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EDITORIAL COMMENT

New Insights Into the Role of GSK-3 in the Regulation of Human Cardiac Electrophysiology*

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Cardiac arrhythmias still represent a serious health issue, leading to adverse outcomes, such as heart failure and sudden death. Several drugs used to treat noncardiac diseases, including antibiotics or antipsychotics, can also be responsible for arrhythmia development. Therefore, a major challenge is a deeper comprehension of the molecular mechanisms regulating cardiac electrophysiology, together with the development of predictive tools aimed at understanding how a specific condition or drug can favor arrhythmic events.

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine kinase regulating several cellular processes and signaling cascades in the heart. GSK-3 plays a pivotal role in heart pathophysiology in different stress conditions, such as pressure overload and ischemic injury, exerting both detrimental and protective effects in a context-dependent manner. GSK-3 represents one of the main components of the Wnt pathway. In the absence of the Wnt ligand, GSK-3 phosphorylates β-catenin, allowing its ubiquitination and degradation. When dephosphorylated, β-catenin accumulates in the cytoplasm and then translocates in the nucleus, where it acts as a transcription factor.[1] β-catenin also participates in the regulation of cell adhesion properties of cardiomyocytes, as it is a component of desmosomes and adherent junctions, which also represent important connections for propagating electrical impulses through the myocardial tissue. Previous work demonstrated that Wnt/β-catenin activation reduces cardiac conduction velocity, owing to the decreased activity of the major cardiac sodium channel subunit $Na_v1.5$ and of the gap junction isoforms connexin-40 and -43.[2] Other reports showed that pharmacological inhibition of GSK-3 with the molecule SB216763 prevents arrhythmias in animal models of arrhythmogenic cardiomyopathy.[3] These data suggest that GSK-3 inhibition may reprogram cardiac electrophysiology by activating the Wnt/β-catenin pathway.

In this issue of *JACC: Basic to Translational Science*, Li et al[4] investigated for the first time the effects of acute GSK-3 inhibition by means of the compound SB216763 (SB2) on cardiac conduction and electrophysiology in human hearts, by using a combined experimental and computational approach. They observed a reduction of impulse conduction velocity in human left ventricular slices obtained from nonfailing donor hearts subjected to acute treatment with SB2 (at 3 and 24 hours), along with a decreased action potential upstroke velocity, indicative of an altered excitability. These effects were associated with the reduction of $Na_v1.5$ protein levels, but with unaltered gene expression, suggesting the involvement of posttranscriptional mechanisms leading to decreased $Na_v1.5$ levels in response to acute GSK-3 inhibition. β-catenin protein levels were also found to be increased, along with its chromatin-bound nuclear accumulation, in response to SB2 treatment. By using a computational platform of virtual cardiac organotypic slices, the authors demonstrated that the decreased conduction velocity and action potential excitability are associated with reduced sodium channel conductance and tissue conductivity, consistently with the experimental data.

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In order to better understand the pathophysiological relevance of GSK-3 inhibition and β-catenin level increase in the actual predisposition to arrhythmia development, Li et al conducted a parallel set of experiments in adult mice with inducible cardiomyocyte-specific β-catenin level augmentation, owing to deletion of β-catenin exon 3, which prevents its degradation. Increased ventricular arrhythmias and reduced conduction velocity were observed in the hearts of these mice, consistently with human data. In addition, the authors found that $Na_v1.5$ protein levels decrease, along with up-regulation of *Axin2* and down-regulation of *Scn5a* ($Na_v1.5$ encoding gene), 2 target genes of Wnt signaling. Of note, the expression of *Axin2* and *Scn5a* genes was not found to be altered in human cardiac slices exposed to acute SB2 treatment, suggesting that the impact of acute GSK-3 inhibition or β-catenin stabilization on cardiac electrophysiology may involve Wnt-independent mechanisms. On the other hand, the transcriptional modulation of Wnt target genes probably occurs only in response to chronic β-catenin activation, as observed in mice. The evidence that *Scn5a* gene expression is down-regulated in mice with β-catenin exon 3 deletion also suggests that $Na_v1.5$ protein reduction may involve transcriptional mechanisms in response to chronic β-catenin accumulation.

Overall, the results obtained by Li et al[4] significantly extend our understanding about the acute effects of GSK-3 inhibition in human cardiac electrophysiology. However, several important aspects remain to be clarified. Previous work demonstrated that chronic SB2 administration reduces arrhythmias and myocardial abnormalities in animal models of arrhythmogenic cardiomyopathy.[3] These divergent results are likely to be attributable to different duration of SB2 treatment and different experimental models. For example, an important difference between ex vivo and in vivo data may reside in the lack of neurohormonal and inflammatory factors in cardiac slices, which are instead present in animal models.

Chronic SB2 treatment may reduce arrhythmia development, by indirectly attenuating myocardial fibrosis, hypertrophy, remodeling and mitochondrial dysfunction, as also observed in previous studies testing the effects of GSK-3 inhibition in cardiomyocytes in response to ischemia-reperfusion, chronic myocardial infarction, and pressure overload.[1] SB2 treatment may also have different effects in cardiac specimens from normal hearts with respect to failing or pathological hearts. To overcome this issue in the future, SB2 treatment should also be tested in cardiac samples derived from patients with inherited and noninherited forms of cardiac diseases associated with arrhythmia development. The effects of SB2 in human cardiac slices should also be tested at longer time points.

Future studies are also needed to better dissect the mechanisms underlying the electrophysiological effects of GSK-3 inhibition. It would be important to check whether the altered electrophysiological properties following acute SB2 treatment in human cardiac slices are abrogated by concomitant inhibition of β-catenin. In this regard, acute GSK-3 inhibition may also modulate other Wnt/β-catenin-independent molecular mechanisms affecting cardiac electrophysiology. GSK-3 is a known inhibitor of the mTORC1 signaling pathway. In addition, the Hippo pathway also concurs to the pathogenesis of arrhythmogenic cardiomyopathy and modulation of Wnt/β-catenin signaling.[5] Transcriptomic and proteomic analyses in human cardiac slices undergoing GSK-3 inhibition should help clarifying this issue.

The molecular mechanisms through which acute GSK-3 inhibition leads to $Na_v1.5$ protein, but not messenger RNA, down-regulation also require additional clarifications. Previous work demonstrated that $Na_v1.5$ levels are affected by autophagy and ubiquitination, 2 processes regulated by GSK-3 and Wnt/β-catenin signaling pathways. In addition, it was recently demonstrated that SB2 treatment increases the localization of $Na_v1.5$ at the intercalated disc by affecting EB1-CLASP2 complex. It will be interesting to check in the future whether $Na_v1.5$ trafficking affects its protein levels, impulse conduction velocity, and tissue excitability.

Finally, GSK-3 exists in 2 isoforms: GSK-3α and GSK-3β. These isoforms may regulate different cellular functions and may exert in some cases opposite effects in response to cardiac stress, such as pressure overload.[1] The specific effects of GSK-3α and GSK-3β on cardiac electrophysiology and arrhythmia development should be clarified in the future.

Although these are open questions, the study by Li et al[4] provides important translational outlooks. Cardiac slices represent a suitable model for the study of cardiac electrophysiology, as they maintain the intercellular connections and myocardial native properties, such as contractility and electrophysiology. This represents an advantage compared with isolated cardiomyocytes or induced pluripotent stem cell-derived cardiomyocytes, which are also used for the study of cardiac electrophysiology. The

integration of experimental data with computational simulations of virtual cardiac organotypic slices may also be adopted as a predictive tool for the screening of drugs that may affect cardiac electrophysiology, especially in the field or cardio-oncology, thereby helping the development of personalized therapies. Conversely, a limit of these approaches relies on the fact that they do not take into account pharmacokinetics and bioavailability of a specific drug.

In conclusion, the study by Li et al[4] demonstrates that acute GSK-3 inhibition may lead to a proarrhythmic phenotype, raising an alert with the clinical use of GSK-3 inhibitors, such as lithium, which is routinely used in psychiatric disorders.

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