Role of ryanodine receptor as a Ca\(^{2+}\) regulatory center in normal and failing hearts

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Summary Abnormal Ca\(^{2+}\) cycling is important in various cardiac diseases. Evidence has accumulated that dysregulation of Ca\(^{2+}\) release from the ryanodine receptor (RyR2) plays a significant role in cardiac dysfunction. Spontaneous Ca\(^{2+}\) release through RyR2 during diastole decreases sarcoplasmic reticulum (SR) Ca\(^{2+}\) content, and also induces delayed after depolarization (DAD) as a substrate for lethal arrhythmia. Several disease-linked mutations in the RyR have been reported in patients with catecholaminergic polymorphic ventricular tachycardia (CPVT) or arrhythmogenic right ventricular cardiomyopathy type 2 (ARVC2). The unique distribution of these mutation sites has produced the concept that the interaction among the putative regulatory domains within the RyR may play a key role in regulating the channel opening, and that there seems to be a common abnormality in the channel disorder between heart failure and CPVT/ARVC2. We review here the considerable body of evidence regarding defective channel gating of RyR2 in the pathogenesis of heart failure and lethal arrhythmia.

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Heart failure (HF) is a complex disorder characterized by contractile dysfunction and activation of neurohumoral factors [1]. Among a variety of factors, abnormal intracellular Ca\(^{2+}\) handling by the sarcoplasmic reticulum (SR) plays a key role in the pathogenesis of HF [2,3]. In particular, abnormal SR Ca\(^{2+}\) release has been reported to play a critical role in abnormal Ca\(^{2+}\) handling in HF [2,3]. This review focuses on the underlying mechanism of the disease-linked channel disorder of the ryanodine receptor (RyR).

**Structure of RyR2 as a scaffolding protein**

The RyR exists as a scaffolding protein bound with many accessory proteins, producing a huge macromolecular complex [4] (Fig. 1). About 90% of the RyR polypeptide chain forms a bulky cytoplasmic domain that modulates the channel function. The remaining 10% of the RyR sequence (C-terminal region) forms transmembrane and channel pore regions. The RyR is a huge tetrameric protein, and each monomer is constituted of \(\sim 5000\) amino acids and has a molecular weight of 565 kDa. There are three RyR isoforms in mammals. RyR1 is predominant in skeletal muscle [5] and RyR2 is predominant in cardiac muscle [6]. RyR3 was first cloned from rabbit brain [7]. The amino acid sequences of these three RyR isoforms reveal a high degree of sequence homology (\(\sim 70\%\)). Many regulatory proteins (i.e. calmodulin (CaM), FKBP12.6 (calstabin2), protein kinase A (PKA), protein phosphatase 1 (PP1), and protein phosphatase 2A (PP2A)) bind to the RyR2, forming a huge macromolecular complex [4]. Both junctin and triadin, which anchor calsequestrin bind to the luminal site of the RyR2 [4].

**Role of various accessory proteins of RyR2 in channel gating**

**FKBP12.6**

FKBP12.6 is one of the important accessory proteins of RyR2. FKBP12.6 binds tightly to RyR2 and stabilizes the channel [8]. The stoichiometry of FKBP12.6 binding to RyR2 is approximately four FKBP molecules per RyR tetramer (or one FKBP12.6 per RyR subunit) [8]. Marx et al. [9] demonstrated that chronic and excess activation of beta-adrenergic receptor induces PKA-mediated hyperphosphorylation of RyR2 at serine 2808, which in turn dissociates FKBP12.6 from RyR2, thereby causing Ca\(^{2+}\) leak. This diastolic Ca\(^{2+}\) leak not only depresses the SR Ca\(^{2+}\) load, but also serves as a substrate for DAD, leading to lethal arrhythmia which can trigger cardiac arrhythmia and lead to sudden death. In pacing-induced canine HF model, we found (1) that spontaneous SR Ca\(^{2+}\) leak was mediated through the conformational change of RyR2, and (2) that the stoichiometry of FKBP12.6 to RyR2 was significantly decreased in association with PKA-hyperphosphorylation of RyR2 [10]. Long-term hyperphosphorylation of RyR2 in failing hearts seems to be maintained through a reduction in RyR2-coupled PP1, PP2A [9], and PDE4D3 [11].

However, several researchers have challenged the role of FKBP12.6 as a channel stabilizer in various experimental conditions. Phosphorylation at serine2808 did not dissociate FKBP12.6 from RyR2 [12], and the constitutive phosphorylation of serine2808 by mutations (S2808D) failed to disrupt the FKBP12.6—RyR2 interaction [13]. Chen and his co-workers emphasized the significant role of PKA phosphorylation of another site (Ser-2030) on hyper-sensitized channel to activation by luminal Ca\(^{2+}\) (SOICR) in disease-linked Ry2 channel disorder [14–16]. Further investigation is still needed to clarify the role of phosphorylation on RyR2 function.

**Calmodulin**

Calmodulin is a 17-kDa ubiquitous Ca\(^{2+}\)-binding protein that regulates channel opening by its direct binding to the RyR. CaM is composed of \(\sim 150\) amino acid residues arranged in two globular lobes. The Ca\(^{2+}\) binding EF-hand motifs are arranged in pairs: one pair per lobe. The Ca\(^{2+}\) free and Ca\(^{2+}\) bound forms of CaM are termed apoCaM and CaCaM, respectively. In RyR1, CaM enhances the channel activity at low [Ca\(^{2+}\)], whereas CaM inhibits
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Figure 1  RyR2 macromolecular complex. Cardiac RyR (RyR2) and satellite proteins are indicated. Calmodulin (CaM), FKBP12.6 (calstabin2), protein kinase A (PKA), phosphatase 1 (PP1), and phosphatase 2A (PP2A) bind to the cytoplasmic region of the RyR. Both junctin and triadin, which anchor calsequestrin to RyR2 depending on the SR Ca2+ concentration, bind to the luminal side of RyR2.

Calsequestrin

Calsequestrin (CASQ) is a major intra-SR Ca2+-binding protein. CASQ is highly acidic with 119 Glu and Asp residues (net 64 negative charge) and each molecule binds ∼20 Ca ions with low affinity [25].
CASQ forms a complex with the RyR2, triadin, and junctin. Both skeletal and cardiac CASQ can bind up to 800 nM of Ca$^{2+}$ per mg of protein (40 ions per molecule) with a Kd of about 1 mM [25]. Upon Ca$^{2+}$ binding, CASQ undergoes major conformational changes, which enables high capacity Ca$^{2+}$ binding by CASQ [26]. In addition, its Ca$^{2+}$ buffering action, calsequestrin has been shown to regulate RyR2 function as a luminal Ca$^{2+}$ sensor of RyR2 (via protein–protein interactions involving triadin and junctin) [27]. Namely, it is suggested that the open probability of RyR2 is inhibited by CASQ at low intra-SR [Ca$^{2+}$], but that this inhibition is relieved at high intra-SR [Ca$^{2+}$], contributing to luminal [Ca$^{2+}$] sensing of RyR2 [27]. CASQ overexpression in mouse hearts revealed increases in SR Ca$^{2+}$ capacity, but decreases in Ca$^{2+}$-induced SR Ca$^{2+}$ release, leading to depressed contractility [28]. Excessive Ca$^{2+}$ buffering by high levels of CASQ2 may inhibit the channel activity of RyR2. On the other hand, several mutations in CASQ2 have been found to be linked with CPVT [29–32]. Recently, a mouse model in which CASQ2 is lacking [33] or mutated [34], has been engineered. Interestingly, these mice showed almost normal cardiac function, but developed polymorphic ventricular tachycardia on infusion of catecholamines, in association with significant polymorphic ventricular tachycardia (CPVT) and catecholaminergic polymorphic ventricular tachycardia (CPVT) and arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD/C2) [3]. All these mutations are not randomly distributed, but cluster into specific regions that correspond to three malignant hyperthermia (MH)/central core disease (CCD) mutable domain of RyR1: N-terminal domain (1–600), central domain (2000–2500) and channel forming, C-terminal domain. Mutations at different positions in either domain seem to result in hyperactivation of the Ca$^{2+}$ channel and hypersensitization to agonists. From these observations, Ikemoto and Yamamoto [36] proposed an inter-domain interaction concept that the two domains (N-terminal: 1–600 and central domain: 2000–2500) interact with each other to act as a regulatory switch for channel-gating activity, with a tight zipping of the interacting domains serving to stabilize the channel. A mutation in either domain weakens the inter-domain interaction, thus increasing the tendency towards unzipping, which causes activation and leakiness of the Ca$^{2+}$ channel. We demonstrated that the domain unzipping of the interacting domains have already taken place in failing hearts, concurrently with an abnormal Ca$^{2+}$ leak, suggesting that defective inter-domain interaction between the regulatory domains may be a key mechanism for the development of the various problems with RyR2 in the failing heart [37]. The previous observation by single particle analysis that the N-terminal and central mutation regions are closely neighboring in the three-dimensional structure (domains 5 and 6 in the clamp region, respectively) [38] strongly supports the inter-domain interaction concept. More recently, Meng et al. [39] reported that Ser-2808 is three-dimensionally located at the boundary between the domain 5 and 6 at the clamp region, suggesting that PKA phosphorylation at Ser-2808 may possibly interrupt the inter-domain interaction between N-terminal and central domains, causing domain unzipping. RyR2 I-domain (3722–4610) was also found to communicate the cytoplasmic domain with the transmembrane domain, thereby modulating RyR sensitivity to caffeine activation [40]. Interestingly, I-domain (human CPVT-type) mutations occurring in domains III (N4104K) and IV (R4497C), and a distinct mutation occurring in domain II (S2246L), promoted equivalent augmentation in channel instability [40]. These findings suggest that defective inter-domain interaction may be a key pathogenic mechanism of HF and lethal arrhythmia.

Defective inter-domain interaction within RyR2 in diseased hearts

More than 70 RyR2 miss-sense mutations have been found to be linked with two inherited forms of sudden cardiac death: i.e. catecholaminergic polymorphic ventricular tachycardia (CPVT) and arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD/C2) [3]. All these mutations are not randomly distributed, but cluster into specific regions that correspond to three malignant hyperthermia (MH)/central core disease (CCD) mutable domain of RyR2: N-terminal domain (1–600), central domain (2000–2500) and channel forming, C-terminal domain.
Correction of defective inter-domain interaction as a new treatment for diseased hearts

Both beta-blockers and angiotensin II receptor blockade (ARB) were found to suppress the hyperadrenergic state, thereby reversing PKA-mediated hyperphosphorylation of the RyR2, restoring the FKBP12.6-mediated stabilization and inhibiting the Ca^{2+} leak [44–46]. Furthermore, we previously reported that a 1,4-benzothiazepine derivative K201 (JTV519), improved contractility and prevented the development of HF, probably by sealing Ca^{2+} leak from RyR2 [47]. JTV519 was found to restore the zipped state of interactions between domains within RyR2, which prevented Ca^{2+} leak from occurring in failing hearts [37]. We recently identified the K201-binding site as domain 2114–2149 of the ryanodine receptor (RyR2), and the binding of K201 to this domain corrects the defective inter-domain interaction between N-terminal (1–600) and central regions (2000–2500) of RyR2 in pacing-induced failing hearts [48]. Using FKBP12.6+/− mice, Wehrens et al. [49] also demonstrated that JTV519 increased the affinity of FKBP12.6 for the RyR2, which stabilized the closed state of the RyR2 and prevented the Ca^{2+} leak that triggers arrhythmias. Taken together, the stabilization of the RyR2 represents a new molecular target for the treatment or prevention of lethal arrhythmia and HF as well.

Conclusions and perspectives

RyR2, as a scaffolding Ca^{2+} regulatory protein, plays a crucial role in the pathogenesis of HF and cardiac arrhythmia. Several accessory proteins; i.e. FKBP12.6, calmodulin, calsequestrin, PP1, PP2A, etc., act as an important modulator for maintaining normal Ca^{2+} cycling. Defective regulation of channel gating by these key proteins may render the channel leaky. Defective inter-domain interaction within the RyR2 also induces the de-stabilized channel gating, as a common key pathogenic mechanism of HF and lethal arrhythmia. Correction of the defective domain–domain interaction would be a possible new strategy against these diseased states.

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