Calcium/calmodulin-dependent protein kinase II (CaMKII) inhibition ameliorates arrhythmias elicited by junctin ablation under stress conditions

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BACKGROUND Aberrant calcium signaling is considered one of the key mechanisms contributing to arrhythmias, especially in the context of heart failure. In human heart failure, there is significant down-regulation of the sarcoplasmic reticulum (SR) protein junctin, and junctin deficiency in mice is associated with stress-induced arrhythmias.

OBJECTIVE The purpose of this study was to determine whether the increased SR Ca^{2+} leak and arrhythmias associated with junctin ablation may be associated with increased calcium/calmodulin-dependent protein kinase II (CaMKII) activity and phosphorylation of the cardiac ryanodine receptor (RyR2) and whether pharmacologic inhibition of CaMKII activity may prevent these arrhythmias.

METHODS Using a combination of biochemical, cellular, and *in vivo* approaches, we tested the ability of KN-93 to reverse aberrant CaMKII phosphorylation of RyR2. Specifically, we performed protein phosphorylation analysis, *in vitro* cardiomyocyte contractility and Ca^{2+} kinetics, and *in vivo* ECG analysis in junctin-deficient mice.

RESULTS In the absence of junctin, RyR2 channels displayed CaMKII-dependent hyperphosphorylation. Notably, CaMKII inhibition by KN-93 reduced the *in vivo* incidence of stress-induced ventricular tachycardia by 65% in junctin null mice. At the cardiomyocyte level, KN-93 reduced the percentage of junctin null cells exhibiting spontaneous Ca^{2+} aftertransients and aftercontractions under stress conditions by 35% and 37%, respectively. At the molecular level, KN-93 blunted the CaMKII-mediated hyperphosphorylation of RyR2 and phospholamban under stress conditions.

CONCLUSION Our data suggest that CaMKII inhibition is effective in preventing arrhythmogenesis in the setting of junctin ablation through modulation of both SR Ca^{2+} release and uptake. Thus, it merits further investigation as promising molecular therapy.

KEYWORDS Arrhythmia; Junctin; Calcium/calmodulin-dependent protein kinase II; Isoproterenol; Ryanodine receptor

ABBREVIATIONS **BDVT** = bidirectional ventricular tachycardia; **CaMKII** = calcium/calmodulin-dependent protein kinase II; **CPVT** = catecholaminergic polymorphic ventricular tachycardia; **CSQ** = calsequestrin; **DAD** = delayed afterdepolarization; **ECG** = electrocardiography; **JKO** = junctin knockout; **PKA** = protein kinase A; **PLN** = phospholamban; **PVC** = premature ventricular contraction; **RyR** = ryanodine receptor; **SCD** = sudden cardiac death; **SERCA** = sarco-endoplasmic reticulum calcium ATPase; **SOICR** = store overload–induced Ca^{2+} release; **SR** = sarcoplasmic reticulum; **SVT** = sustained ventricular tachycardia; **VT** = ventricular tachycardia; **WT** = wild-type

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Introduction

Cardiac arrhythmia symptoms vary from mild disturbances in the cardiac rhythm to severe life-threatening complications, such as sudden cardiac death (SCD). SCD is the cause of more than 60% of all deaths from cardiovascular disease, which is the leading cause of death worldwide.[1] Ventricular fibrillation is the mechanism underlying most sudden cardiac arrest episodes, but other forms of arrhythmias such as tachycardia, bradycardia, or pulseless electric activity can

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trigger malignant forms of arrhythmia. These, in turn, may result in immediate cessation of cardiac mechanical activity and asystole, providing a direct link between arrhythmia trigger mechanisms and SCD.[1] Although arrhythmias and SCD are more frequent among patients with organic heart diseases, such as ischemia and dilated or hypertrophic cardiomyopathy, they also occur in individuals without any detectable underlying cardiac pathology, such as patients with catecholaminergic polymorphic ventricular tachycardia (CPVT) or other genetic arrhythmia syndromes.[2] Despite the complex nature of arrhythmia-triggering mechanisms, it is widely accepted that perturbation in cardiac Ca^{2+} homeostasis contributes to arrhythmogenesis.[3] Consequently, correcting impaired Ca^{2+} cycling represents a highly promising, yet poorly explored, therapeutic target.[4]

Among the key players in cardiac Ca^{2+} cycling is the ryanodine receptor (RyR2), the major Ca^{2+}-release channel located in the sarcoplasmic reticulum (SR) membrane. Diastolic Ca^{2+} leak through RyR2 is one of the central pathophysiologic problems in failing hearts. Spontaneous Ca^{2+} release events via defective RyR2 during diastole may trigger delayed afterdepolarizations (DADs), a common precursor mechanism of lethal arrhythmias.[5] Although the underlying mechanisms for this leak have not been completely elucidated, human mutations in RyR2, calsequestrin 2 (CSQ2), and triadin, which alter RyR2 gating and Ca^{2+} release, have been associated with lethal cardiac arrhythmias, such as CPVT.[6–8] Furthermore, the phosphorylation status of RyR2 as well as interactions with other proteins in the macromolecular channel complex can directly affect opening probability and, if dysregulated in heart failure, contribute to Ca^{2+} leak.[9,10]

Junctin is an essential member of the SR Ca^{2+} release complex. It interacts with RyR2, similarly to triadin, and mediates the anchoring of CSQ2, the major Ca^{2+} binding protein of the SR, to RyR2.[11] Genetic ablation of junctin has revealed that it is essential for maintenance of normal RyR2 activity and Ca^{2+} release. Specifically, the junctin knockout (JKO) mouse presents with ventricular arrhythmias under stress conditions and an overall phenotype reminiscent of classic CPVT symptoms, including aftercontractions, DADs, and an increased propensity for spontaneous SR Ca^{2+} release.[12,13] Consistent with human CPVT pathophysiology, approximately 25% of junctin null mice die suddenly by 3 months of age with structurally normal hearts, suggesting acutely occurring fatal arrhythmias.[12] The trigger mechanism for arrhythmias in the JKO model is speculated to be the aberrant activity of RyR2, which leads to diastolic Ca^{2+} leak from the SR, exacerbated by beta-adrenergic activation. Importantly, in patients with heart failure, junctin protein expression is highly downregulated to the extreme of undetectable protein levels.[14] These patients often develop cardiac mechanoelectrical instability and fatal arrhythmias, especially during increased beta-adrenergic stress.[15] This highlights the critical role of the RyR2 macromolecular complex and its interactions with junctin in normal cardiac function and its potential as a therapeutic target in the context of arrhythmias.

In terms of therapeutic strategies, calcium/calmodulin-dependent protein kinase II (CaMKII) emerges as a molecule of particular interest because it has been associated with the hyperphosphorylated RyR2 complex and increased diastolic Ca^{2+} leak.[16] A novel finding of this study is that the JKO model presents with CaMKII-mediated RyR2 hyperphosphorylation, which can be linked to arrhythmogenesis due to excessive SR Ca^{2+} leak. It is postulated that junctin ablation removes a protective "break" on RyR2 opening and increases SR Ca^{2+} leak, which further activates CaMKII and exacerbates diastolic Ca^{2+} leak, leading to arrhythmias. Thus, our hypothesis was that CaMKII inhibition could be a promising approach for the treatment of arrhythmias, in the JKO model, especially under stress conditions. Further support of this idea comes from evidence that CaMKII suppression inhibits the onset of DADs and prevents fatal arrhythmias in animal models of heart failure[17] and CPVT.[18] Therefore, we proceeded to assess the effects of CaMKII inhibition on life-threatening arrhythmias in the JKO mouse model of stress-induced arrhythmias. We demonstrate that CaMKII inhibition is an effective means of preventing malignant arrhythmogenesis in these mice, both *in vivo* and *in vitro*. These findings suggest the potential benefits of targeted therapy to prevent arrhythmias in the setting of junctin deficiency and may have valuable therapeutic implications.

Methods

Expanded methods can be found in the Online Supplemental Material.

Animals

The generation of the JKO mice was previously described.[12]

Cardiomyocyte mechanics, Ca^{2+} kinetics, and SR Ca^{2+} content

Cardiomyocyte mechanics/aftercontractions, Ca^{2+} transients/aftertransients, and SR Ca^{2+} load were measured as previously described.[12]

In vivo electrocardiography

Three-lead electrocardiography (ECG) electrodes were placed subcutaneously on anesthetized mice, and ECG recordings were obtained under stress conditions as previously described.[19]

Western blot analysis

Wild-type (WT) and JKO cardiac homogenates were subjected to western blot analysis using the antibodies and conditions described in the Online Supplementary Methods.

Statistical analysis

All data are expressed as mean ± SE. The Student *t* test and the χ^2 test were used for statistical analysis. $P < .05$ was considered significant.