRCRESAHA.116.304678

Wallace RH, Hodgson BL, Grinton BE, Gardiner RM, Robinson R, Rodriguez-Casero V, Sadleir L, Morgan J, Harkin LA, Dibbens LM, Yamamoto T, Andermann E, Mulley JC, Berkovic SF, Scheffer IE (2003) Sodium channel alpha1-subunit mutations in severe myoclonic epilepsy of infancy and infantile spasms. Neurology 61(6):765–769

Wang GK, Brodwick MS, Eaton DC (1985) Removal of sodium channel inactivation in squid axon by the oxidant chloramine-T. J Gen Physiol 86(2):289–302

West JW, Numann R, Murphy BJ, Scheuer T, Catterall WA (1991) A phosphorylation site in the Na+ channel required for modulation by protein kinase C. Science 254(5033):866–868

West JW, Patton DE, Scheuer T, Wang Y, Goldin AL, Catterall WA (1992) A cluster of hydrophobic amino acid residues required for fast Na(+)-channel inactivation. Proc Natl Acad Sci U S A 89(22):10910–10914

Yarov-Yarovoy V, DeCaen PG, Westenbroek RE, Pan CY, Scheuer T, Baker D, Catterall WA (2012) Structural basis for gating charge movement in the voltage sensor of a sodium channel. Proc Natl Acad Sci U S A 109(2):E93–102. https://doi.org/10.1073/pnas.1118434109

Yu FH, Catterall WA (2003) Overview of the voltage-gated sodium channel family. Genome Biol 4(3):207

Zeidman R, Jackson CS, Magee AI (2009) Protein acyl thioesterases (review). Mol Membr Biol 26(1):32–41. https://doi.org/10.1080/09687680802629329

Zhang T, Maier LS, Dalton ND, Miyamoto S, Ross J Jr, Bers DM, Brown JH (2003) The deltaC isoform of CaMKII is activated in cardiac hypertrophy and induces dilated cardiomyopathy and heart failure. Circ Res 92(8):912–919. https://doi.org/10.1161/01.RES.0000069686.31472.C5