Sarcoplasmic reticulum calcium leak and cardiac arrhythmias

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Abstract

Ventricular arrhythmias deteriorating into sudden cardiac death are a major cause of mortality worldwide. The recent linkage of a genetic form of cardiac arrhythmia to mutations in the gene encoding RyR2 (ryanodine receptor 2) has uncovered an important role of this SR (sarcoplasmic reticulum) calcium release channel in triggering arrhythmias. Mutant RyR2 channels give rise to spontaneous release of calcium (Ca^{2+}) from the SR during diastole, which enhances the probability of ventricular arrhythmias. Several molecular mechanisms have been proposed to explain the gain-of-function phenotype observed in mutant RyR2 channels. Despite considerable differences between the models discussed in the present review, each predicts spontaneous diastolic Ca^{2+} leak from the SR due to incomplete closure of the RyR2 channel. Enhanced SR Ca^{2+} leak is also observed in common structural diseases of the heart, such as heart failure. In heart failure, defective channel regulation in the absence of inherited mutations may also increase SR Ca^{2+} leak and initiate cardiac arrhythmias. Therefore inhibition of diastolic Ca^{2+} leak through SR Ca^{2+} release channels has emerged as a new and promising therapeutic target for cardiac arrhythmias.

Introduction

Ventricular arrhythmias deteriorating into sudden cardiac death are a major cause of mortality in the Western world. Recently, some types of ventricular arrhythmias have been associated selectively with mutations in the gene coding for the RyR2 (ryanodine receptor 2), the principal calcium (Ca^{2+}) release channel on the SR (sarcoplasmic reticulum) in cardiac myocytes [1]. It has been demonstrated that gain-of-function mutations in RyR2 interfere with the channel's ability to remain closed during diastole, when Ca^{2+} is normally removed from the cytosol [2,3]. The ensuing spontaneous release of Ca^{2+} from the SR Ca^{2+} stores has emerged as the initiator of triggered cardiac arrhythmias in patients with inherited RyR2 mutations [2]. Moreover, defective regulation of RyR2 in cardiomyopathy and CHF (congestive heart failure) also increases the amplitude of SR Ca^{2+} leak, which may contribute to increased arrhythmogenesis observed in structural heart diseases. In the present review, we will discuss recent studies on the molecular and cellular mechanisms underlying abnormal intracellular Ca^{2+} release, spontaneous impulse generation, and triggered ventricular arrhythmias.

Key words: calcium leak, cardiac arrhythmia, catecholaminergic polymorphic ventricular tachycardia, diastole, ryanodine receptor 2 (RyR2), sarcoplasmic reticulum.

Abbreviations used: CaM, calmodulin; CaMKII, Ca^{2+}/CaM-dependent protein kinase II; CASQ2, calsequestrin 2; CHF, congestive heart failure; CICR, Ca^{2+}-induced Ca^{2+} release; CPVT, catecholaminergic polymorphic ventricular tachycardia; DAD, delayed afterdepolarization; EC coupling, excitation–contraction coupling; FKBP12.6, 12.6 kDa FK506-binding protein; HEK-293 cells, human embryonic kidney cells; NCX, Na^+/Ca^{2+}-exchanger; PDE4D3, phosphodiesterase 4D3; PKA, protein kinase A; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; RyR2, ryanodine receptor; SOICR, store overload-induced Ca^{2+} release; SR, sarcoplasmic reticulum; VT, ventricular tachycardia.

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EC (excitation–contraction) coupling in cardiac myocytes

During an action potential, Ca^{2+} enters the myocyte primarily through voltage-activated L-type Ca^{2+} channels, which induces a much larger release of Ca^{2+} from the SR via RyR2 during a process called Ca^{2+}-induced Ca^{2+} release (referred to as CICR) [4]. The increase in cytosolic Ca^{2+} concentrations activates the myofilaments, leading to myocyte contraction. Diastolic relaxation occurs after Ca^{2+} is removed from the cytosol by SERCA2a (sarcoplasmic/endoplasmic-reticulum Ca^{2+}-ATPase) and the NCX (Na^+/Ca^{2+}-exchanger). This process of EC coupling is highly co-ordinated, and determines the force of contraction by circulating Ca^{2+} from the SR to the myofilaments and back into the SR. On the other hand, irregular or non-uniform Ca^{2+} releases may cause contractile defects, and Ca^{2+} transients that could initiate arrhythmias.

Genetic mutations in the *RyR2* gene associated with cardiac arrhythmias

Genetic analysis has shown that autosomal-dominant missense mutations in the *RyR2* gene cause a malignant clinical entity known as catecholaminergic polymorphic ventricular tachycardia (referred to as CPVT) [5]. CPVT is characterized by stress-induced syncope and sudden cardiac death and occurs in the absence of structural heart disease. An estimated 30–50% of CPVT patients die before they reach the fourth decade of life [6], and the lethal episodes of arrhythmias are typically triggered by physical or emotional stress. Two patterns of VT (ventricular tachycardia) are observed in patients with CPVT: bidirectional VT and polymorphic VT [6]. Interestingly, this unique pattern of

arrhythmias is also found in patients with intracellular Ca^{2+} overload as a result of digitalis intoxication [7].

Additional evidence for a link between RyR2 dysfunction and cardiac arrhythmias has been obtained in knockin mice carrying CPVT-associated mutations in *RyR2*. Two human mutations, R176Q in the N-terminal and R4496C in the C-terminal domains of the channel respectively, have been engineered into the mouse *RyR2* gene. Mice heterozygous (+/–) for either of these *RyR2* mutations develop phenotypic manifestations similar to those observed in patients with CPVT, in the absence of structural heart disease [8,9]. In agreement with diagnostic tests in patients, exercise-stress testing and catecholamine administration triggered bidirectional VT in both $R176Q^{+/-}$ and $R4496C^{+/-}$ mice [9,10]. Taken together, these studies provide a causal link between RyR2 defects and cardiac arrhythmias.

Evidence of SR Ca^{2+} leak as the trigger for ventricular arrhythmias

Knockin mouse models of CPVT have enabled studies of mutant RyR2 channels in isolated cardiac myocytes. Cardiomyocytes isolated from $R176Q^{+/-}$ mice exhibit a higher incidence of spontaneous SR Ca^{2+} releases compared with control mice [9]. Additional evidence for spontaneous Ca^{2+} oscillations was obtained from the $R4496C^{+/-}$ mice [10]. Action potential recordings in $R4496C^{+/-}$ myocytes revealed evidence for (diastolic) DADs (delayed afterdepolarizations), in particular at faster pacing frequencies or after administration of isoprenaline [10]. DADs are oscillations of the membrane potential that occur after the repolarization phase of an action potential, and are initiated by aberrant Ca^{2+} release from the SR. The diastolic Ca^{2+} release activates the NCX, resulting in a transient inward current (I_{TI}) that may depolarize the cell membrane, causing the afterdepolarizations [11,12]. If the depolarization threshold is reached, the I_{TI} may trigger an action potential, which could be propagated into a ventricular arrhythmia [13]. In agreement with the above, DADs have been demonstrated in patients with CPVT [14]. Nakajima et al. [14] were the first to show DADs in an adolescent Japanese diagnosed clinically with CPVT. Subsequent studies in 15 patients with confirmed mutations in RyR2 (P2328S, Q4201R and V4653F) have shown the presence of DADs in four patients with CPVT [15].

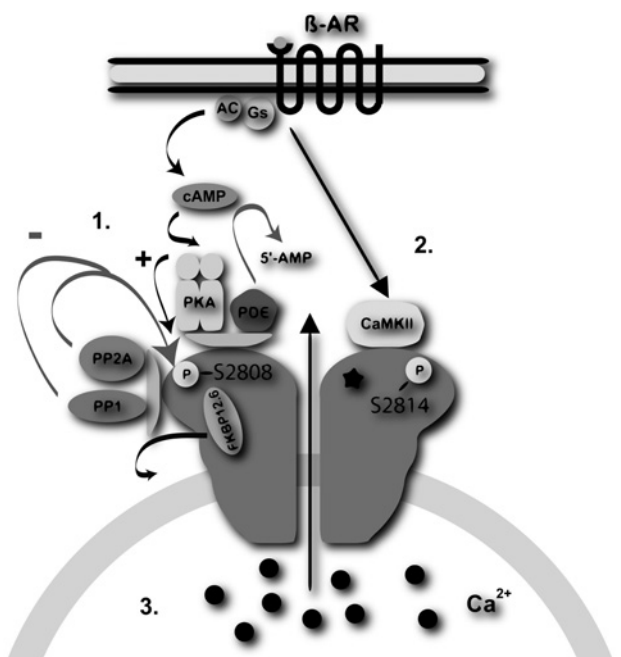
These studies demonstrate that both patients and mice heterozygous for RyR2 mutations exhibit an increased propensity to SR Ca^{2+} leak under conditions that increased SR filling (e.g. β-adrenergic stimulation and increased heart rates). Multiple studies have investigated the molecular and cellular mechanisms underlying abnormal RyR2 activity associated with SR Ca^{2+} leak and DADs. Several of these mechanisms will be discussed in the following paragraphs, and additional information can be found in [2,3,10,16,17].

Molecular mechanisms underlying defective RyR2 regulation and SR Ca^{2+} leak

RyR2 channels are composed of four pore-forming monomers, comprising a relatively small C-terminal transmembrane domain and a large N-terminal domain that protrudes into the cytosol (Figure 1). The cytoplasmic domain serves as a scaffold for multiple regulatory proteins: CaM (calmodulin), FKBP12.6 (12.6 kDa FK506-binding protein) (calstabin2), PKA (protein kinase A), CaMKII (Ca^{2+}/CaM-dependent protein kinase II), PDE4D3 (phosphodiesterase 4D3), and PP1 (protein phosphatase 1) and PP2A (protein phosphatase 2A) [13]. FKBP12.6 (calstabin2) stabilizes the closed conformational state of the channel and is essential for channel closure during diastole [18]. Kinases PKA and CaMKII phosphorylate distinct sites on RyR2, which modulates channel gating properties [19,20]. On the luminal

Figure 1 | Models of defective regulation of RyR2 associated with SR calcium leak and cardiac arrhythmias

Inherited mutations in RyR2 (symbolized by star symbol) cause gain-of–function defects in RyR2 that may be activated by one or more of the following mechanisms: (1) Activation of the β-adrenergic receptor (β-AR) pathway by catecholamines leads to the production of cAMP, which in turn activates PKA bound to RyR2. PKA phosphorylates Ser^{2808}, which leads to a reduction of FKBP12.6 binding to RyR2 and enhanced RyR2 activity. Genetic mutations in RyR2 decrease the binding affinity of FKBP12.6 to RyR2, resulting in incomplete channel closure during diastole. These catecholamine-induced effects may be enhanced by decreased binding of protein phosphatases (PP1 and PP2A) or reduced PDE4D3 activity, which are common findings in patients with CHF. (2) Activation of β-adrenergic receptors may also activate CaMKII, which phosphorylates Ser^{2814} on RyR2. Recent studies suggest that inhibition of CaMKII may decrease SR Ca^{2+} leak and suppress cardiac arrhythmias, although the role of CaMKII modulation of RyR2 in CPVT is still unknown. (3) Genetic mutations in RyR2 may reduce the threshold for diastolic Ca^{2+} release in response to enhanced SR filling following β-adrenergic receptor activation.



side, RyR2 binds junctin and triadin, which anchor the Ca^{2+}-buffering protein CASQ2 (calsequestrin 2) [21].

To date, more than 60 missense mutations in RyR2 have been linked to CPVT. Most of the CPVT mutations occur in three distinct regions of the RyR2 gene (N-terminal, central and C-terminal), which led to the hypothesis that these domains may play an important role in channel regulation. Yamamoto and Ikemoto [22] have proposed that interactions between the N-terminal and central domains of RyR2 stabilize the closed state of the channel and that CPVT mutations disrupt these interdomain interactions, leading to 'unzipping' and uncontrolled Ca^{2+} release. Structural studies of RyR2 using cryoelectron microscopy support this theory. The N-terminal [23] and central [24] domains have been mapped to a region of RyR2 that, similarly to RyR1, undergoes significant structural changes concurrent with the pore opening [25]. George et al. [26] have proposed that CPVT mutations may also alter additional interactions between the N-terminal and C-terminal domains of RyR2. Although it is likely that these domains indeed interact within the three-dimensional folded structure of the RyR2 channel, it remains to be established how stress might induce these changes in interdomain interactions.

Enhanced FKBP12.6 dissociation during stress induces SR Ca^{2+} leak

Arrhythmias in patients with mutations in RyR2 occur almost exclusively during stress or exercise, suggesting that stress-induced intracellular signalling events induce or potentiate RyR2 gating defects that underlie diastolic SR Ca^{2+} leak. Wehrens et al. [2] demonstrated that CPVT-mutant RyR2 channels exhibit increased open probability in single-channel recordings only after PKA phosphorylation, a consequence of β-adrenergic receptor activation during exercise. Moreover, CPVT-mutant RyR2 channels displayed a decreased binding affinity for FKBP12.6, suggesting that excessive loss of FKBP12.6 from the channel complex following PKA phosphorylation might result in RyR2 channels not able to close completely during diastole [2]. It is very possible that the reduced FKBP12.6 affinity results from altered interdomain interactions, although direct evidence for this theory is currently not available. The observation that FKBP12.6-deficient mice exhibit ventricular arrhythmias only after exercise or isoprenaline administration strongly supports this theory [2,27]. Moreover, increasing FKBP12.6 binding to RyR2 either using the experimental drug JTV519 [27] or using transgenic overexpression [28] reduces RyR2 activity and suppresses cardiac arrhythmias. Thus the decreased FKBP12.6 affinity in CPVT-mutant RyR2 potentiates the exercise-induced augmentation of RyR2 activity, resulting in uncontrolled diastolic SR Ca^{2+} release, which can trigger arrhythmias.

Reduced FKBP12.6 binding to RyR2 has also been reported in patients and animals with CHF [29,30]. In this disease state, reduced FKBP12.6 affinity is not caused by genetic mutations in RyR2, but by chronic alterations in the subunit composition of the RyR2 channel complex, which leads to chronically phosphorylated RyR2 subunits [1]. Initially it was proposed by Marks [31] that chronically elevated plasma catecholamine levels underlie sustained activation of PKA, causing hyperphosphorylation of Ser^{2808} on RyR2. Recent studies have revealed that alterations in the protein subunit composition of the RyR2 macromolecular complex may create a local environment that supports chronically increased phosphorylation of Ser^{2808}. In failing hearts, PP1 and PP2A levels are reduced [32], and PDE4D3 binding to RyR2 is also reduced by 50% [33]. Chronic PKA hyperphosphorylation is associated with decreased FKBP12.6 binding to RyR2, which increases the susceptibility to SR Ca^{2+} leak and triggered ventricular arrhythmias.

CaMKII enhances SR Ca^{2+} leak

Another intracellular signalling pathway implicated in the induction of SR Ca^{2+} leak and ventricular arrhythmias is the multifunctional CaMKII [34]. CaMKII was shown to bind and to phosphorylate RyR2 [20,35]. Wehrens et al. [20] demonstrated that CaMKII phosphorylates a unique residue (Ser^{2814}), distinct from the PKA phosphorylation site Ser^{2808}. The functional effect of CaMKII phosphorylation of Ser^{2814} is to increase the open probability of RyR2 at the single channel level [20]. These findings are consistent with the observation that overexpression of CaMKII in cardiac myocytes enhances the frequency of Ca^{2+} sparks [36]. Activation of CaMKII and subsequent phosphorylation of RyR2 leads to enhanced CICR, which plays an important role in the frequency-dependent augmentation of cardiac contractility [20].

In heart failure, CaMKII is activated and leads to enhanced CaMKII phosphorylation of RyR2 [37]. The chronic phosphorylation of RyR2 at Ser^{2814} may cause diastolic Ca^{2+} leak from the SR through a signalling mechanism distinct from the PKA-dependent pathway (Figure 1). CaMKII can be activated as a result of increased diastolic Ca^{2+} levels at faster heart rates, or due to direct stimulation by β-adrenergic receptors during exercise or stress [38]. Elegant studies by Curran et al. [39] have revealed that SR Ca^{2+} leak in rabbit ventricular myocytes induced by β-adrenergic stimulation could be inhibited by CaMKII antagonists but not by PKA inhibitors. Thus it is certainly possible that CaMKII-dependent phosphorylation of CPVT-mutant RyR2 induces or potentiates SR Ca^{2+} leak to levels sufficient to induce premature contractions and triggers ventricular arrhythmias during catecholaminergic stress.

SOICR (store overload induced Ca^{2+} release)

A third mechanism that may contribute to enhanced SR Ca^{2+} leak in patients with CPVT is based on the notion that mutant RyR2 channels are more prone to spontaneous Ca^{2+} release when the SR filling is increased during β-adrenergic receptor signalling [40]. It has been proposed that under normal conditions, the threshold for spontaneous SOICR is not achieved in carriers of CPVT mutant RyR2. In contrast, during catecholaminergic stress, the SR Ca^{2+} loading is

increased to a level above the threshold for SOICR, setting the stage for unevoked SR Ca^{2+} releases and Ca^{2+} leak [16,40]. Jiang et al. [40] provided evidence for an increased sensitivity to luminal Ca^{2+} in HEK-293 cells (human embryonic kidney cells) overexpressing C-terminal CPVT-mutant RyR2 channels. Compared with wild-type channels, CPVT-mutant RyR2 exhibited spontaneous SR Ca^{2+} release events at lower luminal Ca^{2+} levels [40]. Single channel recordings in planar lipid bilayers also revealed increased sensitivity to Ca^{2+}-dependent activation of mutant RyR2 channels. These findings were subsequently extended to mutations in the N-terminal and central domains of RyR2 [16]. At present, it remains unresolved how mutations in the cytoplasmic domain of RyR2 affect the sensitivity to luminal Ca^{2+} levels. Moreover, the SOICR mechanism has only been tested in HEK-293 and HL-1 cell culture experiments, in which protein overexpression levels are uncontrolled and the structural requirements for CICR are not present due to the absence of SR function [17]. Thus the relative importance of the SOICR hypothesis remains to be tested in the available animal models.

Genetic evidence, however, does indicate that abnormal luminal Ca^{2+} handling may lead to increased SR Ca^{2+} leak and cardiac arrhythmias. Inherited mutations in the luminal Ca^{2+} buffering protein CASQ2 have been linked to CPVT-2 (CPVT type 2) [41]. Calsequestrin is the major Ca^{2+} storage protein of the SR, and binding of intraluminal Ca^{2+} to calsequestrin during diastole prevents Ca^{2+} precipitation in the SR and permits maximal SR Ca^{2+} re-uptake during each cardiac cycle [42]. Mutations in CASQ2 may increase the likelihood of diastolic SR Ca^{2+} releases due to a lowered Ca^{2+} buffering capacity or due to altered interactions with RyR2, thereby relieving its inhibitory effects on RyR2 during diastole [21]. Transgenic mice overexpressing the CPVT mutation D307H in *CASQ2* displayed DADs and spontaneous action potentials [43], consistent with an increased leakage of Ca^{2+} through RyR2 during diastole. It is interesting that the complete ablation of CASQ2 resulted in significantly more spontaneous premature Ca^{2+} releases under basal conditions and after isoprenaline administration despite normal SR Ca^{2+} store levels [44]. These results suggest that disrupted CASQ2–RyR2 interactions rather than reduced Ca^{2+}-buffering capacity contribute more to SR Ca^{2+} leak in patients with CPVT.

Conclusions

The identification of mutations in RyR2 provided the unique opportunity to study the role of diastolic SR Ca^{2+} leak in the initiation of lethal ventricular arrhythmias. Two mouse models of inherited mutations in RyR2 closely resemble the phenotypic characteristics observed in patients with CPVT including exercise-induced ventricular arrhythmias. Cardiac myocytes isolated from these knockin mouse models have provided evidence for diastolic SR Ca^{2+} leak as a trigger for arrhythmias. Multiple studies have evaluated the molecular mechanisms underlying spontaneous SR Ca^{2+} release and triggered arrhythmias. Moreover, it has become clear that spontaneous SR Ca^{2+} leak may also contribute to arrhythmogenesis in failing hearts despite differences in the molecular mechanisms causing the Ca^{2+} leak. Taken together, these studies provide strong evidence that defects in RyR2 increase the propensity for diastolic openings of the channel, which has opened new avenues for RyR2-based anti-arrhythmic therapies.

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