Post-translational modifications of the cardiac Na channel: contribution of CaMKII-dependent phosphorylation to acquired arrhythmias

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Herren AW, Bers DM, Grandi E. Post-translational modifications of the cardiac Na channel: contribution of CaMKII-dependent phosphorylation to acquired arrhythmias. Am J Physiol Heart Circ Physiol 305: H431–H445, 2013. First published June 14, 2013; doi:10.1152/ajpheart.00306.2013.—The voltage-gated Na channel isoform 1.5 (NaV1.5) is the pore forming α-subunit of the voltage-gated cardiac Na channel, which is responsible for the initiation and propagation of cardiac action potentials. Mutations in the SCN5A gene encoding NaV1.5 have been linked to changes in the Na current leading to a variety of arrhythmogenic phenotypes, and alterations in the NaV1.5 expression level, Na current density, and/or gating have been observed in acquired cardiac disorders, including heart failure. The precise mechanisms underlying these abnormalities have not been fully elucidated. However, several recent studies have made it clear that NaV1.5 forms a macromolecular complex with a number of proteins that modulate its expression levels, localization, and gating and is the target of extensive post-translational modifications, which may also influence all these properties. We review here the molecular aspects of cardiac Na channel regulation and their functional consequences. In particular, we focus on the molecular and functional aspects of Na channel phosphorylation by the Ca/calmodulin-dependent protein kinase II, which is hyperactive in heart failure and has been causally linked to cardiac arrhythmia. Understanding the mechanisms of altered NaV1.5 expression and function is crucial for gaining insight into arrhythmogenesis and developing novel therapeutic strategies.

Na channel; CaMKII; phosphorylation; heart failure; arrhythmia

THE CARDIAC Na channel, encoded by the gene SCN5A, is activated at negative membrane voltages and is responsible for the generation of the rapid upstroke of the cardiac action potential (AP). The α-subunit of the voltage-gated cardiac Na channel isoform (NaV1.5) forms a macromolecular complex through interactions with many accessory proteins (75), regulatory proteins (132), and other ion channels (77, 105) that alter its expression, trafficking, localization, and gating [reviewed in Abriel (1)] (Fig. 1). Recent evidence also suggests that multiple pools of NaV1.5 may exist in cardiomyocytes, and these channel populations may be differentially regulated (94, 109). Whereas most of the Na channels inactivate rapidly (within a few milliseconds), prolonged membrane depolarization during the AP plateau causes slow/intermediate (hundreds of milliseconds) inactivation of some channels, which recover to a closed conformation as the cell membrane repolarizes during diastole. In physiological situations, Na channel activation and inactivation properties are tightly regulated, thus maintaining cardiac excitability and ensuring propagation of the electrical impulse. However, alterations in channel function (e.g., due to gene mutations or post-translational modifications) may profoundly affect cardiac electrophysiology and arrhythmogenesis. Herein, we review post-translational regulation of NaV1.5. While we describe the molecular and functional aspects of post-translational regulation by PKA, PKC, oxidative stress, Ca, and CaM, we focus on Na channel phosphorylation by Ca/CaM-dependent protein kinase II (CaMKII). We further use CaMKII modulation as a model to understand how Na channel dysfunction may be implicated in acquired arrhythmias.

Na Channel Alterations in Inherited SCN5A Channelopathies and Acquired Diseases

A number of mutations in the gene SCN5A have been described, which cause enhanced or reduced channel function and are linked to cardiac disorders including long QT syndrome (LQTS), Brugada syndrome (BrS), conduction defects, sinus dysfunction, and familial atrial fibrillation (AF) (101). These have tremendously aided our understanding of the regulation of expression, localization, and function of NaV1.5 and suggest that its role is not limited to AP initiation and conduction, but it may also have more subtle functions and be involved in repolarization abnormalities. In addition, mutations disrupting Na channel protein interactions (within the macromolecular signaling complex modulating the localization and biophysical properties of NaV1.5) have also been associated with LQTS and BrS [reviewed by Wilde and Brugada (132)]. These channelopathies provided useful models for understanding the molecular mechanisms whereby pharmacological
interventions or disease states provoke cardiac arrhythmias. For example, LQT3 syndrome is caused by gain-of-function defects in Na channel inactivation, which lead to delays in ventricular repolarization that result in the prolongation of the QT interval. Numerous mutations induce a sustained (late Na current, $I_{Na,L}$) component of inward current (1% of the peak current) throughout the depolarization period. The role of this small current in prolonging repolarization and generating cardiac arrhythmias has been validated in quantitative models of the AP (27) and is further illustrated in the next subsection.

On the other hand, BrS is associated with loss-of-function mutations and is characterized by normal QT intervals and ST segment elevation in the right precordial leads, which mimics ischemic ECG manifestations and predisposition to malignant ventricular tachyarrhythmias. The pathophysiological mechanism is thought to involve heterogeneous loss of the AP dome between the epicardium and endocardium, which creates a transmural voltage gradient during ST segment (36, 137) or conduction delay in the right ventricular outflow tract (76). SCN5A mutations have also been reported leading to combinations of these phenotypes, known as overlap syndromes (100). Intriguingly, a single human mutation at 1795InsD in SCN5A is linked to simultaneous LQT3 and BrS features (124).

Mutation-induced slowing of $I_{Na}$ recovery from inactivation, increase in intermediate inactivation, and hyperpolarizing shift in channel availability all reduce $I_{Na}$ (loss of function) and underlie the BrS-like symptoms (slow conduction) of patients at higher heart rates. In addition, this 1795InsD mutation causes an increase in $I_{Na,L}$. At slow heart rates where $I_{Na}$ recovery from inactivation may be more complete and APs are intrinsically longer, $I_{Na,L}$-dependent AP prolongation is responsible for LQTS (28).

Emerging evidence also suggests that Na channel gating alterations are involved in widespread acquired diseases, e.g., drug-induced LQTS, cardiac ischemia, heart failure (HF), and AF. For example, CaMKII, which is upregulated and more active in HF (5), has been shown to regulate Na channel gating and almost exactly phenocopies the spectrum of gating changes seen for the combined LQTS/BrS phenotype due to 1795InsD (see Fig. 2). Also, the generation of reactive oxygen species (ROS) in conditions of increased oxidative stress (such as during ischemia, HF, and AF) correlates with CaMKII-mediated reduced $I_{Na}$ availability, leading to impairment of cardiac conduction following myocardial infarction (25, 46) and enhancement of late $I_{Na}$ and consequent arrhythmias (128). In this context, post-translational modifications of NaV1.5 may constitute an acquired arrhythmogenic Na channel defect that could affect millions of people (as compared with the relatively small number of individuals affected by the mechanistically informative genetic Na channel mutations).

**Late Na Current**

The late Na current is carried by a fraction of Na channels that remain active throughout the AP plateau, rather than quickly inactivating as the majority of Na channels do. These channels undergo special modes of gating (burst and late-scattered openings) that confer $I_{Na,L}$ slow inactivation and
contrasting results on the modulation of normal and failing canine myocytes (78). However, there are cases of function or gain of function of cardiac Na channel isoforms to fully understood. A significant (example, Maltsev et al. (66) showed that the NaV1.5 by slowing its decay and increasing its amplitude slower than normal hearts (69, 71). NaV1.5 alteration may be acute and result from post-translational modification of NaV channels, or chronic, and involve changes in expression of NaV and its modulatory proteins.

The molecular details of Na,L and its modulation are not fully understood. A significant (~50%) contribution of noncardiac Na channel isoforms to Na,L has been reported in healthy canine myocytes (15), and it has been proposed that its enhancement in pathological conditions could be due to an increase in neuronal Na channel isoforms with higher fractional Na,L. (135). In fact, specific NaV1.8 inhibition in mice and rabbits is antiarrhythmic (138). Modulation of Na channels could also underlie augmentation of Na,L in disease. For example, Maltsev et al. (66) showed that the β1-subunit (but not β2) modulates Na,L produced by heterologously expressed Nav1.5 by slowing its decay and increasing its amplitude relative to the peak current. Recently, they showed that reduced expression of either β1- or β2-subunits caused a significant loss of function or gain of function of Na,L, respectively, in both normal and failing canine myocytes (78). However, there are contrasting results on the modulation of Na,L by β-subunits in heterologous and native systems and whether they modulate similarly cardiac and noncardiac isoforms. Furthermore, interaction with the cytoskeleton, regulatory kinases (as discussed here) and phosphatases, trafficking proteins, and extracellular matrix proteins may all modulate Na,L in different pathological conditions (71).

Although small in magnitude compared with the peak Na current (~1%), Na,L may disrupt the delicate balance between depolarizing and repolarizing currents during the AP plateau, leading to AP prolongation and increasing the propensity to ventricular arrhythmia (79). Indeed, a pathological increase in Na,L has been associated with arrhythmogenic phenotypes in inherited and acquired cardiac diseases including LQT3, HF, myocardial infarction, and AF. Physiological Na,L has been shown to contribute to reverse-rate dependence of AP duration (APD) and beat-to-beat variability caused by K current inhibitors in rabbit hearts (134). It has also been suggested that its inhibition may diminish the rate-dependent prolongation of the APD/QT interval caused by either drugs or pathological conditions that decrease rapidly activating K current and may decrease the occurrence of slow rate- or pause-triggered cardiac arrhythmias. Conversely, Na,L enhancement in HF has been predicted to exacerbate reverse frequency dependence of APD (115), which may increase the proarrhythmic risk. In addition, the increased Na influx via Na,L may partly be responsible for altered global Na and Ca homeostasis in cardiac hypertrophy (29) and failure (111), although the precise mechanism (longer APD vs. higher intracellular Na concentration) and the quantitative aspects of Na,L contribution are debatable (38, 128). In fact, a tetrodotoxin-sensitive increase in intracellular Na concentration has been reported in HF even in resting cardiac myocytes (where the Na channels are supposedly closed) (31).

Post-translational Modulation of Nav1.5

Regulation by PKA and caveolin-3 complex. Initial reports on β-adrenergic regulation of cardiac Na,L were controversial. Several groups reported a decrease of cardiac Na,L in response to PKA activation (22). However, subsequent studies convinc-
ingly demonstrated that PKA potentiates $I_{\text{Na}}$, possibly involving both a fast saturable and a slow unsaturable component. The fast component involves direct channel phosphorylation events regulating the kinetics and voltage dependence of channel gating. The most consistent fast effects were a negative shift in both steady-state inactivation (SSI) and activation and slowed recovery from inactivation (less available but more active channels) (141). As proposed by Zhou et al. (141), the negative shift in inactivation and use of more depolarized holding potentials (where Na channels may not have been fully available) could explain the discrepancies in early results. Subsequent studies that used more negative holding potentials (to ensure full availability of Na channels) observed potentiation of $I_{\text{Na}}$. These observations may have important physiological consequences for the increase in cardiac conduction velocity observed with sympathetic stimulation (where $I_{\text{Na}}$ is thought to be increased) and the genesis of re-entrant arrhythmias in ischemic myocardium, which is often associated with depolarized diastolic membrane potentials (86).

The slow component of PKA-dependent $I_{\text{Na}}$ potentiation is due to enhanced trafficking and insertion of additional functional channels into the membrane, as revealed in a series of studies with recombinant channels expressed in oocytes by the Murray group (41, 140, 141). Although it was demonstrated that the I–II cytoplasmic linker loop is critical for this effect (141), mutation of five putative PKA sites in this loop (including S483/S571/S593) failed to abolish PKA-dependent potentiation of rat $I_{\text{Na}}$ (107, 140). Murphy and colleagues identified two sites on rat Nav1.5 at positions S526 (S525 in human) and S529 (S528 in human) that are phosphorylated by PKA both in vitro and in vivo (83) (Fig. 1). PKA potentiation of $I_{\text{Na}}$ was subsequently determined to be dependent on PKA phosphorylation of S525 and S528 and the presence of three endoplasmic reticulum (ER) retention signals on the I–II cytoplasmic linker loop (140). A S525A/S528A double phosphomutant or mutation of all three putative ER retention signals (with Arg-X-Arg loop (140). A S525A/S528A double phosphomutant or mutation of five putative PKA sites in this loop (including S483/S571/S593) failed to abolish PKA-dependent potentiation of rat $I_{\text{Na}}$ (107, 140). Murphy and colleagues identified two sites on rat Nav1.5 at positions S526 (S525 in human) and S529 (S528 in human) that are phosphorylated by PKA both in vitro and in vivo (83) (Fig. 1). PKA potentiation of $I_{\text{Na}}$ was subsequently determined to be dependent on PKA phosphorylation of S525 and S528 and the presence of three endoplasmic reticulum (ER) retention signals on the I–II cytoplasmic linker loop (140). A S525A/S528A double phosphomutant or mutation of all three putative ER retention signals (with Arg-X-Arg loop (140.5 at S53–S535 playing the most prominent role) abolished PKA effects on $I_{\text{Na}}$ (140)). This favors a model whereby PKA phosphorylation of S525 and S528 (and possibly other proteins) recruits binding of protein partners that mask the ER retention signals of intracellular channel reserves to facilitate forward trafficking of channels to the membrane.

This concept of intracellular storage pools of Na channels was originally proposed by Catterall and colleagues (106) for neurons (where PKA has opposite effects). It is supported by studies in mammalian cells, including dog cardiomyocytes, which demonstrated that intracellular pools of Nav1.5 exist in the ER and subsarcolemmal space that can be recruited to the membrane in response to PKA activation (41, 142). The intracellular pool hypothesis is further substantiated by the observation that Nav1.5 and β-adrenergic receptors colocalize to caveolin domains that participate in membrane trafficking (139). Indeed, it has been shown that Nav1.5 associates with caveolin 3 (Cav3, Fig. 1) (139), and this interaction facilitates direct (PKA independent) Goα protein stimulation of cardiac $I_{\text{Na}}$ (62, 89). Furthermore, mutations in Cav3 that disrupt its interaction with Nav1.5 are reported to result in increased $I_{\text{Na,L}}$, which is the basis for arrhythmogenic LQT9 (123).

**Regulation by PKC and glyceraldehyde-3-phosphate dehydrogenase 1-like protein complex.** Analogous to neuronal Nav1.2 channels, PKC decreases cardiac $I_{\text{Na}}$ in native and heterologous cell systems (98). This $I_{\text{Na}}$ decrease is dependent on voltage and phosphorylation of S1505 (rodent; S1503 human) in the Nav1.5 III–IV inactivation loop (Fig. 1) (99). In cell attached current recordings of Chinese hamster cells expressing rat Nav1.5, the PKC activator 1-oleoyl-2-acetylglycerol (10 μM) resulted in a voltage-dependent decrease in $I_{\text{Na}}$ and a 15mV negative shift in SSI that were reversed by a PKC inhibitor peptide or mutation of S1505 to a nonphosphorylatable alanine (99). The combined effects of a decrease in maximal $I_{\text{Na}}$ and a negative shift of the SSI curve likely explain the voltage dependence of PKC-induced $I_{\text{Na}}$ reduction, with a greater decrease observed at depolarized potentials.

The importance of PKC in regulating $I_{\text{Na}}$ was further demonstrated through the discovery that inherited mutations in glyceraldehyde-3-phosphate (G3P) dehydrogenase 1-like protein (GPD1L) are linked to the reduction in $I_{\text{Na}}$ amplitude seen in some forms of BrS [A280V (61)] and Sudden Infant Death Syndrome [mutation E83K (121)] [see (132) for review]. Although the function of GPD1L is unknown, the related enzyme GPD1 catalyzes the NAD+–dependent reversible conversion of G3P to dihydroxyacetone phosphate and is an important metabolic link between glycolysis and triglyceride synthesis. The A280V and E83K mutations cause a loss of GPD1L enzymatic activity and decrease $I_{\text{Na}}$ when coexpressed with wild-type (WT) Nav1.5 in HEK293 cells (120). Importantly, both WT and mutant GPD1L-glutathione-S-transferase (GST) fusions pull down Nav1.5, which suggests that the effect of GPD1L mutations on $I_{\text{Na}}$ is through enzymatic function and not binding. The proposed pathway involves PKC, because when WT GPD1L and Nav1.5 are coexpressed in HEK293 cells, 1-oleoyl-2-acylglycerol or G3P (a GPD1 substrate) cause $I_{\text{Na}}$ reduction, which is reversed with the serine/threonine kinase inhibitor staurosporine or mutation of S1503 to nonphosphorylatable alanine. Thus loss of function of GPD1L activity results in accumulation of G3P and phospholipid that activate PKC to phosphorylate S1503 and decrease $I_{\text{Na}}$ (120). Interestingly, NADH mass action block of GPD1L also decreased $I_{\text{Na}}$, while there was no significant change with NAD+.

Studies by Liu et al. (60) further suggested that $I_{\text{Na}}$ might be regulated directly by pyridine nucleotides, such as NADH. They found a twofold increase in intracellular NADH concentration when the A280V GPD1L mutant was adenovirally transfected into HEK293 cells stably expressing Nav1.5. Moreover, there was a dose-dependent decrease in $I_{\text{Na}}$ with intracellular application of NADH delivered by patch pipette. The effect of NADH was rapid (suggesting a post-translational effect), did not appreciably alter $I_{\text{Na}}$ gating or mRNA levels and was reproducible in neonatal myocytes. The NADH-induced $I_{\text{Na}}$ decrease required both PKC and ROS, and ROS was downstream of PKC indicating the channel may be directly oxidized. The reported PKC effects (and the GPD1L-dependent pathway) connect the metabolic state of the cell to $I_{\text{Na}}$ and cardiac excitability and may be crucial in cardiac ischemia and failure, which are characterized by increased metabolic stress (11, 112) and PKC upregulation (130). Of clinical relevance, mouse hearts perfused with external lactate/pyruvate to increase intracellular NADH concentration had enhanced inducible ventricular tachycardia with programmed electrical stimulation. Conversely, increased extracellular NAD+ concentration was antiarrhythmic in SCN5A+/− heterozygous mice.
that have decreased \(I_{\text{Na}}\) and increased propensity to inducible ventricular tachycardia (60).

GPD1L mutations are associated with a PKC-dependent decrease in the number of functional channels at the membrane. Mutation A280V was shown to decrease cell surface expression of Na\(\text{v}1.5\) by immunocytochemistry and confocal microscopy (61). Additionally, flow cytometry measurements of HEK293 cells showed decreased Na\(\text{v}1.5\) cell surface expression when coexpressed with A280V or E83K mutant GPD1L (120). This was reversed by mutation of S1503 on Na\(\text{v}1.5\) to alanine or addition of staurosporine to inhibit PKC.

Analogous PKC-dependent effects were obtained independent of GPD1L mutations. Hallaq et al. (40) monitored the movement of green fluorescent protein (GFP)-tagged Na\(\text{v}1.5\) (COOH-terminus) in HEK293 cells compared with hemagglutinin-tagged Na\(\text{v}1.5\) immobilized at the membrane using confocal microscopy (40). In response to PKC activators, they observed a decrease in Na\(\text{v}1.5\)-GFP at the membrane, which was blocked by PKC inhibition, mutation of S1503 to alanine, or ROS inhibition. Fluorescence recovery after photobleach studies further identified that channels have decreased mobility within the membrane with PKC stimulation and, conversely, an increase with PKA stimulation. Peak \(I_{\text{Na}}\) was decreased at all test potentials after a 30-min exposure to the PKC activator phorbol 12-myristate 13-acetate (40). In contrast to the above experiments with relative long (30 min) drug exposures, Liu et al. (60) did not see a change in Na\(\text{v}1.5\) surface expression, assayed by biotinylation and Western blot of surface Na\(\text{v}1.5\) protein or confocal experiments of Na\(\text{v}1.5\)-GFP at the membrane, upon acute (2–10 min) treatment with pyruvate/lactate (to increase expression of Na\(\text{v}1.5\) by immunocytochemistry and confocal microscopy). Mutation A280V was shown to decrease cell surface decrease in the number of functional channels at the membrane.

ROS can negatively regulate SCN5A gene expression by promoting nuclear localization of the Foxo1 transcription factor and its binding to the SCN5A promoter to suppress transcription (72). This may have implications for the Na\(\text{v}1.5\) mRNA decrease observed in some disease models (see Tables).

Endogenous sources of ROS other than mitochondria also exist in cardiac myocytes, including nitric oxide (NO) produced by NO synthases (NOSs). Interestingly, NO has been associated with an increase in \(I_{\text{Na,LT}}\) that is the proposed arrhythmogenic mechanism behind a missense mutation in syntrophin, A390V, causing LQT12 (116). \(\alpha_1\)-Syntrophin is a dystrophin-associated scaffold protein that contains multiple protein interaction motifs. It associates with the neuronal NOS (nNOS), the plasmalemmal Ca-ATPase (PMCA), and the COOH-terminus of Na\(\text{v}1.5\) through postsynaptic density protein/Drosophila disk large tumor suppressor/zonula occludens-1 protein (PDZ) domain interactions. Communoprecipitations from mouse cardiac homogenates or pull downs with GST tagged Na\(\text{v}1.5\) COOH-terminus as bait suggest nNOS, PMCA, \(\alpha_1\)-syntrophin, and Na\(\text{v}1.5\) interact to form a signaling complex (see Fig. 1). The A390V mutation on syntrophin is in the binding region for PMCA. In HEK293 cells, expression of this mutant syntrophin disrupted pull down of PMCA and a biotin switch assay revealed increased direct \(S\)-nitrosylation of Na\(\text{v}1.5\). Thus it was determined that A390V syntrophin fails to associate with PMCA4b, thus releasing PMCA4b-dependent inhibition of nNOS and promoting \(S\)-nitrosylation of Na\(\text{v}1.5\). NO increases peak and late \(I_{\text{Na}}\), and this was reversed by inhibiting NOS and recapitulated with the NO donors (40, 61). In contrast to the above, Ahmed et al. (4) showed that extracellularly applied NO in native guinea pig and mouse cardiomyocytes decreased \(I_{\text{Na}}\) through combined cGMP (PKG) and cAMP (PKA) pathways (4). However, release of caged intracellular NO in ventricular myocytes increased \(I_{\text{Na,LT}}\), independent of guanylate cyclase in another study (2). Thus direct oxidation or \(S\)-nitrosylation of Na\(\text{v}1.5\) may be partly responsible for the increased \(I_{\text{Na,LT}}\) observed by some groups in diseased human or animal cardiac tissue (see Table 1).

Regulation of CaM. The regulation of Na\(\text{v}\) by Ca, either directly or indirectly through CaM or CaMKII, has been a fervent area of research with groups reporting conflicting results. CaM was the first of these studied. Yeast two-hybrid screens demonstrated that CaM directly interacts with an IQ motif in the COOH-terminus of neuronal Na\(\text{v}1.5\) (81) and cardiac Na\(\text{v}1.5\) (113). Additionally, GST-fusion proteins of the WT COOH-terminal domain of both Na\(\text{v}1.5\) and the skeletal muscle Na\(\text{v}1.4\) pull-down CaM (30), demonstrating CaM binding to these regions, and mutation of the IQ domain prevents CaM pull down (30, 54). As with Ca\(\text{v}1.2\) (95), the Na\(\text{v}1.4\) COOH-terminus binds both Ca/CaM and apoCaM with different affinities (20, 54). In whole cell patch-clamp experiments

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<table>
<thead>
<tr>
<th>Model</th>
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<th>Cell Type</th>
<th>INa, mRNA</th>
<th>INa, Protein</th>
<th>Comments (References)</th>
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<td><strong>HCM</strong></td>
<td>Human</td>
<td>V</td>
<td>↑</td>
<td>↔</td>
<td>Mild to moderate HF, NYHA class II–III (29)</td>
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<tr>
<td>CM/CHD ± HF</td>
<td>Human</td>
<td>V</td>
<td>↔ ↔ ↔ ↔</td>
<td>↔</td>
<td>Window current detected, no Δ in HF (102)</td>
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<tr>
<td>Explanted HF</td>
<td>Human</td>
<td>LV</td>
<td>↑</td>
<td>↔</td>
<td>Myocytes from midmyocardial wall (50)</td>
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<tr>
<td>Explanted HF</td>
<td>Human</td>
<td>LV, RV</td>
<td>↑</td>
<td>↔</td>
<td>No Δ with LVADs; CaMKIIγ and δ ↔ ↔ in HF LVs; CaMKIIβ ↑ twofold in RV; CaMKIIβ ↓ to 40% of controls with LVAD (17)</td>
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<tr>
<td>Explanted end-stage HF</td>
<td>Human</td>
<td>LV, RV</td>
<td>↑</td>
<td>↓</td>
<td>↓ protein expression and current in heterologous system with truncation variants (108)</td>
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<tr>
<td>Explanted HF</td>
<td>Human</td>
<td>V</td>
<td>↑ ↑ ↑ ↑</td>
<td>↓ ICC</td>
<td>↓ Peak reversed by carvedilol; no Δ in β1 mRNA, β1 or β2 protein; ranolazine decreased APD, EADs, APD beat-to-beat variability and dispersion (68, 118)</td>
</tr>
<tr>
<td>Arrhythmogenic cardiomyopathy</td>
<td>Human</td>
<td>LV, RV</td>
<td>↓ ICC</td>
<td>↓ ICC</td>
<td>↓ Peak reversed by carvedilol; no Δ in β1 mRNA, β1 or β2 protein; ranolazine decreased APD, EADs, APD beat-to-beat variability and dispersion (68, 118)</td>
</tr>
<tr>
<td>Explanted/pacing</td>
<td>Human/dog</td>
<td>V</td>
<td>↓ ↑ ↑ ↑</td>
<td>↑ ↓ ICC</td>
<td>No Δ in Na1.1, 1.3, β1- and β2-subunits; ↑ cell capacitance in HF (119)</td>
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<tr>
<td>Explanted/pacing</td>
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<td>V</td>
<td>↑ ↑ ↑ ↑</td>
<td>↓ ↓ ↓ ↓ ↓ ↓ ↓</td>
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<tr>
<td>Multiple coronary microembolizations</td>
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<td>Pacing-induced HF</td>
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<td>↑ ↓ ↓ ↓ ↓ ↓ ↓</td>
<td>↑ Heart weight, longer QRS duration, slower conduction velocity (131)</td>
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<td>↑ ↓ ↓ ↓</td>
<td>↑ ↓ ↓ ↓ ↓ ↓ ↓</td>
<td>↑ Heart weight, longer QRS duration, slower conduction velocity (131)</td>
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<td>EBZ</td>
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<td>↑ ↓ ↓ ↓ ↓ ↓ ↓</td>
<td>↑ Heart weight, longer QRS duration, slower conduction velocity (131)</td>
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<tr>
<td>Pressure/volume overload</td>
<td>Rabbit</td>
<td>V</td>
<td>↔ ↔ ↔ ↔</td>
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<td>↑ Heart weight, longer QRS duration, slower conduction velocity (131)</td>
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<td>Aortic banding</td>
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<td>↑ Heart weight, longer QRS duration, slower conduction velocity (131)</td>
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<td>Post-MI</td>
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<td>Siderotic heart disease</td>
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<td>↑ Heart weight, longer QRS duration, slower conduction velocity (131)</td>
</tr>
<tr>
<td>RV pressure overload</td>
<td>Rat</td>
<td>LV, RV</td>
<td>↑ ↓ ↓ ↓</td>
<td>↑ ↓ ↓ ↓ ↓ ↓ ↓</td>
<td>↑ Heart weight, longer QRS duration, slower conduction velocity (131)</td>
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**Continued**
of TSA201 cells transfected with Na\textsubscript{v}1.5, Tan et al. (113) showed that a peptide antagonist of Ca-dependent CaM binding (peptide 290–309) delivered by patch pipette caused a +6 mV depolarizing shift in inactivation (CaM hyperpolarizes SSI) and decreased intermediate inactivation. Deschenes et al. (30), however, showed that CaM shifted SSI to negative potentials in both a mouse myoblast cell line and HEK293 cells expressing Na\textsubscript{v}1.4, but observed no effect of CaM on Na\textsubscript{v}1.5. Thus the effects of CaM may be Na\textsubscript{v} isoform dependent.

Na\textsubscript{v}1.5 may also be directly regulated by Ca. A pair of EF hand-like domains are present in the Na\textsubscript{v}1.5 COOH-terminus just upstream from the IQ domain (Glu1773-Asp1852) (133). Enhancement of intrinsic tyrosine or tryptophan residue fluorescence in the IQ or EF hand-like domains, respectively, was used as an assay for Ca binding the COOH-terminus and how does this relate to the COOH-terminus/CaM complex in Ca but not in EGTA solutions likely all contribute to these discrepant early studies. In a recent study by the Ahern group (103) captured a crystal structure of the Na\textsubscript{v}1.5 COOH-terminus in complex with the III–IV linker via a Ca/CaM bridge. They further identified two aromatic residues (Tyr1494/95) on the III–IV loop, which are critical for its interaction with the C-lobe of CaM (104). It is interesting to speculate that the Ahern crystal structure may have captured a snapshot of Ca/CaM bound transiently to the III–IV loop before it is displaced to allow fast inactivation to proceed normally through interactions with the COOH-terminus and occlusion of the apo-pore. Displaced Ca/CaM may function to activate CaMKII or remove latent regulation of \(I_{Na}\) inactivation by apoCaM bound to the IQ motif (16). These questions remain unresolved.

What are the potential functional consequences of CaM binding the COOH-terminus and how does this relate to the III–IV loop? Electrophysiological studies of \(I_{Na}\) in Na\textsubscript{v}1.5 COOH-terminal (including the IQ motif) LQTS mutants reveal \(I_{Na,L}\) potentially related to perturbed interactions of the COOH-terminus with the III–IV loop, i.e., fast inactivation gate (37, 54). This might explain the arrhythmogenic phenotype in inherited mutations involving the IQ motif (and possibly also the III–IV loop). As for the potential role of CaM to modulate fast inactivation in physiological conditions, one hypothesis is that high-frequency stimulation that elevates the local [Ca\textsuperscript{2+}] around the channel results in resident apo-CaM binding Ca, CaM lobe switching, and destabilization of inactivation (122) (see Fig. 1). Ca remains bound to CaM to disrupt the interaction of the COOH-terminus with the III–IV loop and impair fast inactivation. Thus the availability curve is shifted to depolarized potentials and more Na channels will be available to drive the next AP upstroke. Although purely speculative, such a mechanism for channel facilitation may be particularly important for repetitive firing during rapid heart rates, e.g., in the response to exercise. It is intriguing to consider the possibility that CaM serves a physiological role in transducing Ca signals to enhance Na channel availability through impairment of fast inactivation, whereas CaMKII plays a pathophysiolog-
ical role in enhancing slow inactivation and stabilizing the inactivated state (as illustrated below).

**Regulation by CaMKII.** A number of studies have aimed at elucidating the functional effects of CaMKII-mediated Nav1.5 phosphorylation (14). In HEK293 cells expressing Nav1.5, inhibition of CaM kinase with 10 μM KN93 (but not its inactive analog KN92) slowed IsNa decay, shifted SSI to depolarized potentials, and slowed entry into inactivated states (30). On the other hand, 100 nM autacamide-2-related inhibitory peptide (AIP) in the pipette had no effect on gating, but intracellular [AIP] might have been limiting (IC50 for CaMKII inhibition ~40 nM). As discussed below, these CaMK effects are consistent with more recent studies analyzing CaMKII effects on IsNa gating.

In a seminal study by Wagner et al. (126), CaMKII was shown to associate with and phosphorylate Nav1.5 in cardiac myocytes. CaMKII phosphorylation, either chronically in transgenic (TG) mice or by acute adenoviral overexpression in rabbit, caused simultaneous gain- and loss-of-function effects on native IsNa. Specifically, in myocytes CaMKII shifted SSI to negative potentials (Fig. 2A), enhanced accumulation of intermediate/slow inactivation (Fig. 2B), slowed recovery from inactivation (Fig. 2C), slowed fast IsNa inactivation, and increased InL (Fig. 2D). Moreover, all of these effects could be acutely reversed with CaMKII inhibition, thus strongly arguing for specific CaMKII-dependent modulation of cardiac Na channels. CaMKII phosphorylating mice also displayed enhanced propensity to tachyarrhythmias. Importantly, these effects phenocopy the inherited A572D mutation on Nav1.5, which causes both LQTS and BrS effects (124). Moreover, Maltev et al. (67) tested the inhibition of CaMKII and found that CaMKII slows the decay of InL more so in failing versus nonfailing canine myocytes.

Aiba et al. (6) subsequently used GST fusion constructs of the intracellular regions of Nav1.5 to narrow down CaMKII-dependent phosphorylation to the I–II loop (6). However, the reported InL functional effects of CaMKII phosphorylation (positive shift in SSI, faster recovery from inactivation, decreased entry into intermediate or slow inactivation, and increased InL) were largely inconsistent with those described by Wagner et al. (126) and other groups (8, 47). These discrepancies may be due to Aiba and colleagues’ use of the CaMKIIα isoform (vs. CaMKIIβC), delivery method (dialysis via patch pipette vs. adenovirus or transgenesis), different Ca buffering, and use of fluoride in pipette solutions.

Through alanine scanning of phosphorylation sites fitting the traditional CaMKII phosphorylation motif, RXXS/T, Hund et al. (47) identified S571 as a CaMKII target site in vitro (47). In HEK293 heterologous cell system expressing Nav1.5, CaMKII shifts the SSI of WT Nav1.5 to negative potentials. This effect on channel inactivation [observed in all studies, except Aiba et al. (6)] was abolished when S571 was mutated to a nonphosphorylatable alanine and mimicked when S571 was mutated to a phosphomimetic glutamate residue. Furthermore, the authors demonstrated a role for β2γ-spectrin in targeting CaMKII to Nav1.5 at the intercalated discs. In a more recent study by the same group (56), it was further demonstrated that S571 is phosphorylated in the border zone of infarcted canine hearts, is slightly increased in human HF, and is phosphorylated in mouse hearts following acute stimulation by isoproterenol (with phosphatase inhibitors), but not in mice overexpressing the CaMKII inhibitor AC3I. Two rare, negatively charged arrhythmogenic point mutations at A572D and Q573E functionally mimic the effect of CaMKII phosphorylation at the adjacent S571 residue, suggesting negative charge within this region may confer similar effects on channel biophysics. Although a direct link between S571 and increased InL has not yet been established, prolonged APs and triggered activity were observed with arrhythmia variants A572D and Q573E, expressed in neonatal myocytes and simulation studies. However, a study by Tester et al. (114) demonstrated that the inherited A572D mutation, although originally identified in LQTS genetic screens, is not functionally different from WT channels and is not proarrhythmic by itself.

Our group was the first to show that autophosphorylated CaMKII binds the I–II loop, and we confirmed this loop as the primary CaMKII phosphorylation target in vitro (8). Using peptide spot arrays of the entire I–II loop, we systematically identified CaMKII phosphorylation sites by P32 incorporation with in vitro kinase assays. We found that S516 and T594 are specifically phosphorylated by CaMKII but found no evidence for phosphorylation at the S571 site identified in the Hund study. In HEK293 cells expressing Nav1.5α, we demonstrated that CaMKII phosphorylates S571 and InL, and more recently that CaMKII augments sodium current, sodium channel activity and Cardiac Death during ischemia-reperfusion or HF. Furthermore, CaMKII phosphorylates numerous Ca handling proteins as well as sarcolemmal K channels (Fig. 4) (13). This in turn can influence myocyte Ca regulation and synergize with InL gating effects in arrhythmogenesis.

**Functional Consequences for Acquired Arrhythmias: CaMKII as a Model**

Because CaMKII can cause both LQTS-like InL and loss-of-function BrS-like effects, we sought to investigate how its effects contribute to arrhythmogenesis (38). Indeed, acquired forms of altered Nav1.5 function attributable to post-translational modifications (e.g., phosphorylation or oxidation) may increase the risk of arrhythmia and sudden cardiac death during ischemia-reperfusion or HF. Furthermore, CaMKII phosphorylates numerous Ca handling proteins as well as sarcolemmal K channels (Fig. 4) (13). This in turn can influence myocyte Ca regulation and synergize with InL gating effects in arrhythmogenesis.

CaMKII-mediated gain-of-function effects, i.e., the late or persistent inward current, may delay repolarization, prolong APD (Fig. 3B), and alter intracellular Na and Ca homeostasis, potentially predisposing to arrhythmogenesis [via early and delayed afterdepolarizations (EADs and DADs, respectively); Figs. 3, A and B, and 4]. Our modeling study (38) predicted that CaMKII-mediated enhancement in InL could cause AP prolongation, especially at slow pacing rates (consistent with a LQT3 phenotype). A mechanistic link between H2O2 and...
(CaMKII mediated)-dependent increase of late $I_{Na}$, AP prolongation, and EADs has been established in experimental (136) and simulation (43) studies. Edwards et al. (33) reported that myocytes from TG mice overexpressing CaMKII$\delta$/H9254C (before pronounced HF remodeling) are susceptible to EADs, which are dependent on sarcoplasmic reticulum (SR) Ca release caused by pronounced isoproterenol-induced increases in Ca transient amplitude and APD. Computational modeling also suggested that the mechanism of AP prolongation and EAD initiation involves recruitment of $I_{Na,L}$ secondary to SR Ca release-dependent augmentation of inward Na/Ca exchange current. Prolonged APs and cellular arrhythmias (both EADs and DADs) have been reported in human cardiomyocytes from patients with hypertrophic cardiomyopathy and attributed to CaMKII-mediated $I_{Na,L}$ increase (29). Disrupting spectrin-mediated molecular anchoring of CaMKII at NaV1.5 has been shown to remove the effects of CaMKII on $I_{Na}$ and reduce EAD propensity (47). By enhancing SR Ca loading and promoting spontaneous intracellular Ca waves, H$_2$O$_2$-induced EADs were also shown to cause DADs (136). Recently, the generation of ROS under conditions of increased oxidative stress, such as during HF, was shown to

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**Fig. 3.** Consequences of CaMKII$\delta$/ hyperactivity on cardiac function. A: delayed afterdepolarizations (DADs). B: early afterdepolarizations (EADs). C: transmural dispersion of repolarization. D: slowing of action potential (AP) rate of rise and decreased AP amplitude. Endo, endocardium; Epi, epicardium; bpm, beats/min.

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**Fig. 4.** Arrhythmogenic mechanisms of CaMKII$\delta$/mediated $I_{Na}$ regulation. Gain (+) and loss (−) of function effects of CaMKII$\delta$/phosphorylation are indicated with upward and downward triangles, respectively. NCX, Na/Ca exchanger; NKA, Na-K-ATPase; NaV1.5, voltage-gated Na channel isoform 1.5; RyR, ryanodine receptor; PLB, phospholamban; SERCA, sarco(endo)plasmic reticulum Ca ATPase; APD, AP duration.
correlate with CaMKII-mediated augmentation of late $I_{Na}$ and consequent arrhythmias (128).

The loss-of-function effects (decreased availability, enhanced intermediate inactivation, and slowed recovery therefrom) may favor a BrS-like phenotype (Fig. 4). Our model (38) predicted that these alterations induced by CaMKII could reduce the velocity of AP upstroke (Fig. 3D) and slow conduction and are likely to be exacerbated at high heart rates (Fig. 3D) whereby the diastolic interval is reduced and Na channels have less time to recover. Increased post-repolarization refractoriness in the infarct border zone [where CaMKII activity is enhanced (25, 46)], attributed to $I_{Na}$ remodeling, has been proposed as a mechanism for slow conduction and arrhythmogenesis (18). Indeed, simulations showed CaMKII hyperactivity in the infarct border zone, due primarily to increased oxidation, causes $I_{Na}$-mediated reduction of AP upstroke (46) and conduction velocity (25), increase in effective refractory period, and increased susceptibility to formation of conduction block at the border zone margin, which predisposes to reentrant arrhythmias (25).

CaMKII also mediates Ca current facilitation, which enhances peak L-type Ca current and slows inactivation (gain of function) and tends to prolong APD (along with $I_{Na,L}$) (38). CaMKII also alters both fast and slow transient outward K currents ($I_{to}$; enhanced recovery from inactivation) and inward rectifier K current (127) in ways that shorten APD (Fig. 4). Simulations predicted that with transmural heterogeneity of $I_{to}$ and $I_{to}$ downregulation in HF, the net effect of CaMKII would be to shorten epicardial APD and prolong endocardial APD, thus accentuating the normal transmural dispersion of repolarization (Fig. 3C) (38), an effect known to be proarrhythmic (7).

As discussed, recent computational myocardial models have incorporated elements of the CaMKII signaling cascade and provided new insights regarding the role of CaMKII in regulating cardiomyocyte contractility and excitability in health and disease. Whereas much progress has been made to determine and model the kinetics of phosphorylation of various CaMKII targets (110), the kinetics of CaMKII-mediated phosphorylation and dephosphorylation of Na channels is unknown. Thus these models are somewhat limited in assuming steady-state fractional phosphorylation of Na channels rather than dynamic transitions between unphosphorylated and phosphorylated Na channels (43). However, the dynamics of CaMKII-dependent Na channel phosphorylation may be critical for its arrhythmogenic consequences and associated potential treatment. Recently, Moreno et al. (80) developed a computational approach to study the interaction of kinetics of the blockers flecainide and lidocaine with cardiac Na channels. They used the model to predict the drug effects on human ventricular cellular and tissue electrical activity and in the setting of one common arrhythmia trigger, spontaneous ventricular ectopy. The model predicted that clinically relevant concentrations of the antiarrhythmic drugs flecainide and lidocaine would exacerbate, rather than ameliorate, arrhythmia. On the other hand, therapies that target late $I_{Na}$, such as block by ranolazine, may have promise in treating patients that are at risk for arrhythmia (65, 125).

Pathological Changes in Na Current

Studies of the $I_{Na}$ changes occurring in cardiac pathologies/disease (summarized in Tables 1 and 2) are less abundant than those characterizing inherited Nav1.5 channelopathies. This is largely due to the difficulty in obtaining human donor transplant tissue suitable for electrophysiology (or biochemistry), as well as the cost and complexity of animal models. Moreover, the results of such studies are often variable and difficult to interpret. For example, human explant studies have wide variability in age, sex, ethnicity, severity of disease, and low sample number and are further complicated by patient history (e.g., pharmacological treatments that can alter $I_{Na}$). Animal models (Table 1) may have important species differences, and TG mice with altered genetics (Table 2) may have compensatory ion channel remodeling effects that complicate the interpretation of $I_{Na}$ functional effects.

Na channel expression and functional alterations in HF are complex and may affect cardiac electrophysiology in various ways. Despite the above limitations, a few common observations in the reported results emerge. First, many studies report a decrease in functional $I_{Na}$ density (9, 10, 57, 68, 97, 118, 119). Second, $I_{Na,L}$ has been reported in both canine (68–70, 118) and human HF (119), as well as in failing mouse cardiomyocytes (126). Both observations could be the result of various complex post-translational modifications of Nav1.5, including phosphorylation by CaMKII. Moreover, both would be expected to contribute to arrhythmias as illustrated in Figs. 3 and 4 for CaMKII-dependent Nav1.5 gain- and loss-of-function effects: the reduced $I_{Na}$ could lead to conduction slowing and ventricular arrhythmias based on reentrant mechanisms, whereas increased $I_{Na,L}$ may delay repolarization, prolong APD, alter intracellular Na and Ca homeostasis, and potentially predispose to arrhythmogenesis (EADs, DADs). As reported for K channels (73), a decrease in functional current density could be the result of either a decrease in peak conductance, an active decrease in channel number at the membrane, or myocyte hypertrophy without compensatory increases in channel expression (85). Most studies do not distinguish between these. Thus the decrease in $I_{Na}$ current densities reported in Tables 1 and 2 must be interpreted cautiously. However, a few studies have reported changes in Nav1.5 protein and/or mRNA levels (Tables 1 and 2) that coexist and are consistent with a decrease in $I_{Na}$ density. More controlled experiments in animal disease models are needed to further refine the molecular mechanisms of $I_{Na}$ changes in cardiac disease and their relative contributions to arrhythmias.

Summary and Concluding Remarks

We reviewed here the molecular and functional aspects of cardiac Na channel modulation and its causal link to cardiac arrhythmias. Interestingly, PKA, PKC, and CaMKII similarly shift $I_{Na}$ availability to negative potentials. Although not yet identified, it is probable that additional putative PKC sites exist on the Nav1.5 I–II loop (as for PKA and CaMKII) that also decrease availability. Thus kinase phosphorylation may act in a concerted manner through the I–II loop to enhance inactivation under various stimuli. A negative shift in $I_{Na}$ availability would be particularly detrimental in ischemic conditions, which are associated with depolarized resting membrane potentials (86).

Chronic phosphorylation by either PKA or PKC alters channel surface levels, but in antagonistic ways. In the case of PKA, the increase in myocardial conduction velocity associated with
Table 2. \( I_{Na} \) changes in TG mice with HF

<table>
<thead>
<tr>
<th>Model</th>
<th>Cell Type</th>
<th>( I_{Na} )</th>
<th>SSI SSA</th>
<th>( I_{Na} )</th>
<th>SCN5A mRNA</th>
<th>Na.1.5 Protein</th>
<th>Comments (References)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaMKII ( \delta )-OE</td>
<td>V</td>
<td>↔ ↔ ↔ ↔</td>
<td>↑</td>
<td>Slower recovery from inactivation and enhanced development of inactivation</td>
<td>↑</td>
<td>Trend to ↓ ( I_{Na} ) density; TG mice exhibit longer QRS intervals and monomorphic and polymorphic VT upon programmed electrical stimulation (126)</td>
<td></td>
</tr>
<tr>
<td>Muscle LIM protein MLP ( ^{-/-} )</td>
<td>V</td>
<td>↓ ↔ ↔</td>
<td>↓</td>
<td>Slowed inactivation</td>
<td>↓</td>
<td>↑ ( I_{Na} ) and EAD propensity, ↓ AP amplitude and rate of depolarization, lower Na.1.5 protein molecular weight (deglycosylation) (117)</td>
<td></td>
</tr>
<tr>
<td>Unilateral nephrectomy/DOCA implantation, salt water substitution</td>
<td>V</td>
<td>↓ ↔ ↔</td>
<td>↔ ↔ →</td>
<td>↓</td>
<td>↓</td>
<td>Reducing mitochondrial ROS by application of NADPH, mitoTEMPO, PKC inhibitors, or PKA activators, restored ( I_{Na} ) (58)</td>
<td></td>
</tr>
<tr>
<td>Inducible CX43-KO</td>
<td>LV, RV</td>
<td>↓ ↔ ↔</td>
<td>→ ↔ →</td>
<td>Slower recovery from inactivation</td>
<td>↔ ICC</td>
<td>Slower conduction and longer QRS in pacing induced VT ( ^{-/-} ) mice (49)</td>
<td></td>
</tr>
<tr>
<td>PKP2 ( \pm ) ARVC</td>
<td>V</td>
<td>↓ ↔ ↔</td>
<td>↑</td>
<td>Slower recovery from inactivation</td>
<td>↔ ↔ ICC</td>
<td>Abnormal ultrastructure; exaggerated ( I_{Na} ) decrease and slowed conduction w/ flecaïnide and SCD (19)</td>
<td></td>
</tr>
<tr>
<td>ACE 8/8-OE</td>
<td>↔ ↔ ↔ ↔</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cardia-specific (( \alpha )-MHC) overexpression; results in fourfold increase in ANG II (48, 52)</td>
<td></td>
</tr>
<tr>
<td>Calcineurin-OE</td>
<td>V</td>
<td>↓ ↓ ↔ ↔</td>
<td>↔ ↔ →</td>
<td>↑</td>
<td>↔ ICC</td>
<td>Hypertrophy, ↓ rate of AP rise, progressive heart block, SCD (39)</td>
<td></td>
</tr>
<tr>
<td>Snail ( \pm ) OE/DCM</td>
<td>V</td>
<td>↓ ↔ ↔</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>DCM, ECG abnormalities, conduction defects; Homoygous mice lethal (44)</td>
<td></td>
</tr>
<tr>
<td>CSQ-OE</td>
<td>V</td>
<td>↓ ↓ ↔ ↔</td>
<td>→ ↔ →</td>
<td>↑</td>
<td>↓</td>
<td>DCM, hypertrophy, ↑ cell capacitance, ↑ PR and QT interval, conduction block (55)</td>
<td></td>
</tr>
<tr>
<td>Mdx (5cv) ( ^{-/-} ) KO</td>
<td>V</td>
<td>↓ ↔ ↔</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>Dystrophin deficient; impaired conduction (35)</td>
<td></td>
</tr>
<tr>
<td>Ank ( ^{-/-} ) KO</td>
<td>V</td>
<td>↓ ↔ ↔</td>
<td>↑</td>
<td>↑</td>
<td>↔ ICC</td>
<td>Prolonged APD; impaired QT-rate adaptation; slowed HR; longer single Na channel open time (23)</td>
<td></td>
</tr>
</tbody>
</table>

ACE, angiotensin-converting enzyme; ANG II, angiotensin II; ARVC, arrhythmogenic RV cardiomyopathy; CSQ, calsequestrin; DCM, dilated cardiomyopathy; KO, knockout; MHC, myosin heavy chain; MLP, muscle LIM protein; OE, overexpression; PKP2, plakophilin-2; TG, transgenic; VT, ventricular tachycardia.

Sympathetic stimulation implicates an increase in channel density at the membrane. In contrast, PKC decreases the number of functional channels at the membrane, and this may be particularly relevant for chronic channel remodeling as in HF. In fact, PKC isoforms are upregulated with increased activity in HF (88, 130). More studies are needed to confirm this regulation in cardiomyocytes and determine if CaMKII similarly alters channel surface expression.

Additionally, PKC regulation appears to be intimately intertwined with metabolism, since mutations in GPD1L that increase both NADH and ROS decrease \( I_{Na} \) in a PKC-dependent manner. It is still unclear which mode of PKC activation (increased G3P and phospholipids or direct activation by NADH) is the most relevant, but there is good evidence that direct phosphorylation of NaV1.5 by PKC at S1503 (and likely other sites) decreases \( I_{Na} \). Likewise, the exact mode of regulation that leads to the decrease in \( I_{Na} \) observed with PKC activation is unclear (ROS vs. direct PKC phosphorylation). Is the effect of ROS direct or indirect via activation of other downstream proteins, such as CaMKII? Indeed, it is conceivable that increased PKC activity fuels an increase in mitochondrial ROS, which constitutively activate CaMKII to regulate \( I_{Na} \). Both CaMKII and PKC appeared to be involved in the Ca-dependent increase in \( I_{Na,L} \) observed in rabbit cardiomyocytes (63).

Interestingly, the PKA phosphorylation sites at S525/S528 and the ER retention motif at 533–535 all neighbor a CaMKII phosphorylation site identified at S516 (8) and a methylated Arg at position 513 (12) (of the same CaMKII motif). The close proximity of these PKA and CaMKII phosphorylation motifs and their similar functional consequences for channel inactivation suggest that this region of the I–II loop is important for channel gating and conformationally/structurally accessible to regulation in the fully folded channel complex. Although the Catterall group (92) recently solved the crystal structure for a bacterial voltage-gated Na channel, this channel lacks the regulatory cytoplasmic loops present on mammalian channels. Regarding CaMKII sites identified on NaV1.5 thus far, a recent phosphoproteome study of unstimulated, native mouse NaV1.5\( _{5\alpha} \) showed basal phosphorylation of S571 and no phosphorylation at T594 or S516 (74). Although the S516 site is conserved in mouse, the critical Arg at P-3 (R513) of the CaMKII motif is not (14). Peptides containing T594 were not recovered by mass spectrometry. Furthermore, PKA and CaMKII regulation of NaV1.5 both appear to require multiple phosphorylation events on the channel, analogous to the graded regulation that has been observed for some K channels (90).

Collectively, these studies highlight the important role post-translational modifications play in both acute \( I_{Na} \) gating changes and chronic regulation of NaV1.5 surface expression. Much work remains to fully unravel the complexities of post-translational regulation of Nav1.5 and its role in cardiac physiology and disease.
GRANTS
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AUTHOR CONTRIBUTIONS
A.W.H. and E.G. conception and design of research; A.W.H. and E.G. interpreted results of experiments; A.W.H. and E.G. prepared figures; A.W.H. and E.G. drafted manuscript; A.W.H., D.M.B., and E.G. approved final version of manuscript; D.M.B. edited and revised manuscript.

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POST-TRANSLATIONAL MODIFICATIONS OF NaV1.5


