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CaMKII Regulation of the Cardiac Ryanodine Receptor and SR Calcium Release

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Abstract

Spontaneous release of Ca^{2+} from the sarcoplasmic reticulum has emerged as a mechanism underlying triggered activity and cardiac arrhythmias. Recent studies suggest an important role for increased Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII)-mediated phosphorylation of ryanodine receptors (RyR2) in the induction of arrhythmias. This article briefly reviews the mechanisms underlying CaMKII regulation of RyR2 and discusses directions of current and future research.

Keywords

arrhythmias; calmodulin kinase II; heart failure; phosphorylation; ryanodine receptors

In recent years, Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) has emerged as an important regulator of excitation-contraction coupling, the cellular process by which an action potential triggers contraction of cardiac myocytes. It has become clear that CaMKII-mediated phosphorylation of Ca^{2+} cycling proteins plays an important role in intrinsic regulatory pathways in the heart, such as the force-frequency response¹. Moreover, emerging data suggest that chronic activation of CaMKII in diseased hearts leads to abnormal intracellular Ca^{2+} handling, which could promote cardiac arrhythmias^{2, 3}. This viewpoint article in the mini series on 'CaMKII in the heart' will focus on the effects of CaMKII on intracellular Ca^{2+} release channels known as ryanodine receptors type 2 (RyR2) in relationship to cardiac arrhythmia mechanisms.

Contraction of cardiac myocytes is preceded by the influx of Ca^{2+} into the cytosol via voltage-gated L-type Ca^{2+} channels, which triggers a larger release of Ca^{2+} from the sarcoplasmic reticulum (SR) through RyR2⁴. The ensuing Ca^{2+} transient leads to myocyte contraction, which quickly ends when Ca^{2+} is removed from the cytoplasm due to SR Ca^{2+} reuptake through the SR Ca^{2+} -ATPase (SERCA2a) and is extruded via the $\text{Na}^{+}/\text{Ca}^{2+}$ -exchanger (NCX). In healthy hearts, RyR2 remain closed during diastole to facilitate SR refilling before the subsequent systolic Ca^{2+} release. In contrast, heart failure is associated with spontaneous openings of RyR2 during diastole, which can interfere with SR Ca^{2+} loading, decreased systolic Ca^{2+} release and cardiac contractility⁴. Moreover, it has been

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shown that diastolic Ca^{2+} releases from the SR can initiate arrhythmogenic afterdepolarizations, as will be discussed below.

The RyR2 channel comprises a macromolecular complex consisting of 4 RyR2 monomers each binding several accessory proteins (e.g., FKBP12.6, calmodulin, calsequestrin) that regulate channel activity⁵. Moreover, RyR2 open probability is also modulated by posttranslational modifications such as phosphorylation, nitrosylation, and oxidation. It has been demonstrated that phosphorylation of RyR2 increases the channel open probability, and thus SR Ca^{2+} release during both systole and diastole, although it remains somewhat controversial whether RyR2 phosphorylation alters SR Ca^{2+} release in a cellular environment^{1, 6}. The first phosphorylation site identified on RyR2 was serine 2808 (S2808, or S2809 in some species), which is believed to be the primary target of protein kinase A⁶. Subsequently, it was shown that another serine (S2814) is the main substrate of CaMKII phosphorylation on RyR2¹. CaMKII phosphorylation of RyR2 increases the sensitivity to Ca^{2+} -dependent activation and frequency of Ca^{2+} sparks⁷. Since CaMKII can decode the frequency of Ca^{2+} transients, it is believed that CaMKII phosphorylation of RyR2 plays a role in enhancing SR Ca^{2+} release in response to sudden increases in heart rate or adrenergic stress in normal hearts^{1, 8, 9}. However, chronically increased CaMKII activity in diseased hearts can become maladaptive and lead to abnormal diastolic Ca^{2+} release from the SR, associated with triggered arrhythmias¹⁰.

Over the past decade, extensive evidence has emerged linking diastolic RyR2 Ca^{2+} leakage to arrhythmogenesis in patients with atrial and ventricular arrhythmias^{11, 12}. First, inherited mutations in RyR2 were identified in patients with a rare condition known as catecholaminergic polymorphic ventricular tachycardia (CPVT), providing direct evidence that defects in RyR2 can cause life-threatening arrhythmias and sudden cardiac death¹³. CPVT-linked mutations in RyR2 lead to an increased propensity toward spontaneous Ca^{2+} release from the SR, which under certain conditions (such as during exercise or beta-adrenergic stimulation) facilitates the development of delayed afterdepolarizations (DADs) and triggered arrhythmias¹⁴. Clinical observations suggest that CaMKII activation might increase the risk of arrhythmias in patients with CPVT, as arrhythmias typically only occur above specific threshold heart rates (and CaMKII activity is rate-dependent)¹⁵. However, it remains to be determined whether CaMKII plays a role in triggering ventricular arrhythmias in these patients, a hypothesis that could be studied in mouse models of CPVT^{16, 17}. Drs. Priori and Napolitano will discuss these concepts in more detail in their future viewpoint article.

Some evidence suggests that increased CaMKII activity in heart failure could promote diastolic SR Ca^{2+} release and ventricular tachyarrhythmias. In an arrhythmogenic rabbit model of nonischemic heart failure, Ai *et al.*¹⁸ demonstrated that enhanced diastolic SR Ca^{2+} leak could be blocked by CaMKII inhibition. Because CaMKII phosphorylation of RyR2 was elevated in this model, it was proposed that CaMKII-dependent phosphorylation of RyR2 was involved in enhanced SR diastolic Ca leak and thus arrhythmogenesis in HF¹⁸. Recent studies provide further evidence for the involvement of CaMKII phosphorylation of RyR2 in arrhythmogenesis in heart failure, since pharmacological inhibition of CaMKII completely abolished spontaneous Ca^{2+} waves, which are known to activate inward NCX current and DADs¹⁹. Studies in CaMKII- δ c transgenic mice also revealed that increased cytosolic CaMKII activation contributes to cardiac arrhythmogenesis²⁰. Thus, it is likely that increased CaMKII phosphorylation of RyR2 contributes to arrhythmogenesis in failing hearts. However, it remains to be determined whether CaMKII phosphorylation of RyR2 by itself is sufficient and/or necessary to promote arrhythmias in the failing heart.

Defects in RyR2 regulation may also contribute to triggered activity and arrhythmogenesis in patients with atrial arrhythmias¹¹. Several studies have provided evidence for defects in SR Ca²⁺ release in patients with chronic atrial fibrillation (AF)^{21, 22}. Because the amplitude of the L-type Ca²⁺ channel is decreased and SR Ca²⁺ loading is typically unaltered, increased open probability of RyR2 emerged as the potential culprit for Ca²⁺-dependent arrhythmias in patients with AF²¹⁻²³. Indeed, planar lipid bilayer studies provided direct evidence for enhanced RyR2 open probability in dogs with chronic AF¹¹. Whether increased Ca²⁺ sensitivity of RyR2 leads to elevated cytosolic Ca²⁺ levels in atrial myocytes of patients with chronic AF is still controversial. Neef *et al.*²² recently reported that increased RyR2-dependent SR Ca²⁺ leak caused elevated cytosolic Ca²⁺ levels in myocytes from patients with chronic AF. On the other hand, Voigt *et al.*²⁴ demonstrated unaltered cytosolic Ca²⁺ levels despite enhanced RyR2 Ca²⁺ leak in atrial myocytes isolated from patients with chronic AF.

Several alterations within the RyR2 macromolecular complex are thought to underlie the increased open probability of RyR2 in AF. Biochemical studies revealed decreased levels of FK506-binding protein 12.6 (FKBP12.6, or calstabin2), and increased phosphorylation levels of the PKA (S2808) and CaMKII (S2814) sites on RyR2 in dogs and patients with chronic AF^{3, 11}. However, one potential problem with studies of human tissue samples obtained from patients undergoing coronary artery bypass or valve replacement surgery is the co-existent structural heart disease. It will be important but at the same time challenging to explore alternative means of obtaining human atrial tissue from patients with paroxysmal or chronic AF in the absence of ischemic or structural heart disease.

Whereas it is likely that aforementioned modifications of RyR2 indeed contribute to an increased open probability and atrial arrhythmogenesis in patients, studies in genetically-modified mice provided additional insights into potential causal relationships between individual molecular changes associated with AF and an increased arrhythmia risk. Knock-in mice with a gain-of-function mutation in RyR2 (R176Q) were found to be more susceptible to atrial arrhythmias following rapid atrial pacing³. On the other hand, genetic or pharmacological inhibition of CaMKII prevented inducibility of AF in R176Q/+ mice. The specific contribution of CaMKII phosphorylation of RyR2 was studied in RyR2-S2814A knock-in mice, in which the CaMKII site S2814 was genetically inactivated due to alanine substitution. We demonstrated that inhibition of S2814 phosphorylation was sufficient to prevent the induction of pacing-induced AF following carbachol injection in S2814A mice³. Taken together, our studies revealed that increased CaMKII activity is likely to be a pro-arrhythmogenic factor in AF, and that CaMKII phosphorylation of RyR2 may be an important but probably not exclusive downstream target of CaMKII in AF.

Some have argued that increased RyR2 open probability could not be pro-arrhythmogenic because enhanced diastolic SR Ca²⁺ release would deplete SR Ca²⁺ content and prevent subsequent spontaneous SR Ca²⁺ releases²⁵. This is probably true if RyR2 'leak' occurs as an isolated event. However, AF is characterized by extensive remodeling of various ion channels and Ca²⁺ handling proteins²⁶. For example, activated CaMKII also increases the level of PLN phosphorylation at Thr17, which promotes SR Ca²⁺ refilling and could support chronic diastolic SR Ca²⁺ leak, which may cause afterdepolarizations and triggered activity²⁷.

Finally, it has been shown that diastolic SR Ca²⁺ release may activate a transient inward current (I_{TI}) that can trigger delayed afterdepolarizations (DADs) in mouse ventricular myocytes¹⁴. Pharmacological inhibition of RyR2 with JTV519 inhibited I_{TI} and DADs in this particular study, suggesting that excessive Ca²⁺ release via RyR2 can initiate arrhythmogenic DADs. In AF, increased activity of the NCX could further increase the

likelihood that spontaneous SR Ca^{2+} release events initiate pro-arrhythmic events²⁸. Thus, several modifications of Ca^{2+} handling proteins might synergistically promote the induction of AF, including increased CaMKII phosphorylation of RyR2, increased SERCA2a activity due to increased PLN phosphorylation by CaMKII, and enhanced NCX activity. It remains to be determined if CaMKII plays a role in stimulating NCX in patients with AF.

In conclusion, there is emerging evidence that increased CaMKII activity and in particular CaMKII phosphorylation of RyR2 may promote triggered activity and arrhythmia induction in patients with heart failure and atrial fibrillation. Genetically modified mice have emerged as important models to probe the specific contributions of particular CaMKII isoforms or CaMKII phosphorylation sites on downstream targets, including the RyR2 Ca^{2+} release channel. Although increased CaMKII phosphorylation of RyR2 increases diastolic SR Ca^{2+} leak, it is likely that alterations in other Ca^{2+} handling proteins in conjunction with RyR2 modifications are necessary to enable the development of delayed afterdepolarizations that could trigger arrhythmias. These observations suggest that pharmacological targeting of RyR2 and/or CaMKII signaling in the heart may constitute a promising avenue for the treatment of cardiac arrhythmias.

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