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The Ryanodine Receptor in Cardiac Physiology and Disease

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Abstract

According to the American Heart Association it is estimated that the United States will spend close to \$39 billion in 2010 to treat over five million Americans suffering from heart failure. Patients with heart failure suffer from dyspnea and decreased exercised tolerance and are at increased risk for fatal ventricular arrhythmias. Food and Drug Administration -approved pharmacologic therapies for heart failure include diuretics, inhibitors of the renin–angiotensin system, and β -adrenergic receptor antagonists. Over the past 20 years advances in the field of ryanodine receptor (RyR2)/calcium release channel research have greatly advanced our understanding of cardiac physiology and the pathogenesis of heart failure and arrhythmias. Here we review the key observations, controversies, and discoveries that have led to the development of novel compounds targeting the RyR2/calcium release channel for treating heart failure and for preventing lethal arrhythmias.

I. Introduction

In 1883 Sydney Ringer discovered that calcium (Ca^{2+}) is required for cardiac contraction (Ringer, 1883). Twenty four years later Locke and Rosenheim (1907) observed that Ca^{2+} is responsible for linking myocardial excitation with contraction. Following these seminal discoveries important advances have been made toward understanding the molecular determinants of cardiac Ca^{2+} regulation and its role in determining cardiac function.

In cardiomyocytes Ca^{2+} is stored in an intracellular vesicular network called the sarcoplasmic reticulum (SR) (Hasselbach & Makinose, 1961, 1963; Martonosi & Feretos, 1964) and is available for immediate release into the cytosol, where it binds to Troponin C and enables actin–myosin binding and sliding of the myofilaments that results in sarcomere shortening and myocardial contraction (Ebashi & Lipmann, 1962; Otsuka et al., 1964; Weber, 1959). The key roles that cyclical SR Ca^{2+} release and reuptake play in cardiac contraction underscore the importance of exquisite regulation of the proteins involved in these processes.

Cardiac contraction can be divided into electrical (excitation) and contractile phases. The electrical phase begins with depolarization of the sinoatrial node (SAN), situated near the junction of the superior vena cava and the right atrium, which causes a wave of depolarization to spread via the conduction system through the atria and ventricles. On the cellular level current flows between a depolarized cardiomyocyte and its resting neighbor

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through specialized low-resistance channels called gap junctions (Weidmann, 1952) causing depolarization of the membrane potential of the resting cell (Rohr, 2004). As the membrane potential of the resting cell increases from -90 mV (Draper & Weidmann, 1951) to -70 mV voltage-gated Na^+ channels (SCN5A) begin to open allowing an influx of sodium ions into the myocyte, further depolarizing the cell to $\sim +10$ mV (Gibbons & Zygmunt, 1992). As the membrane potential rises above -40 mV L-type calcium channels (Cav1.2) on the sarcolemma begin opening leading to an influx of Ca^{2+} into the myocyte (Bean, 1985; Gibbons & Zygmunt, 1992). At ~ 0 mV voltage-gated K^+ channels (e.g., KCNH2 and KCNQ1) open allowing K^+ to efflux from the cell (Oudit et al., 2004). The influx of Na^+ and Ca^{2+} balanced by the efflux of K^+ causes the membrane potential to plateau at ~ 0 mV. Na^+ channels and L-type calcium channels inactivate as a function of time, membrane potential, and $[\text{Ca}^{2+}]$ (Campbell et al., 1988) which reduces inward current leaving the unopposed efflux of potassium to repolarize the membrane to resting potential.

Ca^{2+} that enters the cell through $\text{Ca}_v1.2$ during the excitation phase initiates contraction by binding to and activating the ryanodine receptor (RyR2), the major cardiac SR Ca^{2+} release channel, to release Ca^{2+} from the SR into the cytosol. As cytosolic Ca^{2+} levels increase from less than 100 nM to ~ 1 μM Ca^{2+} binds to troponin C, a component of the myofilaments, causing a conformational change in the troponin/tropomyosin complex, which enables myosin to interact with actin leading to myofilament shortening. Ca^{2+} is subsequently pumped back into the SR through the sarco/endoplasmic reticulum ATPase (SERCA2a) and is removed from the cell through the sarcolemmal sodium–calcium exchanger (NCX) and plasmalemmal Ca^{2+} ATPase (Fig. 1).

II. Ryanodine Receptors

Ryanodine receptors were originally identified using the plant alkaloid ryanodine, isolated from *Ryania speciosa* found in Central and South America. At the time ryanodine was being tested as a potential insecticide (Rogers et al., 1948) owing to its paralyzing effect on insects (Edwards et al., 1948). Ryanodine was subsequently found to induce profound paralysis of cardiac and skeletal muscle and that it bound to a component of the SR (Jenden & Fairhurst, 1969). Ryanodine was used as a high-affinity ligand to track the purification of its receptor from SR preparations. The purified ryanodine receptors were shown to be Ca^{2+} release channels in skeletal (Fleischer et al., 1985; Meissner, 1986) and cardiac (Meissner & Henderson, 1987) muscles. Ryanodine was shown to lock the channel open in a characteristic subconductance state resulting in an SR Ca^{2+} leak that provided a mechanism for the paralytic action of the drug (Fill & Copello, 2002; Fleischer et al., 1985).

The cardiac ryanodine receptor isoform, RyR2, is a homotetramer comprising four ~ 565 kDa monomers. Each monomer contains a transmembrane segment located at the carboxy terminus that is formed by $\sim 10\%$ of the linear sequence, whereas the remaining 90% of the protein sequence encodes an enormous cytoplasmic domain that serves as a scaffold for regulatory subunits and enzymes that modulate the function of the channel (Zalk et al., 2007). It is estimated that each monomer contributes 6–8 transmembrane segments that form the pore region of the channel (Du et al., 2002). Cryo-electron microscopic (cryo-EM) analysis of the RyR2 pore combined with predictions based on sequence and structural similarities with the bacterial K^+ channels suggests that the luminal loops of the RyR2 monomers contribute to the pore structure (Balshaw et al., 1999; Samso et al., 2009) and that amino acids Glu4832, Ile4829, Gly4826, and Gln4881 (Rabbit RyR2) directly mediate Ca^{2+} passage through the pore (Welch et al., 2004)

RyR2, first cloned from rabbit heart in 1990 (Nakai et al., 1990; Otsu et al., 1990), shares close to 70% homology with two other mammalian RyR isoforms: RyR1 and RyR3. RyR1

is found predominantly in skeletal muscle where it is activated directly by the L-type calcium channel (Cav1.1) to release SR Ca^{2+} stores during skeletal muscle contraction. All three isoforms have been identified in smooth muscle and in the brain, though the physiologic role of RyR in these tissues has not been fully elucidated. All three channels share similar permeation properties with large conductance for both monovalent and divalent cations and relatively low Ca^{2+} selectivity (Fill & Copello, 2002). Under physiologic conditions RyR2 predominantly conducts Ca^{2+} because Ca^{2+} is present at millimolar concentrations in the SR.

RyR2 is normally closed at low cytosolic diastolic $[\text{Ca}^{2+}]$ (~100–200 nM) (Copello et al., 1997). At submicromolar cytosolic $[\text{Ca}^{2+}]$ Ca^{2+} binds to high-affinity binding sites on RyR2 which increases the open probability (P_o) of the channel. Channel activity is maximal at $[\text{Ca}^{2+}]_{\text{cyto}} \sim 10 \mu\text{M}$ and elevating $[\text{Ca}^{2+}]_{\text{cyto}}$ beyond this point leads to a reduction in P_o (Copello et al., 1997; Laver et al., 1995) possibly due to Ca^{2+} binding to low-affinity inhibitory binding sites (Fill & Copello, 2002). Regulation of RyR2 P_o by luminal $[\text{Ca}^{2+}]$ between 10 μM and 1 mM has also been demonstrated (Ching et al., 2000). However, whether this regulation is due to Ca^{2+} flow through the channel and binding to cytosolic Ca^{2+} -binding sites remains uncertain (Fill & Copello, 2002; Gyorke & Gyorke, 1998; Liu et al., 2010; Tripathy & Meissner, 1996).

Studies employing single-particle cryo-EM of RyR1 have revealed conformational changes which occur when the channel opens including expansion of the clamp domain (situated between domains 9 and 10 and depicted as the “corners” of the RyR1 tetramer), rotation of the transmembrane domains relative to the cytoplasmic region, and expansion of the pore to 18 Å (Orlova et al., 1996; Samso et al., 2005, 2009). Cryo-EM has also been useful for identifying binding sites for several RyR modulatory proteins which interact with the cytoplasmic domain of the channel (Samso et al., 2005).

A. Modulatory Proteins

Many proteins complex directly and indirectly with the N-terminal cytoplasmic domain of RyR2 including the 12.6 kDa FK506-binding protein (calstabin2 or FKBP12.6) (Timerman et al., 1994), protein kinase A (PKA) (Marx et al., 2000), calcium/calmodulin-dependent kinase II (CaMKII) (Wehrens et al., 2004b), phosphodiesterase 4D3 (PDE4D3) (Lehnart et al., 2005), calmodulin (CaM) (Meissner & Henderson, 1987), protein phosphatases 1 and 2A (PP1 and PP2A) (Marx et al., 2000), and Sorcin (Farrell et al., 2004). Calsequestrin, junctin, and triadin have been proposed to complex with the C-terminus of RyR2 (Gyorke & Terentyev, 2008) (Fig. 1).

1. Calstabin2 (FKBP12.6)—Calstabin2 (FKBP12.6) is a 12.6 kDa immunophilin originally identified by its ability to bind to FK506, a common immunosuppressant used in organ transplantation. FKBP12.6 has peptidyl-prolyl *cis/trans*-isomerase (rotamase) activity that is highly conserved among all FKBP isoforms (Marks, 1996). FKBP12 and FKBP12.6 have been renamed calstabin1 and calstabin2 reflecting their roles as RyR1 and RyR2 Ca^{2+} channel stabilizers, respectively (calcium channel-stabilizing binding proteins) (Wehrens & Marks, 2003).

In 1992 calstabin1 (FKBP12), which is 85% similar to calstabin2 (FKBP12.6), was found to be associated with RyR1 in skeletal muscle (Jayaraman et al., 1992). Co-expressing calstabin1 with RyR1 stabilized the closed state of the channel and eliminated subconductance states of single RyR1 channels reconstituted in planar lipid bilayer (Brillantes et al., 1994). Calstabin1 also increased the probability of coupled gating, a phenomenon whereby RyR1 channels exhibit simultaneous openings and closings (Marx et al., 1998). Pharmacologic depletion of calstabin1 from RyR1 shifted the Ca^{2+} dependence of

the channel leftward resulting in increased P_o (Brillantes et al., 1994). Calstabin2 was subsequently shown to colocalize with RyR2 in the heart (Timerman et al., 1994) and dissociating calstabin2 from RyR2 enhanced channel P_o (Kaftan et al., 1996; Xiao et al., 1997). Genetic deletion of calstabin2 enhanced SR Ca^{2+} release in isolated cardiomyocytes (Xin et al., 2002).

Cryo-EM studies on RyR1 have demonstrated that calstabin1 binds to the channel at least 130 Å away from the pore (Samsó et al., 2009), and it has been proposed that calstabin1 does not directly interact with the pore. The calstabin2-binding site on RyR2 is controversial. Using yeast two-hybrid and site-directed mutagenesis Ile2427 and Pro2428 were identified as the peptidyl–prolyl bond to which FKBP12.6 binds (Marx et al., 2000). Ile2427 was proposed to be responsible for the selectivity of RyR2 for calstabin2, as replacing this residue with a valine, which is naturally present in RyR1, causes calstabin1 to bind to RyR2 instead of calstabin2 (Gaburjakova et al., 2001). This selectivity may explain why calstabin2 colocalizes with RyR2 in the heart despite the higher concentration of calstabin1 in heart muscle cytosol. However, others using serial carboxyl terminal deletions identified a region between amino acids 1815 and 1855 as the calstabin2-binding site on RyR2 (Masumiya et al., 2003). A crystal structure of the ryanodine receptor providing atomic resolution may be necessary before consensus can be achieved. Isomerase activity does not appear to be necessary for the RyR stabilizing properties of these immunophilins (Huang et al., 2006; Timerman et al., 1995).

Others have challenged the findings that calstabin2 stabilizes the closed state of RyR2 (Timerman et al., 1996; Xiao et al., 2007a). Chen and colleagues observed that ryanodine binding to cell lysates of HEK293 cells expressing RyR2 was similar whether or not RyR2 was co-expressed with calstabin2 (Xiao et al., 2007a). Additionally, the P_o of recombinant RyR2 reconstituted in planar lipid bilayer was similar in the absence and presence of calstabin2 (Xiao et al., 2007a). Methodological differences between single channel studies including the use of different charge carriers (K^+ vs. Ba^{2+} vs. Ca^{2+}), absence or presence of potential difference across the bilayer membrane, [ATP] and $[\text{Mg}^{2+}]$, $[\text{Ca}^{2+}]$ in the *cis* and *trans* compartments may partially explain these conflicts. However, many other studies now suggest that calstabin2 modulates RyR2 activity (Chen et al., 2010; Gellen et al., 2008; Hu et al., 2010; Noguchi et al., 2008; Zhang et al., 2008, 2009).

2. PKA and CaMKII—The PKA holoenzyme comprises two catalytic and two regulatory subunits that is targeted to the RyR2 channel via binding to the muscle A kinase anchoring protein (mAKAP) (Marx et al., 2000). mAKAP binds to leucine/isoleucine zipper domains on RyR2 at amino acids 3003–3039 (Marx et al., 2001). PKA becomes activated when cyclic adenosine monophosphate (cAMP) binds to the regulatory subunits causing them to dissociate from the catalytic subunits. CaMKII is a dodecameric holoenzyme activated by Ca^{2+} -bound CaM (Couchonnal & Anderson, 2008). Activated PKA and CaMKII phosphorylate Ser2809 and 2815, respectively, on RyR2 (Marx et al., 2000; Wehrens et al., 2004b).

Ser2809 on canine and human RyR2 (Ser2808 on murine RyR2) was originally identified as a CaMKII phosphorylation site (Witcher et al., 1991). However, in these experiments the RyR2 peptide that was identified as being phosphorylated by endogenous CaMKII included both Ser2809 and Ser2815 (Ser2814 in murine RyR2). Moreover, experiments using phosphospecific antibodies have suggested that both PKA and CaMKII phosphorylate Ser2809 (Rodriguez et al., 2003). However, studies using site-directed mutagenesis of recombinant RyR2 have demonstrated that PKA specifically phosphorylates Ser2809 (Marx et al., 2000; Wehrens et al., 2004b, 2006) and CaMKII specifically phosphorylates Ser2815 (Kushnir et al., 2010b; Wehrens et al., 2004b). The observations that PKA but not CaMKII

phosphorylates RyR1 and that RyR1 contains only a serine residue corresponding to Ser2809 (Ser2845) but not to Ser2815 (Witcher et al., 1991) further support the proposed specificity of these phosphorylation sites. Other reports suggest that serine 2030 (murine RyR2) is the major PKA phosphorylation site on RyR2 and that phosphorylation at this site increases the sensitivity of the channel to luminal Ca^{2+} (Xiao et al., 2005, 2007b). Studies supporting (Benkusky et al., 2007) and challenging (Huke & Bers, 2008; Wehrens et al., 2006) Ser2030 as a physiological RyR2 PKA phosphorylation site have been reported.

PKA phosphorylation of RyR2 activates the channel (Valdivia et al., 1995), at least in part by increasing the sensitivity of RyR2 to cytosolic Ca^{2+} (Marx et al., 2000). It has been proposed that PKA phosphorylation of RyR2 causes dissociation of calstabin2 from the channel complex as a result of steric repulsion between the negatively charged phosphate group that is covalently linked to RyR2 and Asp37 on calstabin2 (Huang et al., 2006; Marx et al., 2000). Supporting this hypothesis, recombinant mutant RyR2 engineered with aspartic acid in place of Ser2808 (RyR2-S2808D, murine RyR2) has reduced binding of calstabin2 and increased Po at diastolic $[\text{Ca}^{2+}]$ (Wehrens et al., 2006).

The observation that calstabin2 depleted channels have increased Po which can be further enhanced by PKA phosphorylation (Wehrens et al., 2003) supports the proposal that PKA phosphorylation of RyR2 also enhances RyR2 activity independent of calstabin2 depletion. CaMKII phosphorylation of RyR2 at Ser2815 increases the Po of RyR2 by sensitizing the channel to cytosolic Ca^{2+} , though unlike PKA this does not dissociate calstabin2 from the channel (Wehrens et al., 2004b). Cellular evidence that CaMKII phosphorylation of RyR2 enhances the activity of the channel is based on the observation that Ca^{2+} transient amplitude is reduced by pharmacologic inhibition of CaMKII with KN-93, under conditions of matched $\text{I}_{\text{CaV1.2}}$ (L-type Ca^{2+} channel current) and SR Ca^{2+} load (Li et al., 1997). Furthermore, cardiomyocytes isolated from cardiac-specific CaMKII over-expressing mice have enhanced fractional release of SR Ca^{2+} (Maier et al., 2003).

Studies measuring small spontaneous RyR-mediated Ca^{2+} release events, Ca^{2+} sparks, in permeabilized cardiomyocytes have suggested that PKA phosphorylation of RyR2 does not play a role in isoproterenol (Iso) enhancement of Ca^{2+} release (Li et al., 2002). However, in this study the investigators artificially clamped cytosolic $[\text{Ca}^{2+}]$ at either 10 or 50 nM in order to reduce spark frequency to facilitate their measurements (Li et al., 2002).

While some groups have demonstrated that PKA phosphorylation of RyR2 causes depletion of calstabin2 from the channel (Blayney et al., 2010; George et al., 2003) others have not (Stange et al., 2003; Xiao et al., 2004). Recently it has become apparent that nitrosylation (Aracena et al., 2005; Bellinger et al., 2009) and oxidation (Zissimopoulos et al., 2007) of RyR also affect the binding of calstabin to the channel. Additionally variations in the molar ratios of RyR2 and calstabin2 in heterologous systems can influence the amount of calstabin2 bound to PKA phosphorylated channels. Thus, differences in these parameters, which are sometimes not assessed, may explain the divergent findings that have been reported.

Cryo-EM studies have reported a 105–120 Å distance between the PKA phosphorylation site, Ser2808, and the putative calstabin2-binding site (Meng et al., 2007). This challenges the hypothesis that phosphorylation at one site directly interferes with binding at the other. Similar analysis has demonstrated that Ser2030 is too far from the calstabin2-binding site for phosphorylation at this site to affect calstabin2 binding (Jones et al., 2008). However, while these studies are certainly informative, in the absence of an atomic resolution structure, they fall short of providing definitive assignments for the locations of these regulatory sites on

the RyR2 channel cytoplasmic domain. These results do not rule out the possibility that PKA phosphorylation at Ser2808 indirectly modulates calstabin2 binding to RyR2.

3. Phosphodiesterase 4D3 and Protein Phosphatases 1 and 2a—PDE4D3

degrades cAMP and plays an important role in regulating spatial and temporal cAMP levels (Zaccolo & Pozzan, 2002). Both PDE4D3 and PKA bind to mAKAP (Dodge et al., 2001) as part of the RyR2 macromolecular complex (Lehnart et al., 2005). PKA phosphorylation of PDE4D3 at Ser54 and Ser13 increases enzymatic activity twofold (Sette & Conti, 1996) and enhances the affinity of PDE4D3 to mAKAP (Carlisle Michel et al., 2004), respectively. This may provide negative feedback for modulating the activating effects of PKA. The localization of PDE4D3 to RyR2 enables regulation of local cAMP levels near the channel (Lehnart et al., 2005).

PP1 and PP2a indirectly associate with RyR2 through spinophilin and PR130, respectively, and regulate channel activity by dephosphorylating phosphorylated channels (Marx et al., 2001). It has been proposed that PP1 is the predominant phosphatase which dephosphorylates Ser2808 and Ser2814 and that PP2a contributes to dephosphorylating Ser2814 (Huke & Bers, 2008).

4. Calmodulin—CaM is a ubiquitously expressed, highly conserved, 17 kDa protein that contains four Ca^{2+} -binding EF hands, two on each end of the protein. CaM preferentially inhibits RyR2 at $[\text{Ca}^{2+}] < 10 \mu\text{M}$ by binding to a region on RyR2 comprising amino acids 3583–3603 (Yamaguchi et al., 2003). CaM may function to assist closing RyR2 following SR Ca^{2+} release in EC-coupling (Xu & Meissner, 2004). Supporting this hypothesis, cardiomyocytes isolated from mice engineered with RyR2 lacking the CaM-binding site have prolonged Ca^{2+} transients (Yamaguchi et al., 2007).

5. Sorcin—Sorcin is a 22 kDa Ca^{2+} -binding protein localized to the dyadic space between RyR2 and $\text{Ca}_v1.2$, which associates with RyR2 when $[\text{Ca}^{2+}]$ are elevated. In planar lipid bilayers sorcin reduces the P_o of RyR2 (Lokuta et al., 1997) and dialyzing sorcin into cardiomyocytes reduces the amplitude of SR Ca^{2+} release without affecting L-type Ca^{2+} channel current (Farrell et al., 2003). These data suggest that sorcin, similar to CaM, may inhibit RyR2 following SR Ca^{2+} release during EC coupling to prevent the formation of positive feedback loops.

6. Calsequestrin, Triadin, and Junctin—Calsequestrin, triadin, and junctin have been proposed to form a complex with RyR2 in the SR lumen with triadin and junctin linking calsequestrin to the channel (Gyorke & Terentyev, 2008). Calsequestrin is a low-affinity, high-capacity Ca^{2+} -binding protein which sequesters Ca^{2+} in the SR. According to a current model elevation of luminal Ca^{2+} weakens the interactions between calsequestrin, triadin, and junctin causing an increase in RyR2 P_o which normalizes SR Ca^{2+} load (Zhang et al., 1997).

III. Regulation of RyR2 in the Cardiac Response to β -adrenergic Receptor Activation

Early studies using radiolabeled ryanodine to detect channel openings noted that phosphorylation of RyR2 by PKA (Takasago et al., 1989) and CaMKII (Takasago et al., 1991) increased channel activity. Subsequent studies in planar lipid bilayer demonstrated that PKA phosphorylation of Ser2809 and CaMKII phosphorylation of Ser2815 caused a leftward shift in the sensitivity of RyR2 to cytosolic Ca^{2+} (Wehrens et al., 2004b). Similarly, PKA phosphorylation of RyR2 was reported to increase channel P_o to ~ 1.0 following flash

photolysis-induced exposure of the channel to 10 μM cytosolic Ca^{2+} compared to a P_o of ~ 0.75 for nonphosphorylated channels (Valdivia et al., 1995).

Cellular studies analyzing the effect of PKA phosphorylation on RyR2 function have been controversial. In 2004 Bers and colleagues (Ginsburg & Bers, 2004) demonstrated that under conditions where $I_{\text{Cav}1.2}$ trigger and SR Ca^{2+} load were constant, addition of the β -adrenergic receptor (AR) agonist Iso enhanced the kinetics of SR Ca^{2+} release but did not induce any changes in peak systolic Ca^{2+} transient amplitude. These findings were challenged by Niggli and colleagues (Ogrodnik & Niggli, 2010) who demonstrated that Iso increased the kinetics and Ca^{2+} transient amplitude in cells with matched Ca^{2+} trigger and SR Ca^{2+} load. Niggli and colleagues used UV-flash photolysis to rapidly uncage Ca^{2+} in order to activate RyR2, while Bers and colleagues used patch clamp to depolarize the sarcolemma to induce SR Ca^{2+} release. Additionally, Bers and colleagues used phospholamban-S16A mice that cannot be phosphorylated by PKA to maintain comparable SR Ca^{2+} loads in the absence and presence of Iso while Niggli and colleagues varied their pre-pacing SR loading protocols. Contrasting these two studies highlights the technical challenge associated with isolating the contribution of RyR2 modulation to SR Ca^{2+} release during β -adrenergic stimulation.

An intriguing possibility has been proposed that PKA phosphorylation of RyR2 contributes to synchronizing SR Ca^{2+} release (Lakatta, 2004; Wang & Wehrens, 2010). PKA phosphorylated RyR2 has a P_o of ~ 1.0 in the moments following a Ca^{2+} trigger compared to ~ 0.75 for nonphosphorylated channels (Valdivia et al., 1995), which would facilitate the likelihood of two channels opening simultaneously following membrane depolarization. Cellular studies employing a high-affinity Ca^{2+} indicator combined with EGTA (a slow Ca^{2+} buffer) to elicit Ca^{2+} spikes demonstrated that Iso enhances the simultaneous release of Ca^{2+} from multiple RyR2 clusters (Song et al., 2001). PKA phosphorylation of RyR2 may increase the speed and fidelity with which RyR2 releases Ca^{2+} in response to $I_{\text{Cav}1.2}$ (Zhou et al., 2009).

Similarly, synchronization studies in SAN cells (SANC) have suggested that PKA phosphorylation of RyR2 during a β -adrenergic response may contribute to heart rate regulation (Lakatta, 2004). Small Ca^{2+} release events have been observed during the diastolic depolarization phase in SANC and are hypothesized to activate inward NCX current and contribute to the rate of depolarization (Vinogradova et al., 2002). Taken together these studies suggest that PKA phosphorylation of RyR2 may contribute to enhancing cardiac chronotropy and inotropy during a β -adrenergic response.

Two recent studies, however, have challenged the potential role of PKA phosphorylation of RyR2 in the cardiac response to β -adrenergic activation (Benkusky et al., 2007; MacDonnell et al., 2008). In these studies mice engineered with RyR2 that cannot be PKA phosphorylated (RyR2-S2808A) were reported to have a normal inotropic and chronotropic response to Iso treatment using *in vivo* and *ex vivo* cardiac function studies (MacDonnell et al., 2008). Of note, cardiomyocytes isolated from the RyR2-S2808A mice had a blunted enhancement of systolic Ca^{2+} transient amplitude at 3 Hz but not at lower frequencies (Benkusky et al., 2007).

CaMKII activity and subsequent phosphorylation of Ser2815 on RyR2 are increased at faster heart rates (Chelu et al., 2009; Wehrens et al., 2004b) due to the “ Ca^{2+} memory” properties of CaMKII, whereby during each contraction a fraction of the CaMKII monomers become activated and that faster frequencies cause a stepwise increase in the number of activated monomers (De Koninck & Schulman, 1998). This led to the proposal that CaMKII-dependent phosphorylation of RyR2 contributes to the force-frequency response,

the observation that cardiac contractility increases as a function of heart rate (Li et al., 1997; Wehrens et al., 2004b). This hypothesis has been recently demonstrated in mice engineered lacking the CaMKII phosphorylation site on RyR2 (RyR2-S2814A) which have blunted contractility at faster heart rates (Kushnir et al., 2010b).

Recent studies indicate that β -AR stimulation activates CaMKII, in addition to activating PKA (Ferrero et al., 2007). While the exact mechanism of this phenomenon is unclear it is possible that CaMKII is activated by higher levels $[Ca^{2+}]_{cyto}$ or by activation by another kinase such as PKA or guanine nucleotide exchange protein (Epac) (Pereira et al., 2007). Indeed, transgenic mice with cardiac-specific inhibition of CaMKII have fewer SR Ca^{2+} release events during diastolic depolarization in SANC as well as a blunted heart rate in response to Iso treatment (Wu et al., 2009). This suggests that CaMKII-mediated phosphorylation of RyR2 may contribute to the cardiac response to β -adrenergic activation.

PKA phosphorylation of sorcin relieves its natural inhibition of RyR2. This suggests that phosphorylation of sorcin downstream of β -AR activation may contribute to enhancing RyR2 Ca^{2+} release during EC coupling (Lokuta et al., 1997).

Eisner and colleagues have challenged the hypothesis that modulation of RyR2 Ca^{2+} release contributes to the cardiac “fight or flight” response. Exposing cardiomyocytes to low-dose caffeine, which sensitizes RyR2 to activation by Ca^{2+} , only transiently potentiated SR Ca^{2+} release as SR Ca^{2+} stores are rapidly reduced under these conditions (Trafford et al., 2000). These results, however, also demonstrated that sensitizing RyR2 to activating Ca^{2+} increases the fractional release of Ca^{2+} from the SR (Ca^{2+} released as a function of SR Ca^{2+} load). Thus, under physiologic conditions the combination of enhanced rate of SR Ca^{2+} uptake (as occurs during a β -adrenergic response) and increased fractional release of Ca^{2+} through RyR2 would result in a sustainable elevation in Ca^{2+} transient amplitude.

IV. RyR2 Dysfunction in Heart Disease

A. RyR2 in Heart Failure

Studies in the 1990s reported alterations in intracellular Ca^{2+} handling in cardiomyocytes isolated from hearts at different stages of heart failure (HF). These changes included reduced Ca^{2+} transient amplitude, increased Ca^{2+} transient duration, prolonged Ca^{2+} transient decay time, as well as reduced SR Ca^{2+} load (Hobai & O’Rourke, 2001). This suggested that reduced systolic Ca^{2+} transient amplitude secondary to reduced SR Ca^{2+} stores was responsible for the decreased contractility and reduced cardiac output in HF. Cardiomyocytes isolated from hearts in end-stage HF also had elevated diastolic Ca^{2+} levels, which was not observed in early-stage HF hearts (Zaugg & Buser, 2001). Elevated diastolic Ca^{2+} levels in the cytosol generate transient inward current (I_{ti}) which causes DADs. Additionally, elevated diastolic $[Ca^{2+}]$ induces cell-to-cell uncoupling (Kleber, 1992), which slows the velocity of a propagating impulse, increasing the propensity for reentry circuit formation in the presence of an arrhythmogenic substrate (Fig. 2).

Pathologic reduction in SERCA2a function and expression as well as enhanced NCX activity was originally proposed to explain why SR Ca^{2+} load is lower in HF (Hobai & O’Rourke, 2001; Mercadier et al., 1990; Pogwizd & Bers, 2004). Some suggested that increased NCX activity compensated for the reduced SERCA2a activity in early-stage HF but that continuous uncompensated decline of SERCA2a activity in end-stage HF resulted in elevated baseline Ca^{2+} (Zaugg & Buser, 2001). In fact, over-expressing SERCA2a in cardiomyocytes isolated from patients with failing hearts improves contraction velocity and decreases diastolic $[Ca^{2+}]$ and has been proposed as a possible therapy for HF (del Monte et al., 1999).

Over the past 10 years the role of pathological diastolic Ca^{2+} leak through dysfunctional RyR2 has been recognized as an important contributor to altered Ca^{2+} handling in HF (Marx et al., 2000). The original HF RyR2 Ca^{2+} leak hypothesis was that the chronic hyperadrenergic state observed in HF patients induced chronic PKA hyperphosphorylation of RyR2 at Ser2808, causing depletion of calstabin2 (Marx et al., 2000) from the channel complex. The term hyperphosphorylation describes RyR2 in which 3–4 of the four RyR2 monomers are chronically PKA phosphorylated. PKA hyperphosphorylated/calstabin2 depleted channels are sensitized to cytosolic Ca^{2+} leading to inappropriate Ca^{2+} release during diastole, referred to as a diastolic SR Ca^{2+} leak. Ca^{2+} leak would reduce SR Ca^{2+} stores and activate I_{ti} (Lehnart et al., 2004b).

The HF RyR2 Ca^{2+} leak hypothesis is supported by studies demonstrating that HF patients have PKA hyperphosphorylated and calstabin2 depleted RyR2. Furthermore, patients whose cardiac function was restored by implantable left ventricular assist devices (LVAD) had reduced levels of circulating catecholamines (Estrada-Quintero et al., 1995) and reduced phosphorylation of RyR2 at Ser2809. Normalization of the RyR2 complex is associated with the improved cardiac function observed during the short period post-LVAD explantation (Marx et al., 2000).

The HF RyR2 Ca^{2+} leak hypothesis has been challenged based on the observation that β -AR density on cardiomyocytes is reduced in HF (Bristow et al., 1982) and that this coincides with a global reduction in cytosolic cAMP levels. Accordingly it is unclear how the presence of a chronic hyperadrenergic state in HF can lead to chronic PKA hyperphosphorylation of RyR2. This paradox can be explained by the general remodeling of the RyR2 macromolecular complex and depletion of phosphatases (Reiken et al., 2001) and PDE4D3 (Lehnart et al., 2005) that occurs in HF. Localized depletion of PDE4D3 and phosphatases can induce discrete microdomains of elevated levels of cAMP in the vicinity of RyR2 (Lehnart et al., 2005) and decreased rate of dephosphorylation of a hyperphosphorylated channel.

The HF RyR2 Ca^{2+} leak model provides a novel mechanism to explain the therapeutic efficacy of β -AR blockers in HF: by preventing stimulation of β -ARs and downstream activation of PKA in failing hearts, thereby reducing phosphorylation at Ser2809 and causing calstabin2 to rebind to the channel and reduce SR Ca^{2+} leak. This proposal is supported by studies showing that HF patients taking β -blockers have reduced phosphorylation of RyR2 at Ser2809 and enhanced calstabin2 binding to RyR2 (Reiken et al., 2003). Furthermore, RyR2 isolated from HF patients which was reconstituted in planar lipid bilayer had elevated Po at diastolic $[\text{Ca}^{2+}]$ which was reduced in patients on β -blockers (Reiken et al., 2003).

Evidence from multiple animal models supports a role for PKA hyper-phosphorylation of RyR2 in HF progression (Kushnir et al., 2010a). Mice engineered with an RyR2-S2808A mutation, RyR2 that cannot be PKA phosphorylated, were not depleted of calstabin2 4 weeks post-myocardial infarction (MI) and were protected from the deleterious progression of cardiac dysfunction observed in wild-type (WT) mice 4 weeks post-MI (Wehrens et al., 2006). Mice engineered lacking PDE4D3 develop cardiomyopathy and arrhythmias which coincide with progressive PKA hyperphosphorylation of RyR2 and calstabin2 depletion. These mice also had accelerated decline in cardiac function post-MI. A cross between the PDE4D3 deficient mice and the RyR2-S2808A mice results in mice which are protected from the deleterious effects of PDE4D3 deficiency (Lehnart et al., 2005). These PDE4D3 studies provide a novel mechanism to explain the arrhythmogenic propensity of PDE inhibitors such as theophylline. It should be noted that these drugs have also been reported

to enhance the sensitivity of RyR2 to luminal Ca^{2+} which could also explain their arrhythmogenic potential (Kong et al., 2008).

Direct evidence that calstabin2 depletion from RyR2 contributes to HF progression comes from transgenic mice overexpressing a mutant D37V-calstabin2, which remains bound to PKA phosphorylated RyR2 channels. Cardiac dysfunction post-MI is ameliorated in these mice (Huang et al., 2006). Mice engineered lacking calstabin2 suffer from exercise-induced ventricular tachycardia (VT) which parallels the presence of DADs in cardiomyocytes isolated from these mice (Wehrens et al., 2003). Furthermore, RyR2 isolated from calstabin2-deficient mice exhibits relatively normal Po at baseline which is drastically increased when the mice are exercised.

The HF RyR2 Ca^{2+} leak hypothesis introduces novel therapeutic opportunities for HF. JTV-519 (K201), a 1,4-benzothiazepene derivative, was originally discovered based on its cardioprotective effects in a myofibrillar overcontraction model of myocardial injury, possibly related to the drug's ability to block intracellular Ca^{2+} overload (Kaneko, 1994). While early reports lacked a clear mechanism of action for JTV-519 later studies reported that JTV-519 nonspecifically inhibited transmembrane Na^+ , Ca^{2+} , and K^+ currents (Kimura et al., 1999) and that the cardioprotective effects of the drug were abolished when hearts were pretreated with inhibitors of protein kinase C (Inagaki et al., 2000; Ito et al., 2000) or nitric oxide synthase (Kawabata et al., 2002).

In 2003 Matsuzaki and colleagues, using microsomes isolated from canine failing hearts, reported that JTV-519 stabilized RyR2 by improving binding of calstabin2 to RyR2 (Kohn et al., 2003). *In vivo* HF studies demonstrated that pretreatment with JTV-519 prevented rapid right ventricular pacing-induced HF in canines (Yano et al., 2003). Studies with homozygous and heterozygous calstabin2-deficient mice provided *in vivo* evidence that the cardioprotective effects of JTV-519 depend on stabilizing the interaction of calstabin2 with RyR2. Exercising homozygous and heterozygous calstabin2-deficient mice induces fatal ventricular arrhythmias due to SR Ca^{2+} leak which can be prevented by pretreating heterozygous calstabin2-deficient mice with JTV-519. JTV-519 does not protect the calstabin2-deficient mice (Wehrens et al., 2004a). Furthermore, treating WT mice with JTV-519 ameliorated the decline in cardiac function post-MI while treating calstabin2-deficient mice with JTV-519 did not (Wehrens et al., 2005). The observation that JTV-519 does not alter the gating properties of non-diseased ryanodine receptor channels and that the drug causes no observable effects in healthy dogs and mice substantiates its therapeutic potential (Lehnart et al., 2008; Wehrens et al., 2004a; Yano et al., 2003).

The HF RyR2 Ca^{2+} leak hypothesis has generated significant controversy with reports supporting (Ono et al., 2000; Yano et al., 2000) and challenging it (Benkusky et al., 2007; Jiang et al., 2002; MacDonnell et al., 2008; Xiao et al., 2007a). Chen and colleagues have published a series of reports challenging this hypothesis. In one study, this group reported that RyR2 isolated from human and canine failing hearts had similar levels of associated calstabin2 and similar Ca^{2+} sensitivity as RyR2 from non-failing hearts (Jiang et al., 2002). The investigators concluded that the reduction in SR Ca^{2+} stores was secondary to a reduction in SERCA2a expression. In another study it was reported that JTV-519 suppresses spontaneous Ca^{2+} release independent of calstabin2 binding to RyR2 (Hunt et al., 2007). This group also reported that they were unable to induce arrhythmias in calstabin2-deficient mice using combined administration of epinephrine and caffeine (Xiao et al., 2007a). Valdivia and colleagues demonstrated that the RyR2-S2808A mice are not protected from HF induced by thoracic aortic constriction (Benkusky et al., 2007). Of note, in this study the investigators reported that fractional shortening was reduced in WT mice but not in the RyR2-S2808A mice suggesting that the mutation imparted cardioprotection.

Alternative mechanisms have been proposed to explain SR Ca^{2+} leak in HF. Disulfide oxidation of free cysteine residues on RyR2 increases the sensitivity of the channel to luminal Ca^{2+} in lipid bilayer as well as enhances SR Ca^{2+} leak, manifested by reduced SR Ca^{2+} load, in cardiomyocytes (Terentyev et al., 2008). Matsuzaki and colleagues have proposed that redox modification of RyR2 in HF disrupts the interaction between N-terminal (amino acids 1–600) and central domains (2000–2500) of the channel complex, which causes dissociation of CaM from the channel and diastolic Ca^{2+} leak (Oda et al., 2005; Ono et al., 2010; Yano et al., 2009). Furthermore this group has proposed that JTV-519 binds to RyR2 between amino acids 2114 and 2149 and stabilizes the inter-domain interactions of the channel (Yamamoto et al., 2008). These studies also suggest that enhancing binding of CaM to RyR2 may have therapeutic potential in HF.

Chen and colleagues have proposed that RyR2 has increased sensitivity to luminal Ca^{2+} in HF so that when SR Ca^{2+} load is elevated under stressful conditions (i.e., activation of the β -AR cascade) this leads to inappropriate Ca^{2+} “spillover” during diastole (store overload-induced Ca^{2+} release or SOICR) (Xiao et al., 2007). However, it is unclear according to this model how the sensitivity of RyR2 to luminal Ca^{2+} is modified in HF (Fig. 3).

CaMKII δ levels are elevated in human HF samples (Hoch et al., 1999) suggesting that CaMKII phosphorylation of RyR2 may contribute to the pathogenesis of HF. This hypothesis is supported by reports that there is an increase in CaMKII-dependent phosphorylation of RyR2 in HF, which enhances SR Ca^{2+} leak (Ai et al., 2005). Mice overexpressing CaMKII δ have enhanced Ca^{2+} spark frequency (Maier et al., 2003) and develop cardiomyopathy (Zhang et al., 2003). Additionally, mice expressing the CaMKII inhibitory peptide AC3-I are cardioprotected from ischemic HF (Zhang et al., 2005) and pressure overload-induced HF (Ling et al., 2009). Based on these studies it has been proposed that CaMKII inhibitors may have therapeutic potential for preventing HF progression. However, mice engineered with RyR2 that cannot be phosphorylated by CaMKII (RyR2-S2814A) are not cardioprotected in a model of ischemic HF suggesting that the pathological consequences of CaMKII activation in HF are not a result of CaMKII phosphorylation of RyR2 (Kushnir et al., 2010b).

B. RyR2 in Atrial Fibrillation

Atrial fibrillation (AF) is a major cause of morbidity and mortality and the pathophysiology of this disease has not been elucidated. The clinical signs of AF are an irregularly irregular rhythm with a concomitant loss of P-waves (atrial depolarization) and the presence of F-waves in the electrocardiogram. Fibrillating atria lack unifocal pacing and may consist of multiple small reentry circuits or ectopic triggers (Nattel et al., 2008).

Early studies on canine atrial myocytes reported altered Ca^{2+} handling in cells isolated from atria with AF (Sun et al., 1998). RyR2 isolated from canine atria in AF was found to be PKA hyperphosphorylated (Chelu et al., 2009; Vest et al., 2005), depleted of calstabin2, and had increased Po at diastolic $[\text{Ca}^{2+}]$ (Vest et al., 2005) suggesting a link between SR Ca^{2+} leak and AF. Furthermore calstabin2-deficient mice have an increased propensity for developing AF and atrial myocytes isolated from these mice have enhanced SR Ca^{2+} leak (Sood et al., 2008).

Human and goat atria with AF have elevated levels of activated CaMKII and enhanced phosphorylation of RyR2 at Ser2815 suggesting that CaMKII phosphorylation of RyR2 may be involved in the pathogenesis of AF (Chelu et al., 2009; Neef et al. 2010). This is supported by observations that mice with RyR2 that cannot be phosphorylated by CaMKII (RyR2-S2814A) are protected from developing AF (Chelu et al., 2009). Additionally, Ca^{2+} spark frequency and diastolic $[\text{Ca}^{2+}]$ are both elevated and SR Ca^{2+} stores are depleted in

atrial myocytes isolated from human atria with AF (Neef et al., 2010). Pharmacological inhibition of RyR2 with tetracaine reduces cytosolic Ca^{2+} levels back to baseline confirming that Ca^{2+} leak through RyR2 contributes to the Ca^{2+} abnormalities in these cells (Neef et al., 2010).

The role of RyR2 Ca^{2+} leak in arrhythmogenesis has been challenged based on the observation that increasing RyR2 Po with low-dose caffeine induces Ca^{2+} waves only at high SR Ca^{2+} loads. Accordingly, pathological modification of RyR2 which would induce Ca^{2+} leak would ultimately lead to a reduction in SR Ca^{2+} load to the point where Ca^{2+} waves no longer appear (Venetucci et al., 2007).

C. RyR2 in Catecholaminergic Polymorphic Ventricular Tachycardia

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare, familial, exercise-induced polymorphic VT that occurs in the absence of gross myocardial morphological abnormalities. Approximately 10 years ago two independent groups identified several RyR2 mutations in families of patients with CPVT (Priori et al., 2001; Swan et al., 1999). To date 71 mutations linked to CPVT have been identified in three “hot spot” regions of RyR2 (a current list is available at <http://www.fsm.it/cardmoc>).

Three hypotheses have been proposed to explain how mutations in RyR2 lead to SR Ca^{2+} leak and arrhythmias. Marks and colleagues have observed that CPVT mutants RyR2-S2246L, RyR2-R2474S, and RyR2-R4497C all have reduced affinity for calstabin2 (Wehrens et al., 2003). At rest these channels have similar Po compared to WT channels; however, PKA phosphorylation of these mutant channels results in increased Po at diastolic $[\text{Ca}^{2+}]$. This pathological phenotype can be prevented by addition of calstabin2-D37S, a mutant calstabin2 that binds to phosphorylated RyR2 (Wehrens et al., 2003). Furthermore, treating mutant CPVT channels with JTV-519 restores calstabin2 binding to the channels and reduces Po at diastolic $[\text{Ca}^{2+}]$ (Lehnart et al., 2004a). Mice engineered with the RyR2-R2474S human CPVT mutation have normal sinus rhythm at baseline and develop ventricular arrhythmias when exercised. These arrhythmias can be prevented by treating the mice with S107, a drug similar to JTV-519, which rebinds calstabin2 to the CPVT mutant channels and prevents diastolic SR Ca^{2+} leak (Lehnart et al., 2008).

S107 was discovered as part of a project to identify compounds with similar RyR/calstabin stabilizing properties as JTV-519 that did not significantly interact with other ion channels. Other favorable drug properties, such as oral availability and stability, were also taken into account during this screen (Bellinger et al., 2008). The therapeutic efficacy of S107 was originally demonstrated in skeletal muscle where mice treated with S107 had enhanced binding of calstabin to RyR1 in skeletal muscle and improved muscle force generation and exercise capacity (Bellinger et al., 2008). S107 was also able to reduce the incidence of arrhythmias (Fauconnier et al., 2010) and improve muscle function (Bellinger et al., 2009) in mice with Duchenne muscular dystrophy by reducing pathologic SR Ca^{2+} leak in cardiac and skeletal muscle. Incidentally, the ability for S107 to stabilize dysfunctional RyR2 offers therapeutic opportunities for pathologies in other organ systems that are associated with RyR2 dysfunction. Recently, mutations in RyR2 which reduce the affinity of calstabin2 to RyR2 have been linked to seizures in humans (Johnson et al., 2010) and mice and treating these mice with S107 prevented the seizures (Lehnart et al., 2008). While the role of RyR2 in the brain is unknown this study demonstrates the therapeutic potential of drugs targeting dysfunctional RyR2 in other systems other than cardiac and skeletal muscle.

Chen and colleagues have proposed that CPVT mutations in RyR2 sensitize the channel to luminal (SR) Ca^{2+} such that under baseline conditions, where SR load is normal, there is no Ca^{2+} leak. When β -ARs are activated during a sympathetic response SR $[\text{Ca}^{2+}]$ is elevated

above the reduced threshold, causing Ca^{2+} to leak out of the SR (SOICR). The SOICR-CPVT hypothesis was developed based on the observation that HEK cells expressing recombinant CPVT-mutant-RyR2 had increased sensitivity (manifested as spontaneous Ca^{2+} oscillations) to progressively higher extracellular $[\text{Ca}^{2+}]$ compared to cells expressing WT RyR2 (Jiang et al., 2005).

Matsuzaki and colleagues have proposed that CPVT mutations induce unzipping of the N-terminal and central domains of RyR2 which causes the channel to become leaky (Uchinoumi et al., 2010). This hypothesis is supported by experiments using a small peptide, DPc10, which intercalates with the central domain of RyR2 to cause channel unzipping and Ca^{2+} leak in WT cardiomyocytes. In cardiomyocytes isolated from RyR2-R2474S mice, a CPVT mouse model, Ca^{2+} spark frequency is elevated at baseline and is not amplified by addition of the peptide (Uchinoumi et al., 2010). CaM binding to RyR2 is disrupted by channel unzipping in mutant myocytes but not in WT cells suggesting that depletion of CaM from RyR2 secondary to channel unzipping may contribute to the pathogenesis of CPVT (Xu et al., 2010). In all these studies the zipped state of RyR2 is confirmed by changes in the signal from fluorescent markers incorporated into the channel.

Dantrolene, a drug used to prevent malignant hyperthermia in patients with mutations in RyR1 who have been exposed to volatile anesthetics, has been proposed to have therapeutic potential in heart disease by causing “re-zipping” of the amino and central domains of RyR2 (Kobayashi et al., 2009; Uchinoumi et al., 2010). RyR2 isolated from dogs with HF and mice engineered with a CPVT mutation (RyR2-R2474S) had unzipped RyR2 which could be “re-zipped” by treating the defective channels with dantrolene. Furthermore, dantrolene reduced Ca^{2+} spark frequency in cardiomyocytes isolated from these animal models. The authors in these studies have proposed that dantrolene binds to RyR2 between amino acids 601 and 620 (Kobayashi et al., 2009). This hypothesis has been challenged by reports that dantrolene specifically inhibits RyR1 and RyR3 but does not affect the gating properties of RyR2 (Zhao et al., 2001). However, in this report the investigators studied normal channels. Altogether, these data suggest that dantrolene may specifically stabilize dysfunctional RyR2 without affecting the gating properties of normal RyR2 (Kobayashi et al., 2009).

The Na^+ channel antagonist flecainide has recently been demonstrated to prevent lethal ventricular arrhythmias in mice and humans carrying CPVT mutations (Watanabe et al., 2009). Flecainide has been proposed to reduce Ca^{2+} spark amplitude by inducing brief closures of open RyR2 to subconductance states which reduces burst mass without affecting channel closed time (Hilliard et al., 2010).

D. RyR2 in Sudden Infant Death Syndrome

Sudden infant death syndrome (SIDS) describes idiopathic sudden death in infants less than 1 year of age and is a leading cause of postnatal mortality in developed countries. Mutations R2267H and S4565R in RyR2 have been identified in children that have died from SIDS (Tester et al., 2007). These mutations enhance the sensitivity of RyR2 to cytosolic Ca^{2+} during β -adrenergic stress, similar to mutations in RyR2 that cause CPVT. Mice engineered with the R176Q CPVT mutation have a higher rate of postnatal mortality. Death in these animals is due to abnormal Ca^{2+} release and ectopic activity as determined by isochronal Ca^{2+} and voltage mapping of isolated neonatal hearts (Mathur et al., 2009). Mice engineered with the highly arrhythmogenic R2474S CPVT mutation have increased rate of intrauterine lethality, which can be prevented by treating the pregnant mothers with S107 (Lehnart et al., 2008).

V. Conclusion

Many technical approaches have been used to isolate and study RyR2 function including the planar lipid bilayer, Ca^{2+} fluorescence in HEK cells, permeabilized cardiomyocytes, patch clamping, flash photolysis, and *in vivo* and *ex vivo* cardiac function studies on genetically modified mouse models. Despite many advances in our understanding of RyR2, consensus has not achieved on some fundamental questions: What are the PKA and CaMKII phosphorylation sites on RyR2? What is the effect of phosphorylation on the channel macromolecular complex and channel function? What causes RyR2 dysfunction in HF and CPVT? Notwithstanding these issues many studies now agree that diastolic SR Ca^{2+} leak through dysfunctional RyR2 contributes to the pathogenesis of HF and various arrhythmias. The development of novel experimental techniques and additional genetically engineered mouse models will help clarify many discrepancies that currently exist in the field of RyR2 research and will aid in the development of novel therapeutics to target Ca^{2+} leak in patients suffering from various forms of heart disease.

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Non standard abbreviations

Cav1.2	cardiac L-type calcium channels
CPVT	catecholaminergic polymorphic ventricular tachycardia
FKBP	FK506-binding protein
HF	heart failure
RyR	ryanodine receptor
SAN	sinoatrial node
SR	sarcoplasmic reticulum

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FIGURE 1.

Cardiac action potential: Depolarization is initiated by opening of voltage-gated Na^+ channels followed by the opening of voltage-gated Ca^{2+} and K^+ channels. Influx of Ca^{2+} into the cytosol during phase 2 of the cardiac action potential triggers Ca^{2+} release from the sarcoplasmic reticulum (SR) through the ryanodine receptor (RyR2). Baseline $[\text{Na}^+]$ and $[\text{K}^+]$ are restored by the Na^+/K^+ ATPase (NKA). RyR2 is a homotetrameric macromolecular complex.

FIGURE 2.

Arrhythmias induced by cytosolic Ca^{2+} overload: (A) Elevated cytosolic $[\text{Ca}^{2+}]$ causes transient inward current through the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) which can generate delayed after depolarizations (DADs). (B) Cardiomyocytes eliciting DADs can become ectopic foci of automaticity which can evolve into reentry circuits to cause (C) premature ventricular contractions (PVCs) on the electrocardiogram (ECG). (D) If uncorrected this may deteriorate into ventricular fibrillation (VF) and sudden cardiac death.

FIGURE 3.

Mechanisms of RyR2 Ca^{2+} leak in HF: (A) PKA hyperphosphorylation of RyR2 at Ser2808 and depletion of calstabin2 from the channel causes SR Ca^{2+} leak. (D) The therapeutic efficacy of JTV-519 is based on the ability of the drug to stabilize the interaction between calstabin2 and RyR2. (B) Store overload-induced Ca^{2+} release (SOICR), RyR2 has increased sensitivity to luminal $[\text{Ca}^{2+}]$ which causes Ca^{2+} to leak out of the channel. (E) According to this model JTV-519 stabilizes RyR2 independent of calstabin2. (C) Unzipping of the amino and central domains of RyR2 causes the channel to become leaky. (F) JTV-519 stabilizes the zipped state of the channel.