Na^v Channel Complex Heterogeneity:
New Targets for the Treatment of Arrhythmia?

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Despite major breakthroughs in cardiovascular diagnostics and therapies over the past century, diseases of the heart remain the number one cause of death in the United States, nearing 600,000 deaths per year. Most of these deaths (200,000-400,000/year) are due to cardiac arrhythmia where syncope and sudden death are often the first manifestations of heart disease. Foundational work of Keating and colleagues in the mid-1990’s cemented the critical role of ion channel dysfunction in human arrhythmia. Today, we now know that ~35% of sudden unexplained death and ~20% of sudden infant death syndrome cases may be explained by mutations in cardiac ion channels (“cardiac channelopathies”). Further, defects in ion channel function have widely been observed in common forms of heart failure. This year marks the 25th anniversary of publication of the preliminary Cardiac Arrhythmia Suppression Trial (CAST) findings in New England Journal of Medicine. Here, we discuss new findings from Abriel and colleagues on Na\textsubscript{v} channel macromolecular complexes reported in this issue of Circulation, and reflect on lessons learned in the ensuing years after CAST that may help propel advances in treatment of cardiovascular disease over the next quarter century.

Defects in voltage-gated sodium (Na\textsubscript{v}) channels are among the best characterized of the cardiac channelopathies. Na\textsubscript{v} channel complexes are comprised of a large ~260 kD pore-forming \( \alpha \)-subunit and an associated auxiliary \( \beta \)-subunit. In humans, Na\textsubscript{v} \( \alpha \)-subunits are encoded by nine genes, while four genes encode Na\textsubscript{v} \( \beta \)-subunits. Beyond heart, Na\textsubscript{v} channel gene defects are linked to a host of excitable cell phenotypes including epilepsy and seizures, myotonia, and erythromelalgia. Although multiple Na\textsubscript{v} channel \( \alpha \)-subunits are expressed in heart, Na\textsubscript{v}1.5 (SCN5A) is the primary \( \alpha \)-subunit responsible for conducting inward sodium current (\( I_{Na} \)) at the outset of the action potential (phase 0). Human SCN5A gene defects leading to alterations in Na\textsubscript{v}1.5-depedent \( I_{Na} \) are now linked with many cardiac arrhythmia phenotypes including sick
sinus syndrome, atrial fibrillation, progressive and non-progressive heart block, type 3 long QT syndrome, and Brugada syndrome.

The Na_\text{v}1.5 channel protein consists of four membrane domains (DI-DIV) with each domain comprised of seven transmembrane spanning helices (S1-S6, Figure 1). Each membrane-embedded helix serves specific roles to regulate Na\textsuperscript{+} flux through the channel. For example, S5/S6 helices form the Na\textsuperscript{+} conductance pore, while the S4 helices serve as a voltage sensor to facilitate channel activation.\textsuperscript{9} Cytoplasmic loops connect DI-DIV, with additional intracellular domains at both the N- and C-termini of the protein (Figure 1). To date, the majority of arrhythmia variants are located in regions of the SCN5A gene that affect channel biophysical properties. However, advances in genetics, small animal physiology, signaling, and molecular biology over the past decade have powered new studies highlighting the role of Na_\text{v}1.5-associated proteins in the regulation of \textit{I}_{\text{Na}}\text{c} as well as dysfunction in heart failure and arrhythmia. In fact, these findings are not limited to Na_\text{v}1.5, but have been illustrated for voltage-gated potassium and calcium channels, as well as membrane transporters and non-voltage-gated channels.\textsuperscript{10}

Targeting Na_\text{v}1.5 to prevent arrhythmias has a troubled history, exemplified by CAST where the Na\textsuperscript{+} channel blocking agents encainide and flecainide increased mortality compared to placebo in patients following myocardial infarction.\textsuperscript{7} Despite the fact that 25 years has passed since this landmark report, the field struggles to move beyond lessons learned about pro-arrhythmic potential of anti-arrhythmia drugs. The study from Abriel and colleagues in this issue of \textit{Circulation}\textsuperscript{8} may suggest a way forward by adding to mounting evidence that multiple Na_\text{v}1.5 populations exist within the cardiomyocyte. These populations differ not only by location (e.g. intercalated disc, transverse-tubule, lateral membrane) but by the nature of their interacting
partners, regulation, and likely drug-sensitivity. In fact, multiple Na\textsubscript{v}1.5 macromolecular complexes form as a result of a large number of interactions between Na\textsubscript{v}1.5 and accessory, adapter, cytoskeletal, and regulatory proteins (Figure 1). Importantly, Na\textsubscript{v}1.5 interacts with different partners depending on its location in the cell. Functionally, this contributes to a heterogeneous population of Na\textsubscript{v}1.5 within the cell. At the intercalated disc where cells are electrically and mechanically coupled, the Na\textsubscript{v}1.5 macromolecular complex includes the adapter protein ankyrin-G, as well as calcium/calmodulin-dependent protein kinase II (CaMKII) via interaction with β\textsubscript{IV}-spectrin. Na\textsubscript{v}1.5 is also found at transverse-tubules together with ankyrin-G. In fact, SCN5A mutations that block ankyrin-G binding alter Na\textsubscript{v}1.5 membrane trafficking and are associated with Brugada syndrome. Other studies have identified possibly a second population of channels at the intercalated disc that interact with the adapter protein synapse-associated protein 97 (SAP97) via a PDZ-domain (named for presence in post-synaptic density protein-PSD95, disc large tumor suppressor-Dlg1, zonula occludens1-ZO1) binding motif in the Na\textsubscript{v}1.5 C-terminus. Potential interaction between Na\textsubscript{v}1.5 and both Connexin43 and plakophilin-2 at the intercalated disc has also been reported. At the lateral membrane, recent work from Abriel and colleagues has identified an important role for the syntrophin/dystrophin complex in targeting Na\textsubscript{v}1.5.

The study from Abriel and colleagues in this issue of Circulation provides important new \textit{in vivo} data on the characteristics of distinct Na\textsubscript{v}1.5 complexes at the intercalated disc and lateral membrane, highlighting the structural and functional differences between at least two of the potential Na\textsubscript{v}1.5 populations. Based on previously observed interaction of Na\textsubscript{v}1.5 with PDZ domain-bearing proteins at both the lateral membrane (syntrophin) and intercalated disc (SAP97), the authors developed a knock-in mouse that expresses Na\textsubscript{v}1.5 lacking the PDZ
domain-binding motif (ΔSIV). The authors report a significant decrease in Na⁺ current in ventricular myocytes from the ΔSIV mice compared to WT mice, coupled with a loss of Na⁺,1.5 at the lateral membrane⁸, consistent with the previous reports.¹⁵ Notably, Na⁺,1.5 at the intercalated disc was unaffected in ΔSIV myocytes - an unexpected finding given prior studies in myocytes with acute knockdown of SAP97 expression showed disrupted Na⁺,1.5 intercalated disc targeting.¹⁵,¹⁷ These new in vivo data strongly support a PDZ-domain-dependent interaction for lateral membrane Na⁺,1.5 targeting. Conversely, these in vivo findings clearly demonstrate that Na⁺,1.5 is targeted to the intercalated disc independent of PDZ-domain protein association.

Finally, the authors report a de novo human arrhythmia mutation in the Na⁺,1.5 PDZ-domain binding motif that negatively affects partner interaction and Na⁺ channel function, suggesting a role for this channel population in human cardiovascular disease.

In light of growing evidence that multiple Na⁺ channel complexes exist in the myocyte, can we exploit the unique characteristics of these distinct populations for therapeutic advantage? Currently, Na⁺-channel blocking drugs that target the late (persistent) phase of Na⁺ current (as opposed to the rapid component) are gaining favor as potential agents to treat cardiovascular disease/arrhythmias.¹⁸ For example, the anti-anginal Na⁺ channel blocker ranolazine with unique kinetics that preferentially target the late Na⁺ current has proven effective in preventing arrhythmias/improving outcomes in a number of animal models and are in limited clinical trials for heart failure.¹⁸ Going forward, can we apply these findings to devise new anti-arrhythmia strategies based on the distinct profile of a specific Na⁺ channel population? In other words, are there unexplored avenues for preventing arrhythmias/disease by targeting specific Na⁺ channel complexes? To answer this question, it is important to consider the cellular factors that regulate the cardiac Na⁺,1.5-late current. Mounting evidence supports a central role for the multifunctional
serine/threonine CaMKII in controlling magnitude of the late current through direct phosphorylation of the Na\(^+\) channel.\(^{19,20}\) CaMKII is preferentially targeted to Nav1.5 at the intercalated disc via direct interaction with the actin-associated cytoskeletal protein \(\beta_{IV}\)-spectrin.\(^{13}\) Furthermore, targeted disruption of spectrin/CaMKII interaction decreases late Na\(^+\) current without affecting the peak.\(^{13}\) Together with the new data from Abriel and colleagues\(^{8}\), and prior functional work from Delmar\(^{11}\), these findings suggest that perhaps by targeting intercalated disc Nav1.5 (e.g. alter spectrin levels/interaction with CaMKII) we may preferentially target the pro-arrhythmic component of the Na\(^+\) current, while protecting/maintaining key populations of Na\(_v\)1.5 required for cardiac conduction.

As the 25th anniversary of the CAST publication comes and goes, it is appropriate to reflect on the importance of this work and the many ways it has impacted basic and translational cardiac arrhythmia research. At the same time, it is important to recognize the sea change that has transpired in our understanding of Na\(_v\) channel biology as well as our ability to manipulate channel function. It is our expectation that major therapeutic advances will be made over the next 25 years by focusing on specific Na\(_v\) channel macromolecular complexes to fine tune Na\(_v\) function.

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**References:**


**Figure Legend:**

**Figure 1.** Voltage-gated Na⁺ channel structure and interaction proteins. Naᵥ protein partners include the adapter protein ankyrin-G, the ubiquitin ligase Nedd4-2 (neural precursor cell expressed developmentally down-regulated protein 4), calmodulin, MOG1 (multicopy suppressor of Gsp1), 14-3-3n, PTP-H1 (protein tyrosine phosphatase H1), FGF (fibroblast growth factor homologous factor 12), syntrophin, and SAP97.⁠¹² The PDZ-domain binding motif (SIV) in the C-terminus controls regulation with PDZ-domain containing proteins (e.g. syntrophin and SAP97).
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