Late $I_{Na}$ in the Heart
Physiology, Pathology, and Pathways

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In this issue of Circulation, Glynn and colleagues\(^1\) make an important contribution to our understanding of the physiological and pathophysiological roles of late sodium current ($I_{Na}$) in the heart, with a focus on a key pathways regulating late $I_{Na}$ amplitude. They conducted well-designed and detailed studies with 2 new genetically engineered mouse lines: an S571A mouse that ablates phosphorylation by Ca\(^2+\)/calmodulin-dependent kinase II (CaMKII)\(^2\) selectively at serine 571 (S571) in the cardiac Na channel pore-forming protein Scn5a and an S571E mouse that mimics phosphorylation at S571. S571 was shown previously to be a target for phosphorylation by CaMKII, and this phosphorylation enhanced late $I_{Na}$.\(^3\)

The present studies in “knock-in” mice expressing either S571A or S571E have distinct advantages over earlier studies in heterologous expression systems, including cultured myocyte models, because they allow the study of whole-animal and organ phenotypes and cellular and molecular biophysical properties in a more native environment. These new in vivo studies reveal that, despite the extensive network of CaMKII targets, phosphorylation of S571 selectively regulates late $I_{Na}$ and, in particular, enhanced late $I_{Na}$ in failing heart.

Peak $I_{Na}$ is the large inward current flowing mainly through the cardiac Na channel pore formed by Scn5a, which is part of a larger sodium channel macromolecular complex. Members of this macromolecular complex act to localize the complex and to regulate $I_{Na}$.\(^3\) With the onset of the action potential (AP) in the myocardium, the peak $I_{Na}$ rapidly rises and decays to nearly zero over several milliseconds. This $I_{Na}$ spike underlies excitability and conduction in working myocardium and the Purkinje conduction system. In contrast to peak $I_{Na}$, late $I_{Na}$ is a small inward current, usually ≤3% of peak $I_{Na}$, that flows throughout the AP plateau. Although the amplitude of late $I_{Na}$ is small, it plays a role in maintaining the AP plateau because competing repolarizing potassium currents are also small. Increased late $I_{Na}$ can directly affect cardiac electrophysiology by prolonging refractoriness and predisposing to triggered activity as early afterdepolarizations, observed clinically as long-QT arrhythmia. Because late $I_{Na}$ flows for much longer time than peak $I_{Na}$ (≈300–400 milliseconds for late $I_{Na}$), it is predicted to play a greater role in Na\(^+\) loading than peak $I_{Na}$.\(^4\) Increased Na\(^+\) loading increases intracellular Ca\(^2+\) levels through effects on Na\(^+\)-Ca\(^2+\) exchange and thereby affects contractility and relaxation.\(^5\) Increased intracellular Ca\(^2+\) levels affect the electrophysiology of the cell via a number of mechanisms, including delayed afterdepolarizations. Late $I_{Na}$ is increased under many conditions, including inherited disorders such as long-QT syndromes (LQT3, LQT9, LQT10, LQT12) and acquired conditions such as hypertrophy, heart failure, ischemia, and diabetes mellitus, in which it plays roles in the pathogenesis of arrhythmia, heart failure, and angina,\(^6\) and has attracted much attention as a therapeutic drug target.\(^7–9\) Therefore, understanding the properties and pathways regulating late $I_{Na}$ has the potential to help us understand the pathogenesis and to provide avenues for treatment of many disease processes in clinical cardiology.

In this commentary, we consider key unanswered and partially answered questions about late $I_{Na}$ and discuss how the genetically engineered mice developed and characterized by Glynn and colleagues\(^1\) have addressed or could be used to address them.

What Are the Signaling Pathways That Regulate Late $I_{Na}$? How Do They Interact?

Two pathways that enhance late $I_{Na}$ act by posttranslational modification of Scn5a involve CaMKII-dependent phosphorylation\(^10\) and neuronal nitric oxide synthase–dependent nitrosylation.\(^11\) A third pathway that may involve direct phosphorylation of Scn5a or other regulatory protein involves phosphoinositide 3-kinase (PI3K), which acts to suppress late $I_{Na}$.\(^12\) The CaMKII pathway is currently the most studied and best defined with the key phosphorylation site affecting late $I_{Na}$ known to be S571. The neuronal nitric oxide synthase pathway appears to involve direct nitrosylation of the Scn5a channel, but the Cys sites have not yet been determined. Whether and how these different pathways interact are unknown. Are they independent and additive? Do they share common features? It is not known whether the PI3K pathway acts directly by phosphorylation of Scn5a\(^12\) or whether it may somehow involve the CaMKII or neuronal nitric oxide synthase or other pathways. Although these questions were not directly addressed in the present study, it is interesting to note that the S571A mouse retains a significant proportion of wild-type late $I_{Na}$ (Figure 2 in Glynn et al\(^1\)), suggesting a component of late $I_{Na}$ that is not regulated by the S571 site. The S571 mouse models should be useful for addressing other questions about pathway interactions. For example, would inhibition of the PI3K pathway result in an increase in late $I_{Na}$ in the S571A model? If not, this...
would support the idea that PI3K activation ultimately acts by suppressing phosphorylation of S571. In addition to the above-named pathways, protein kinase C–dependent phosphorylation at S1503 altered $I_{\text{Na}}$ kinetics in a way that enhances a type of late $I_{\text{Na}}$ called window current, and protein kinase C inhibition blocked increased late $I_{\text{Na}}$ caused by calcium loading the cell, suggesting roles for protein kinase C that may interact with the CaMKII pathway. The S571A and S571E models will be useful tools to further define the relationships and relative importance among these pathways.

**What Signaling Pathways Are Involved in Inherited and Acquired Diseases With Increased Late $I_{\text{Na}}$?**

Enhanced late $I_{\text{Na}}$ occurs in numerous inherited cardiac disorders (mutations in the Scn5a complex for LQT3, LQT9, LQT19, LQT12) and in acquired conditions (hypertrophy, heart failure, ischemia, diabetes mellitus) and can arise as a result of changes in metabolites and other molecules (acidosis, carbon monoxide, reactive oxygen species, and drugs [PI3K inhibitors]). The detailed mechanisms for the causes of late $I_{\text{Na}}$ in disease and the pathways involved have been investigated in only a few of these diseases. Activation of the neuronal nitric oxide synthase pathway to increase late $I_{\text{Na}}$ has been implicated in the pathogenesis of LQT9 involving caveolin3 mutations and LQT12 involving α1-syntrophin mutations. An important finding in the present study is that stress-induced heart failure in the S571A mouse failed to phosphorylate the increased late $I_{\text{Na}}$ seen in wild-type mice, strongly supporting the idea that phosphorylation of S571 via the CaMKII pathway is required for the late $I_{\text{Na}}$ in this model of heart failure. Further experiments in this model could extend these important insights into the role of the CaMKII pathway in causes of late $I_{\text{Na}}$. For example, would late $I_{\text{Na}}$ be enhanced by ischemia, carbon monoxide, reactive oxygen species, or PI3K inhibitors in the S571A mouse? If not, this would provide evidence that they all work through the CaMKII pathway.

**What Are the Biophysical and Structure Function Mechanisms for Late $I_{\text{Na}}$?**

Overall, the detailed biophysical mechanisms for late $I_{\text{Na}}$ at the level of Scn5a are not clear. The prevailing idea is that it involves the inactivation gate on the D3-4 linker or the activation receptor involving the S4-5 linker and residues on S5. However, LQT3 mutations occur over much of the Scn5a topology. A lack of “hotspots” suggests complexity in the structures affecting complete inactivation of $I_{\text{Na}}$. In particular, how does phosphorylation of S571 increase late $I_{\text{Na}}$? Is it linked in some way to inactivation structures? Although the present study does not address these structure-function and biophysical issues directly, the finding that late $I_{\text{Na}}$ can be regulated by phosphorylation at S571 independently of other gating changes is of interest and must be accounted for by any proposed structure-function model. Two LQT3 mutations close to S571 were postulated to cause late $I_{\text{Na}}$ by mimicking the charge near this site and provide additional clues to the structure-function of late $I_{\text{Na}}$. Other nearby CaMKII-dependent phosphorylation sites (S516 and T594) affect the kinetics of gating but do not appear to contribute specifically to late $I_{\text{Na}}$. These key and interesting findings might be investigated by additional electrophysiological studies, including single-channel analysis of the S571 knock-in mouse lines.

Another key observation was the rate-dependent decrease in late $I_{\text{Na}}$ in these mice. This property was seen with the canonical LQT3 mutation delta KPQ and has been postulated to be protective. The late $I_{\text{Na}}$ found in some Scn5a mutations such as those found in sudden infant death syndrome do not have this property and may account for greater lethality.

**Is Physiologically Late $I_{\text{Na}}$ (in Contrast to the Enhanced Late $I_{\text{Na}}$ Found in Inherited and Acquired Disorders) Important for Regulating Excitability and Contractility in the Absence of Disease?**

Most studies of late $I_{\text{Na}}$ have been conducted in models of pathologically enhanced late $I_{\text{Na}}$, but late $I_{\text{Na}}$ is also present in normal hearts (usually 0.2%–0.5% of peak $I_{\text{Na}}$). Is this physiological late $I_{\text{Na}}$ important for normal electrophysiology and regulation of contractility? Might drugs that block too much of the late $I_{\text{Na}}$ lead to undesired effects? The recognition that late $I_{\text{Na}}$ plays a role in normal cardiac physiology goes back ≈50 years, when it was shown that the selective Na⁺ channel blocker tetrodotoxin shortened the AP plateau in normal myocytes. However, in normal rabbit and rat hearts, specific late $I_{\text{Na}}$ blockers had no important effects on contractility and conduction. Small but significant decreases in ejection fraction were observed in the S571A mouse, supporting the idea that CaMKII-dependent late $I_{\text{Na}}$ has importance in regulating contractility in the normal heart. However, “S571A APD [AP duration] was not significantly different than WT [wild-type] at baseline,” suggesting modest if any effects on electrophysiology. It is possible that the complex pathways for regulation of late $I_{\text{Na}}$ evolved to deal exclusively with stress or pathological conditions, but it is more likely that these physiological levels of late $I_{\text{Na}}$ are under tight regulatory control for important reasons related to normal cardiac physiology. More studies are needed on this less well-studied issue.

**Can Pathologically Enhanced Late $I_{\text{Na}}$ Be Better Selectively Targeted on the Basis of Pathway Mechanism?**

The authors of the present study emphasized how CaMKII-dependent S571 phosphorylation specifically regulates late $I_{\text{Na}}$ and may represent an attractive target for blocking pathological late $I_{\text{Na}}$. Currently used drugs such as flecainide, amiodarone, and ranolazine are all pore blockers and would presumably block late $I_{\text{Na}}$ regardless of the mechanism generating late $I_{\text{Na}}$. Their selectivity to block late $I_{\text{Na}}$ over peak $I_{\text{Na}}$ appears to derive from state-dependent block. Even more selective blockers of late $I_{\text{Na}}$ are in development. Regulating the phosphorylation S571 could in theory be a novel and important specific regulator of late $I_{\text{Na}}$, but it is not yet clear if small molecules can be found for this target.

**Caveats and Importance**

As the authors of the present study correctly point out, a mouse model may not be completely translatable to human
physiology. Despite this limitation, this study has generated insights into unanswered questions about the regulatory pathways and characteristics of both pathological and physiological late $I_{\text{Na}}^\ell$, and the models developed in these studies have the potential to generate more insights.

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None.

References

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