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Dysregulated sarcoplasmic reticulum calcium release: Potential pharmacological target in cardiac disease

Sandor Györke[a,*] and **Cynthia Carnes**[b]

[a]Department of Physiology and Cell Biology, The Ohio State University, Columbus, OH, United States

[b]College of Pharmacy, Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, United States

Abstract

In the heart, Ca^{2+} released from the intracellular Ca^{2+} storage site, the sarcoplasmic reticulum (SR), is the principal determinant of cardiac contractility. SR Ca^{2+} release is controlled by dedicated molecular machinery, composed of the cardiac ryanodine receptor (RyR2) and a number of accessory proteins, including FKBP12.6, calsequestrin (CASQ2), triadin (TRD) and junctin (JN). Acquired and genetic defects in the components of the release channel complex result in a spectrum of abnormal Ca^{2+} release phenotypes ranging from arrhythmogenic spontaneous Ca^{2+} releases and Ca^{2+} alternans to the uniformly diminished systolic Ca^{2+} release characteristic of heart failure. In this article, we will present an overview of the structure and molecular components of the SR and Ca^{2+} release machinery and its modulation by different intracellular factors, such as Ca^{2+} levels inside the SR as well as phosphorylation and redox modification of RyR2s.We will also discuss the relationships between abnormal SR Ca^{2+} release and various cardiac disease phenotypes, including, arrhythmias and heart failure, and consider SR Ca^{2+} release as a potential therapeutic target.

Keywords

Ryanodine receptor; Calcium release; Calsequestrin; Junctin; Triadin; Cardiomyopathy; Arrhythmias

1. Introduction

Despite significant advances in treatments, cardiac diseases remain the leading cause of mortality and mor!bidity in Western nations. Development of new efficient treatment strategies requires understanding of basic mechanisms underlying cardiac function. Since the classical experiments of Dr. Sydney Ringer, Ca^{2+} has been recognized as a principal agent governing the mechanical activity of the heart (Ringer, 1883). On a beat-to-beat basis, Ca^{2+} derived from the intracellular Ca^{2+} storage site, the sarcoplasmic reticulum (SR), results in activation of contractile filaments and shortening of cardiac myocytes. Alterations in timing and magnitude of SR Ca^{2+} release result in different cardiac diseases such as arrhythmia and heart failure. SR Ca^{2+} release is controlled by specialized molecular machinery, composed of the cardiac ryanodine receptor (RyR2) and a number of accessory proteins, including FKBP12.6, calsequestrin (CASQ2), triadin (TRD) and junctin (JN). This multimolecular Ca^{2+} signaling complex is essential to normal Ca^{2+} cycling and is a site of potential pathologic failure. In this

*Corresponding author. Fax: +1 614 247 7799. E-mail address: sandor.gyorke@osumc.edu (S. Györke).

review, we will present an overview of the molecular organization of the Ca^{2+} release machinery and its modulation by different intracellular factors, such as Ca^{2+} levels inside the SR as well as phosphorylation and redox modification of RyR2s.We will also consider the relationships between abnormal SR Ca^{2+} release and various cardiac disease phenotypes, including, catecholaminergic polymorphic ventricular tachycardia, triggered and reentrant arrhythmias and heart failure. Finally we will consider SR Ca^{2+} release as a potential therapeutic target.

2. The sarcoplasmic reticulum

2.1. Structure and continuity

Striated muscle sarcoplasmic reticulum is a highly specialized form of endoplasmic reticulum (ER), which was described first in detail for both skeletal and cardiac muscle as an extensive network of interconnected cisternae and tubules throughout the sarcoplasm (Fawcett & McNutt, 1969; Sommer, 1995; Franzini-Armstrong & Protasi, 1997). Estimates of the total SR volume in ventricular myocytes of different mammalian species vary between 0.87 and 7.9% of cell volume, and it is commonly assumed to constitute 3.5% of cell volume (Bers, 2001). The SR consists of two distinct regions: junctional SR (jSR), which directly faces the transverse tubules, and free SR (fSR) which faces the myofibrils and does not form junctions with other membrane systems. See Fig. 1A and B. The total SR volume is divided approximately equally between jSR and fSR. In rabbit ventricular myocytes, jSR forms extended, flattened cisternae with an average diameter of 592 nm (Brochet et al., 2005). Each cistern carries 66 RyR2s clustered in one or two release units on the junctional membrane. The jSR contains an electron dense material which is formed by the Ca^{2+} binding protein calsequestrin (CASQ2) (Tijskens et al., 2003). The free SR is devoid of internal content, and its external surface exhibits densely distributed 8 nm particles representing the Ca^{2+} ATPase molecules. Although topologically continuous with the rest of the SR network, the connectivity of an average jSR cistern to fSR is limited to a few narrow passages; therefore, diffusional barriers for the free translocation of Ca^{2+} and other molecules exist between these compartments (Brochet et al., 2005). The restricted connections between jSR and fSR facilitates the generation of local luminal Ca^{2+} gradients, which are thought to play an important role in luminal Ca^{2+} signaling (Gyorke and Terentyev, 2008).

2.2. SR as a Ca^{2+} storage and release organelle

The Ca^{2+} stored in and released from the SR is a critical determinant of cardiac contractility. Additionally intra-SR Ca^{2+} levels play a key role in SR-mediated Ca^{2+} signaling by controlling the functional activity of RyR2s. In mammalian cardiac myocytes, the total SR Ca^{2+} content has been estimated to be in the range of 50–150 μmol/ l in the cytosol (or 1.4–3 mmol/l of SR volume), considering that SR comprises 3.5% of cell volume (Bers, 2001). Due to the presence of substantial amounts of Ca^{2+} binding sites in the SR, only a fraction of this Ca^{2+} is free. According to various estimates, 50 to 90% of the total Ca^{2+} is bound to Ca^{2+} buffering proteins such as CASQ2 (Shannon & Bers,1997; Shannon et al., 2000), and the existence of large reserves of bound Ca^{2+} permits the SR to serve as an efficient intracellular Ca^{2+} storage site despite its minute space. Whereas bound Ca^{2+} contributes to the storage capacity of the SR, free Ca^{2+} plays an important role in store operation by setting thermodynamic limits on Ca^{2+}ATPase Ca^{2+} transport activity, establishing the driving force for SR Ca^{2+} release, and modulating RyR2 activity. As measured directly by low affinity Ca^{2+} dyes loaded into the SR, the resting (diastolic) free $[Ca^{2+}]$SR constitutes 1 to 1.5 mM and becomes only partially depleted (24% to 63%) during contraction (Shannon et al., 2003a). See Fig. 1C and D. Local depletion signals in individual SR cisternae during Ca^{2+} sparks (termed “blinks”) have been measured in myocytes from both normal and diseased hearts (Brochet et al., 2005; Kubalova et al., 2005). The occurrence of local SR Ca^{2+} depletion is consistent with restricted diffusion

of Ca^{2+} from fSR to jSR (described above). Indeed, a rapid refilling of local jSR Ca^{2+} store from the bulk of the SR would be expected to prevent significant local depletion. Interestingly, as determined by luminal Ca^{2+} measurements with Fluo5N, Ca^{2+} diffusion through the SR network along the length of the myocyte is unexpectedly fast (60 $\mu m^2\ s^{-1}$,(Guo et al., 2007). This reinforces the notion that diffusion barriers between jSR and the rest of the SR network must exist to explain jSR Ca^{2+} depletion during localized Ca^{2+} release events.

3. The Ca^{2+} release channel complex

The cardiac Ca^{2+} release channel is a macromolecular complex composed of more than a dozen of proteins centered around the RyR2 (Franzini-Armstrong & Protasi, 1997; Zhang et al., 1997; Marx et al., 2000; Bers, 2004). See Fig. 2. The cardiac RyR2 is composed of four homologous subunits of 560KD that are encoded by a separate gene of three existing genes (Nakai et al., 1990; Fill & Copello, 2002). While a significant portion of the RyR2 extends into the SR lumen, the bulk of the RyR2 homotetramer is situated on the cytosolic side of the SR membrane (visible on EM micrographs and described as the “foot”); it carries multiple sites for cytosolic regulatory molecules (Ca^{2+}, ATP, etc.) as well as docking sites for accessory regulatory proteins (FKBP12.6, calmodulin, sorcin, etc.). On the luminal side, the RyR2 is associated with a number of proteins, including TRD, JN and CASQ2. TRD and JN bind directly to RyR2 and each other and mediate interactions of CASQ with RyR2. The structure of RyR2 at an atomic resolution remains to be resolved. Structure–function studies, and analysis of mutational hot spots revealed several key regions, referred to as domains (Ikemoto & Yamamoto, 2002; George et al., 2007). Interactions between these domains (domain “unzipping”) are thought to underlie transitions between conformations corresponding to open and closed states of the channel, as well as transduction of regulatory events, and pathologic influences from both the cytosolic and luminal side.

3.1. FK binding protein 12.6

FK binding proteins (FKBPs) are immunophilins, and are so named based on binding to the immunosuppressant tacrolimus (originally named FK506) (Brillantes et al., 1994). In cardiac muscle, FKBP12 and FKBP12.6 are present (Jayaraman et al., 1992; Timerman et al., 1993; Timerman et al., 1996), and FKBP12.6 has been put forward as a key modulator of RyR2 function in both health and disease (Marx et al., 2000; Wehrens et al., 2005a; Yano et al., 2005). However, the role of FKBP12.6 in regulating RyR2 function remains highly controversial. FKBP12.6 is preferentially associated with RyR2 due to higher affinity (Timerman et al., 1996). The binding stoichiometry is one FKBP12.6 per RyR subunit, but the FKBP12.6 binding site in the RyR2 linear sequence has not been precisely identified. Dissociation of FKBP12.6 has been reported to result in increased RyR2 activity. It has been suggested that PKA phosphorylation at 2809 results in dissociation of FKBP12.6 from the RyR2 with a subsequent increase in the open probability of the RyR2 (Brillantes et al., 1994; Ahern et al., 1994; Kaftan et al., 1996). However, other studies have failed to detect any changes in RyR2 function upon dissociation of FKBP12.6 (Barg et al., 1997; Xiao et al., 2007). Furthermore, it has been reported that FKBP12.6 remains attached to RyR2 following phosphorylation (Stange et al., 2003; Xiao et al., 2004).

3.2. Calsequestrin

Calsequestrin (CASQ) is an intra-SR protein which has a low affinity and high-capacity for Ca^{2+} binding (MacLennan & Wong, 1971; Campbell et al., 1983; Mitchell et al., 1988; Beard et al., 2004; Gyorke & Terentyev, 2008). In the SR, calsequestrin exists as a mixture of monomers, dimers, and various multimers. When Ca^{2+}concentrations exceed 1–10 mmol/l, CASQ monomers and dimers form linear oligomers, and these polymers (Park et al., 2004) can be detected as electron-dense filamentous matrices both in vitro and in vivo (Somlyo et

al., 1981; Saito et al., 1984; Franzini-Armstrong et al., 1987). CASQ polymers are primarily located in the junctional SR (jSR) in both skeletal and cardiac muscle (isoforms CASQ1 and CASQ2, respectively) (Jorgensen & Campbell, 1984). Due to the proximity of CASQ matrices to RyR2, CASQ2 polymers are thought to provide the stores of releasable Ca^{2+} which activate myocyte contraction. In this regard, condensed CASQ represents the high Ca^{2+} binding capacity form of the protein and possesses at least twice the Ca^{2+} binding capacity of monomeric CASQ in both skeletal and cardiac muscle (Tanaka et al., 1986; Park et al., 2004). CASQ condensation reportedly involves head-to-tail oligomerization that occurs through front-to-front and back-to-back dimerization contacts involving the N-and C-terminal regions of the protein, respectively (Wang et al., 1998; Park et al., 2004). While the precise mechanism(s) of Ca^{2+} sequestration by CASQ is not known, it has been suggested that CASQ polymers provide electrostatically charged surfaces for absorption of Ca^{2+} (Wang et al., 1998).

In addition to serving as a Ca^{2+} storage site, cardiac calsequestrin (CASQ2) has also been shown to regulate RyR2 activity more directly via protein–protein interactions involving triadin (TRD) and junctin (Jn) (Gyorke et al., 2004; Gyorke & Terentyev, 2008; Terentyev et al., 2007). Both the N-terminal (Zhang et al., 1997; Kobayashi et al., 2000) and C-terminal regions of CASQ2 have been suggested as the triadin (and/or junctin) interaction sites (Shin et al., 2000). It has been proposed that CASQ2mediates the responsiveness of the RyR2 channel to luminal Ca^{2+} by serving as a luminal Ca^{2+} sensor (Gyorke et al., 2004; Terentyev et al., 2007; Gyorke & Terentyev, 2008). In particular, we demonstrated that both triadin 1 and junctin exert potentiatory influences on RyR2 activity (Gyorke et al., 2004). Thus, at low luminal Ca^{2+}, CASQ2 is tightly bound to triadin 1 (junctin) and prevents it from activating the RyR2 channel. When luminal Ca^{2+} is increased, the binding of Ca^{2+} to CASQ2 disrupts the interactions of CASQ2with TRD thereby relieving the inhibitory influence of CASQ2 on the RyR2 channel. Thus, luminal Ca^{2+}-dependent termination of Ca^{2+} release could be partly attributed to inhibition of the RyR2 by CASQ2 at lowered $[Ca^{2+}]_{SR}$. At the same time, when there is an increased $[Ca^{2+}]_{SR}$, the increase in the fraction of CASQ2-uninhibited RyR2 channels could contribute to the known enhancement of diastolic SR Ca^{2+} leak in cardiac myocytes. Modulation of channel function seems to be done by the monomer as inhibition occurs at low concentrations when CASQ2 exists as a monomer. In summary, CASQ2 appears to play at least two different roles in cardiac myocytes: as a Ca^{2+} storage reservoir in the SR, and as an active modulator of the Ca^{2+} release process, mediated by monomers.

3.3. Triadin and junctin

Triadin (TRD) and junctin (JN) are transmembrane proteins in the junctional SR that appear to bind directly to the RyR2, and to CASQ2 thereby anchoring CASQ2 to the RyR2 channel (Jones et al., 1995; Kobayashi & Jones, 1999; Kobayashi et al., 2000; Gyorke & Terentyev, 2008). Three isoforms of triadin (35, 40 and 92 kD) have been described of which triadin 1 (40 kD) is the predominant, representing more than 95% of the cardiac protein (Guo et al., 1996; Kobayashi & Jones, 1999). Junctin was identified as a 26-kDa CASQ2 binding protein in cardiac and skeletal muscle (Jones et al., 1995). Triadin 1 and junctin exhibit significant structural similarities. Both proteins are composed of a single membrane spanning domain, a short cytoplasmic N-terminal segment and a long, highly positively charged C-terminal domain situated in the lumen of the SR. The luminal domains of both proteins are characterized by the frequent occurrence of long stretches of alternating positively and negatively charged residues known as KEKE motifs (Jones et al., 1995; Kobayashi et al., 2000). These regions, in general denoted as protein–protein binding motifs, are thought to be involved in interactions between triadin 1 and junctin themselves and in interactions with RyR2 and CASQ2 (Zhang et al., 1997). In lipid bilayers both TRD and JN activate RyR2s [Gyorke 2004], and acute overexpression of TRD also results in increased RyR activity (Terentyev et al., 2005). The

functions of TRD1 and JN have also been studied using genetic mouse models (Zhang et al., 2001; Kirchhefer et al., 2001; Kirchhefer et al., 2003; Kirchhefer et al., 2006; Yuan et al., 2007). However, the interpretation of the physiologic roles of TRD and JN from the transgenic models is complicated by various adaptive and pathological changes (e.g. hypertrophy and heart failure) accompanying chronic protein expression. Our current understanding, as described above (Section 3.2) is that the complex of CASQ, TRD and JN serves to regulate RyR2 activity in a $[Ca^{2+}]_{SR}$-dependent manner.

3.4. Other proteins

A number of other proteins interact with the RyR2 complex, including sorcin (Valdivia, 1998), homer (Pouliquin et al., 2006; Worley et al., 2007), and the histidine-rich Ca^{2+} binding protein (Kim et al., 2003). The specific role of these proteins in SR Ca^{2+} release regulation remains to be defined.

4. Modulation of sarcoplasmic reticulum Ca^{2+} release

4.1. Modulation of sarcoplasmic reticulum Ca^{2+} release by luminal Ca^{2+}

Rather than merely serving as a passive pool of available Ca^{2+}, intra-SR Ca^{2+} plays an active role in controlling the Ca^{2+} release process. With measurements of cell-wide Ca^{2+} release, this regulatory influence manifests itself as an increase in the fraction of total releasable Ca^{2+} from the SR at elevated Ca^{2+} loads and conversely as a decrease of fractional release at reduced SR Ca^{2+} loads (Bassani et al., 1995; Shannon et al., 2000). Increasing SR Ca^{2+} content also enhances the frequency of spontaneous Ca^{2+} release events, Ca^{2+} sparks (Cheng et al., 1996; Lukyanenko et al., 1996). The effects of luminal Ca^{2+} on SR Ca^{2+} release have been attributed to twomechanisms: 1) Activation of RyR2s on the cytosolic side of the channel at sites involved in CICR by Ca^{2+} passing through the channel; and 2) stimulation of RyR2s activity at distinct sites on the luminal side of the channel. The relative contribution of each mechanism to Ca^{2+} signaling in myocytes is difficult to define due to significant interdependence of the two mechanisms.

The stimulatory effects of luminal Ca^{2+} on RyR2 channels have been directly demonstrated in in vitro reconstitution studies(Lukyanenko et al., 1996; Xu & Meissner, 1998; Gyorke & Gyorke, 1998; Ching et al., 2000; Gyorke & Terentyev, 2008). Consistent with the existence of Ca^{2+} regulatory sites on both sides of the channel, two different mechanisms of action of luminal Ca^{2+} upon RyR2 have been described: 1) indirect, “feed-through” activation by luminal Ca^{2+} at the cytosolic activation site of the channel; and 2) direct, “true luminal” activation by Ca^{2+} on the luminal side of the channel. In single reconstituted RyR2s, these two mechanisms are readily distinguishable based on differences in the voltage-dependency of luminal Ca^{2+} effects and the dissimilar impact of luminal Ca^{2+} on channel gating behavior which are characteristic of these two mechanisms. For example, feed-through effects are characteristically larger at negative holding potentials than at positive holding potentials due to the reliance of the stimulatory action of luminal Ca^{2+} on luminal-to-cytosolic fluxes which are intrinsic to this mechanism (Xu & Meissner, 1998). Furthermore, feed-through activation increases RyR2 open probability by prolonging the lifetime of channel openings rather than by increasing their frequency. This is understandable considering that luminal Ca^{2+} can flow through the RyR2 channel and reach the cytosolic activation sites only when the channel is open; once the channel closes, Ca^{2+} accumulated near the cytosolic face of the RyR rapidly dissipates rendering the channel inactive. Compelling evidence for true luminal Ca^{2+} activation of RyR2s has been obtained under experimental conditions that prevent any luminal-to-cytosolic Ca^{2+} fluxes (measurements performed at highly positive holding potentials and millimolar cis $[Ca^{2+}]$) and by using tryptic digestion of the luminal phase of the RyR2 channel to disable the putative luminal Ca^{2+} sensor (Gyorke & Gyorke,1998; Ching et al., 2000).

4.2. Modulation of the Ca^{2+} release channel by protein kinases and phosphatases

In addition to being governed by changes in cytosolic and SR luminal Ca^{2+}, the RyR2 channels are targets for modulation by a number of intracellular ligands and signaling cascades, including phosphorylation pathways. The RyR2 is phosphorylated by PKA and CAMKII at Ser-2809 and by CAMKII at Ser-2815 (Marx et al., 2000; Wehrens et al., 2004). Recently a new PKA phosphorylation site has been reported at Ser-2830 (Xiao et al., 2005; Xiao et al., 2006), although this site has been disputed (Wehrens et al., 2006). In addition to these identified phosphorylation sites, RyR2 is phosphorylated by CAMKII on as many as five sites, based on ^{32}P phosphate incorporation measurements (Rodriguez et al., 2003). The RyR2 is reportedly preassociated with PKA, and the protein phosphatases PP1 and PP2A through the anchoring protein mAKAP (Marx et al., 2000). In addition, RyR2 co-immunoprecipitates with CAMKII (Zhang et al., 2003) and PP2B (calcineurin, CN, (Bandyopadhyay et al., 2000), However, the molecular sites for these interactions remain to be identified. Thus, the molecular components involved in both phosphorylation and dephosphorylation of the RyR2 are anchored directly to the protein.

Despite clear evidence for RyR2 phosphorylation, the physiological consequences of this process remain highly controversial. PKA-dependent phosphorylation has been shown to either increase or decrease steady-state RyR2 open probability in different reports (Bers & Guo, 2005; Benkusky et al., 2004; Wehrens et al., 2004). Marks and collaborators have reported that PKA phosphorylation at Ser-2809 leads to dissociation of FKBP12.6 from the RyR2, in turn resulting in enhanced channel activity (Marx et al., 2000); however, other investigators find that FKBP12.6 still binds to RyR2 after PKA phosphorylation (Stange et al., 2003; Xiao et al., 2004). In intact myocytes, PKA stimulation has been shown to enhance SR Ca^{2+} release, an effect that could be attributed to phosphorylation of PLB and increased SR Ca^{2+} load as well as increased I_{Ca}. Interestingly, in PLB knock-out myocytes, RyR2 phosphorylation by PKA produced no effects on spontaneous local Ca^{2+} release signals [i.e. Ca^{2+} sparks, (Li et al., 2002)], indicating that the PKA-dependent increase in Ca^{2+} spark frequency is entirely attributable to PLB phosphorylation and the resulting increase in SR Ca^{2+}load. A recent study showed that RyR2 is stimulated only during extreme hyperphosphorylation (i.e. all 4 subunits of the tetramer are phosphorylated at Ser-2809) (Carter et al., 2006). CAMKII, expressed as the delta isoform, has also been shown to produce disparate effects. Some authors have reported that exogenous CaMKII increases RyR2 activity (Witcher et al., 1991; Hain et al., 1994), whereas others have found that exogenous (Lokuta et al., 1995) and endogenous CaMKII reduces RyR2 opening or ryanodine-RyR binding (Takasago et al., 1991). Similarly, in intact voltage clamped myocytes some investigators have reported that both endogenous and exogenous CAMKII increase the amplitude of Ca^{2+} transients at a given SR Ca^{2+} content and I_{Ca} trigger (Bers & Guo, 2005), whereas others have reported that endogenous CAMKII and constitutively active CAMKII suppress Ca^{2+} transients and Ca^{2+} sparks (Wu et al., 2002; Yang et al., 2007). Thus, this remains an open area of investigation at the present time.

The action of protein kinases is opposed by dephosphorylating phosphatases. Three types of protein phosphatases, including PP1, PP2A and PP2B (calcineurin), have been shown to influence cardiac performance (Neumann, 2002). Overall, in the majority of studies, phosphatases reportedly downregulate SR Ca^{2+} release and contractile performance (Neumann et al., 1993; duBell et al., 1996, [2002]; Carr et al., 2002; Santana et al., 2002). Application of PP1 and PP2A to permeabilized myocytes caused an acute activation of Ca^{2+} sparks followed by depletion of the SR Ca^{2+} content (Terentyev et al., 2003a), suggesting that depression of EC coupling by phosphatases may involve depletion of the SR Ca^{2+} stores due to increased activation of RyR2s. Consistent with this possibility, phosphatases have been shown to increase RyR2 activity in some (Lokuta et al., 1995; Terentyev et al., 2003a; Carter et al., 2006) but not all single channel studies (Hain et al., 1994). Overall, as summarized in this brief overview,

the mechanisms and functional consequences of RyR2 phosphorylation remain to be conclusively elucidated.

4.3. Redox modulation

Ryanodine receptor channels are subject to modifications by oxidizing and reducing agents capable of interacting with thiolcontaining cysteine residues of the channel protein. The cardiac RyR contains approximately 90 cysteines per subunit, of which approximately 20 are in reduced state (Xu et al., 1998). These free cysteines are targets for several types of redox modifications, including disulfide crosslinking, *S*-nitrosylation and *S*-glutathionylation. In general, oxidizing conditions increase RyR2 activity and therefore stimulate SR Ca^{2+} release (Abramson & Salama, 1988; Eager et al., 1997; Zima & Blatter, 2006), while these effects are reversed by reducing reagents such as dithiothreitol (DTT). Although different classes of oxidizing agents tend to increase RyR2 activity, the underlying effects on channel gating are not uniform. For example, whereas oxidation increases the sensitivity of the channel to Ca^{2+} activation, *S*-glutathionylation decreases the sensitivity of the channel to inhibition by Mg^{2+} (Donoso et al., 2000). There is also evidence of time-dependent effects whereby prolonged exposure to high doses of oxidizing agents, after an initial stimulation of activity, can cause irreversible inactivation of RyRs (Eager et al., 1997; Marengo et al., 1998). Thus, the functional consequences of RyR2 thiol modification are dependent on the concentration of the oxidizing agent, exposure time and the nature of the chemical reaction involved in the modification. These different effects are likely to reflect participation of different sets of cysteine residues under different oxidizing conditions. The role of specific cysteine residues in RyR redox modifications has only started to be elucidated. It has been shown that redox modification involves only 12 of the total 100 cysteines on RyR1 (Aracena-Parks et al., 2006). Whereas some of these cysteines are non-specific targets for different redox agents, other sites are exclusively *S*-nitrosylated (Cys-1040, Cys-1303) or *S*-glutathionylated (Cys-1591 and Cys-3193). Endogenous oxidizing agents (reactive oxygen species or ROS) are continuously generated in the heart and are likely to exert tonic influences on intracellular targets/processes including SR Ca^{2+} release. ROS production has been shown to increase in various pathological settings such as ischemia-reperfusion and heart failure. Growing evidence suggests that modification of RyR2 by ROS contributes to the abnormal Ca^{2+} handling associatedwith these disease states (see section 6.7 below).

5. The Ca^{2+} release-uptake cycle

5.1. Activation of sarcoplasmic reticulum Ca^{2+} release

Myocardial excitation–contraction (EC) coupling begins with membrane depolarization activating voltage-dependent Ca^{2+} channels in the plasma membrane which allows a relatively small amount of Ca^{2+} to enter the cell. This Ca^{2+} serves as a trigger signal to activate Ca^{2+} release channels present in large clusters in the junctional sarcoplasmic reticulum (jSR). See Fig. 3A. The release of Ca^{2+} via groups of RyR2s has been visualized as Ca^{2+} sparks (Cheng et al., 1993). Sparks have been estimated to involve coordinated openings of 6 to 30 RyR2s (Bridge et al., 1999;Lukyanenko et al., 2000;Wang et al., 2001). Ca^{2+} sparks amplify the initial Ca^{2+} influx trigger signal and combine to produce an elevation of cell-wide myoplasmic $[Ca^{2+}]$, the Ca^{2+} transient. This increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) leads to activation of the contractile proteins and hence to generation of the heartbeat. Under certain conditions, Ca^{2+} release can be triggered by other mechanisms such as Ca^{2+} entry via reverse mode NCX (Sipido et al., 1997). Moreover, Ca^{2+} entry via these Ca^{2+} transport mechanisms can interact synergistically resulting in activation of large SR Ca^{2+} release at positive membrane potentials where I_{Ca} by itself is small. (Viatchenko-Karpinski et al., 2005;Sobie et al., 2008).

5.2. Termination of sarcoplasmic reticulum Ca^{2+} release

Following its rapid activation, SR Ca^{2+} release robustly terminates leaving a substantial Ca^{2+} reserve of releasable Ca^{2+} in SR. The exact underlying causes of Ca^{2+} release termination remain to be defined (Stern & Cheng, 2004). Several mechanisms have been proposed, including inactivation of the RyR2 channel by elevated cytosolic Ca^{2+} (i.e. Ca^{2+}-dependent inactivation); obligatory shift from high- to low modes of activity (i.e. adaptation); and RyR2 deactivation caused by decline in intra-SR $[Ca^{2+}]$ (i.e., luminal-Ca^{2+}-dependent deactivation). In recent years, several lines of evidence have pointed to a prevailing role of luminal Ca^{2+} in controlling SR Ca^{2+} release (Terentyev et al., 2002; Sobie et al., 2002; Zima et al., 2008; Terentyev et al., 2008). For example, buffering $[Ca^{2+}]$SR using low affinity exogenous Ca^{2+} buffers such as citrate or maleate prolonged the duration of Ca^{2+} release during both Ca^{2+} sparks and global Ca^{2+} transients (Terentyev et al., 2002). Subsequently, similar results were obtained by varying the expression level (increasing or decreasing) of the endogenous Ca^{2+} buffer CASQ2 in cardiac myocytes (Terentyev et al., 2003b). Recently, the role of this mechanism has received further support from the observation that Ca^{2+} sparks terminate at a constant free $[Ca^{2+}]_{SR}$ regardless of the extent of $[Ca^{2+}]_{SR}$ buffering by CASQ2 (Terentyev et al., 2008). Moreover, mutations in CASQ2 that enhance the responsiveness of RyR2s to luminal Ca^{2+} reduced the $[Ca^{2+}]_{SR}$ threshold for local Ca^{2+} release termination. Thus, mounting experimental evidence suggests that luminal Ca^{2+}-dependent changes in RyR2s contribute to SR Ca^{2+} release termination in cardiac myocytes.

5.3. Refractoriness of Ca^{2+} signaling

After activation, a shift in the gating mode of RyR2s closes the channels, which then remain closed in a time-dependent manner. There is substantial evidence indicating that changes in luminal Ca^{2+} govern not only termination of CICR, but also induce a refractory state that persists until recovery of $[Ca^{2+}]_{SR}$. (Terentyev et al., 2002, 2003b; Szentesi et al., 2004; Sobie et al., 2005). Thus, experimental interventions to slow SR-Ca^{2+} reuptake (either by SERCA-pump inhibitors or by increasing the intra-SR Ca^{2+} binding sites with either exogenous or endogenous buffers) slow recovery of Ca^{2+} signaling from refractoriness (Szentesi et al., 2004; Terentyev et al., 2002, 2003b). Moreover, accelerating $[Ca^{2+}]_{SR}$ recovery following Ca^{2+} release either by stimulation of the SERCA pump or by reducing endogenous CASQ levels accelerated recovery of RyR2 refractoriness. Interestingly, restitution of refractoriness appears to lag behind $[Ca^{2+}]_{SR}$ recovery (Wang et al., 2001; Sobie et al., 2005) suggesting that the relationship between recovery and luminal Ca^{2+} is non-linear and/or may involve some intermediate steps. Consistent with the latter possibility, modulation by luminal Ca^{2+} may involve a group of intermediate proteins including CASQ2, triadin and junctin (see Section 3.2 above).

5.4. Sarcoplasmic reticulum Ca^{2+} reuptake

During relaxation, rapid reuptake of Ca^{2+} into the SR is achieved by the SR Ca^{2+} ATPase (SERCA2a). The activity of SERCA is modulated by the phosphoprotein phospholamban (PLB) in a phosphorylation-dependent manner (Brittsan & Kranias, 2000). Dephosphorylated PLB inhibits SERCA activity whereas phosphorylation relieves the inhibition. Phosphorylation occurs at two sites, Ser 16 and Thr 17 by PKA and CAMK2 respectively. Within the SR a large fraction of Ca^{2+} is bound to CASQ, and this binding accelerates uptake by keeping free $[Ca^{2+}]_{SR}$ at low levels. The ability of SERCA to attain a maximum thermodynamically allowable $[Ca^{2+}]_{SR}$ is limited by the leak though RyRs which increases as a function of $[Ca^{2+}]_{SR}$ as described below (Lukyanenko et al., 2001).

5.5. Luminal Ca^{2+}-dependent leak

The SR exhibits a substantial diastolic Ca^{2+} leak in the form of spontaneous Ca^{2+} sparks. The frequency of sparks and the amount of leak is increased at elevated SR Ca^{2+} content and conversely is reduced at lowered SR Ca^{2+} content (Lukyanenko et al., 2001; Shannon et al., 2002). This leak limits the ability of the SR Ca^{2+} pump to attain the maximum thermodynamically allowable $[Ca^{2+}]_{SR}$ and plays an important role in setting the SR Ca^{2+}content. Mechanistically, the SR Ca^{2+} leak is a consequence of activation of RyRs by increased $[Ca^{2+}]_{SR}$. Increased SR Ca^{2+} leak plays an important role in various pathological states such as HF, in which it results in reduced SR Ca^{2+} content and weakened contractility (see below).

5.6. Balance of trans-sarcolemmal and sarcoplasmic reticulum Ca^{2+} fluxes

In order to maintain Ca^{2+} homeostasis, an equivalent amount of Ca^{2+} must enter the myocyte via I_{Ca} and then be removed from the myocyte on a beat-to-beat basis. Most of this Ca^{2+} is removed by the electrogenic Na^+–Ca^{2+} exchanger that exchanges three Na^+ ions for one Ca^{2+} ion. At the same time, the amount of Ca^{2+} released from the SR must be balanced by the amount of Ca^{2+} taken back up by SERCA. Deviation from the balance of *trans*-sarcolemmal and SR Ca^{2+} fluxes will lead to either gain or loss of Ca^{2+} in the cell and in the SR. This requirement for the homeostatic balance of cellular and SRCa^{2+} fluxes is an important factor in determining the SR Ca^{2+} content and release, and in shaping the response of SR Ca^{2+} release to pharmacological and/or pathophysiologic modulation.

By studying the effects of low concentrations of the RyR2 channel modulators (caffeine and tetracaine) on systolic Ca^{2+} release, Eisner and coworkers demonstrated that the SR Ca^{2+} release mechanism in cardiac myocytes exhibits an ability for self-regulation which allows myocytes to maintain a consistent amplitude of systolic Ca^{2+} transients, even when interventions alter RyR2 activity (Overend et al., 1998; Trafford et al., 2000). They attributed this self-regulation to a feedback loop consisting of SR Ca^{2+} release causing cellular Ca^{2+} extrusion; Ca^{2+} extrusion influencing SR Ca^{2+} load; and SR Ca^{2+} load affecting SR Ca^{2+} release (Eisner et al., 1998; Trafford et al., 2002). As susequently shown, the dependence of RyR gating on luminal Ca^{2+} is another important factor in determining Ca^{2+}cycling, and is expected to both accelerate and increase the extent of restitution of Ca^{2+} transient amplitude after a change caused by a perturbed open probability of RyR2s (Lukyanenko et al., 2001; Shannon et al., 2002). It is to be noted, however, that the capacity of a myocyte to restore its initial Ca^{2+} transient amplitude following alterations in open probability of RyRs only operates over a limited range. Thus, if RyRs are hyperstimulated the SR becomes completely depleted and there is no SR Ca^{2+} release, and consequently only low amplitude steady-state Ca^{2+} transients. This situation occurs in certain pathological states such as HF and is examined in detail below.

6. Pathologies of cardiac sarcoplasmic reticulum Ca^{2+} signaling

6.1. Overview

Abnormal RyR2-mediated SR Ca^{2+} release has been implicated in a spectrum of cardiac disease states ranging from triggered and reentrant arrhythmias to HF (Wehrens et al., 2004; George et al., 2007; Ter Keurs & Boyden, 2007; Laurita & Rosenbaum, 2008). A classification of cardiac diseases related to abnormal SR Ca^{2+} release and their hypothetical relationship to defective function is presented in Fig. 4. Ca^{2+}-dependent arrhythmias of both triggered and reentry types are generally associated with instability of SR Ca^{2+} release whereas HF is associated with diminished systolic SR Ca^{2+} release. In general, loss of control of CICR can arise in two ways. Firstly, in a variety of conditions that lead to increased SR Ca^{2+} load (i.e. “Ca^{2+} overload”), sensitization of RyR2s by enhanced luminal Ca^{2+} contributes to generation

of spontaneous Ca^{2+} releases or instabilities of SR Ca^{2+} release that manifest themselves as Ca^{2+} alternans (Fig. 3B; Fig. 4). Whereas spontaneous Ca^{2+} releases causes DADs and triggered arrhythmia, Ca^{2+} alternans can provide basis for heterogeneities in electrical repolarization and substrate for reentry arrhythmia. Secondly, acquired or genetic defects in RyR2s enhance the responsiveness of RyR2 to luminal Ca^{2+} such that the channel becomes hyperactive even at reduced SR Ca^{2+} content. This state of "perceived" Ca^{2+} overload (Gyorke & Terentyev, 2008) can also lead to Ca^{2+}-dependent arrhythmias and has been implicated familial tachyarrhythmias associated with mutations in RyR2 or CASQ2 (Fig. 3C). Diminished SR Ca^{2+} release (e.g. as in HF) can result either from failure of EC coupling or exhaustion of SR Ca^{2+} stores due to an excessive leak via RyR2s (Fig. 3D). Thus, different diseases could result from different degrees of alteration in the same underlying mechanism, namely increased RyR activity such that a relatively small change could result in destabilization of CICR whereas a more severe deviation could lead to SR Ca^{2+} leak and SR store depletion. Clearly, other factors besides abnormal RyR function, such as changes in SERCA and NCX activity, also contribute to different disease phenotypes. The role of these factors has been extensively reviewed (e.g. Kranias and Bers, 2007; Periasamy et al., 2008) and is not discussed here.

6.2. Ca^{2+} overload and triggered arrhythmia

Excess intracellular Ca^{2+} (i.e. Ca^{2+} overload) is a characteristic feature of various pathological states such as metabolic inhibition, ischemia/reperfusion and digitalis toxicity. Ca^{2+} overload is associated with triggered afterdepolarizations, which can initiate sustained tachyarrhythmias (Ferrier et al., 1973; Kass et al., 1978; Pogwizd & Bers, 2004; Maltsev et al., 2006; Venetucci et al., 2008). See Fig. 3B. The causal relationships between Ca^{2+} overload and triggered activity are known to include the following sequence of events: (1) SR Ca^{2+} overload leads to generation of spontaneous Ca^{2+} release from the SR, (2) elevated Ca^{2+} induces depolarizing membrane currents that give rise to delayed afterdepolarizations (DADs), (3) DADs activate ectopic action potentials, and (4) ectopic action potentials can then initiate tachyarrhythmias. A brief discussion of these steps follows below.

6.3. Spontaneous Ca^{2+} release

Since the initial studies of Fabiato in skinned myocytes (Fabiato & Fabiato, 1972), spontaneous Ca^{2+} release or Ca^{2+} waves have been described by many investigators in various preparations. Ca^{2+} waves in general are associated with increased SR Ca^{2+}content (Stern et al., 1988; Pogwizd & Bers, 2004; Venetucci et al., 2008). Moreover, it has been shown with both total SR Ca^{2+} content determinations (Diaz et al., 1997) and more directly using SR-entrapped Ca^{2+} indicators (Kubalova et al., 2004) that Ca^{2+} waves arise when $[Ca^{2+}]_{SR}$ reaches a certain threshold value. See Fig. 3B. As visualized by confocal Ca^{2+} imaging, Ca^{2+} waves are initiated by local release events, Ca^{2+} sparks, that occur spontaneously in resting myocytes, and then propagate through saltatory activation of adjacent release sites (Cheng et al., 1996; Lukyanenko et al., 1999). The mechanisms for the transition from Ca^{2+} sparks to self-propagating Ca^{2+} waves have been examined. One possibility is that Ca^{2+} waves start and propagate when Ca^{2+} efflux via individual release sites becomes sufficient to raise Ca^{2+} at adjacent release sites above the threshold for CICR from a Ca^{2+}-overloaded SR. However, this mechanism may not be sufficient by itself to account for Ca^{2+} wave generation. Experimental studies combined with mathematical modeling have suggested that propagation of Ca^{2+} waves relies on stimulatory effects of elevated luminal Ca^{2+} on RyR activity (Lukyanenko et al., 1999). As to the specific role of luminal Ca^{2+}, two hypotheses have been considered: (1) increased luminal Ca^{2+} sensitizes the Ca^{2+} release channels to cytosolic Ca^{2+}, thus enhancing the ability of cytosolic Ca^{2+} to activate adjacent release sites via CICR, and (2) Ca^{2+} transported from the wave-front into the adjacent SR elements raises luminal Ca^{2+} above a critical threshold level, resulting in activation of the release channels at a luminal site. Examination of the effects of SERCA inhibitors, such as thapsigargin, showed that Ca^{2+} waves elicited by local application

of caffeine in myocytes preloaded with Ca^{2+} not only did not stop but even accelerated after rapid inhibition of SR Ca^{2+} uptake (Lukyanenko et al., 1999). At the same time, Ca^{2+}waves could be elicited following sensitization of RyRs by agents such as caffeine — even in the absence of Ca^{2+} overload. These finding supported the role of CICR in wave propagation with luminal Ca^{2+} serving to sensitize RyRs. However, recently (Keller et al., 2007) reexamined the role of SERCA-mediated uptake in Ca^{2+} wave propagation using a photo-releasable SERCA inhibitor. Following inhibition of SR Ca^{2+} uptake these authors observed a reduction in Ca^{2+} wave velocity in contrast to previous findings. The reasons for these discrepant results remain undefined and additional experimentation will be required to resolve this issue. In summary, evidence exists to suggest that the effects of luminal Ca^{2+} on RyR2s play at least a sensitizing role in the generation of Ca^{2+} waves by promoting CICR. The intriguing possibility that $[Ca^{2+}]_{SR}$ plays a more direct triggering role in Ca^{2+} wave generation will require further verification.

6.4. Afterdepolarizations and triggered arrhythmia

Triggered arrhythmias originate from afterdepolarizations and are so named as they are "triggered" by either abrupt or sustained abnormal variations in heart rate. Afterdepolarizations occur either during the action potential (early afterdepolarizations or EADs) or after termination of the action potential (delayed afterdepolarizations or DADs). Classically, EADs are considered to occur at slow heart rates, DADs at rapid heart rates.

Early afterdepolarizations are often associated with delayed cardiac repolarization (action potential prolongation or QT/QTc prolongation on the electrocardiogram), and have been implicated in the genesis of Torsades de Pointes arrhythmias (see below). It has been suggested that repolarization abnormalities either directly cause or provide a foundation for EADs [reviewed by (Roden, 1993)]. Postulated ionic mechanisms responsible for EADs include K^+ channel block, enhanced late sodium current, reactivation of L-type calcium current during prolonged action potential plateau. However, most relevant to this review, abnormal release of calcium from intracellular SR stores during repolarization can lead to inward current via Na^+/Ca^{2+} exchange and activation of EADs, even during rapid heart rates. This has been demonstrated in computer simulations (Huffaker et al., 2004) and in isolated myocytes when SR Ca^{2+} is increased (Spencer & Sham, 2003). In addition to NCX, window I_{Ca} has also been implicated in the development of EADs; however, SR Ca^{2+} release has been implicated as an important modulator of I_{Ca} inactivation and EADs, in a canine model of TdP (chronic AV block) (Antoons et al., 2007).

Delayed afterdepolarizations are typically attributed to "calcium overload" of cardiac myocytes, with the classic examples being inhibition of Na^+–K^+-ATPase with cardiac glycosides (e.g. digoxin) or beta-adrenergic stimulation (Rosen & Legato, 1985; Rosen, 1985). DADs are attributed to spontaneous Ca^{2+} release from the sarcoplasmic reticulum (SR), resulting in subsequent activation of a transient inward current via the Na^+/Ca^{2+} exchanger. Elevations of cytosolic Ca^{2+} resulting from spontaneous SR Ca^{2+} release causes inward current to flow across the sarcolemma, producing transient depolarizations or DADs (Lederer & Tsien, 1976; Allen et al., 1984; Wier & Hess, 1984; Marban et al., 1986). These inward currents are predominantly due to the electrogenic NCX exchanging 1 Ca^{2+} for 3 Na^+ ion (Schlotthauer & Bers, 2000). In most cases DADs are not of sufficient amplitude to reach the threshold to initiate an AP (see Fig. 5). This is because a Ca^{2+} wave at any time occupies only a fraction of the cell; therefore only a fraction of the total NCX molecules are exposed to elevated Ca^{2+}. However, when several waves arise in the cell simultaneously (i.e. multifocal waves), the summation of the inward currents can depolarize the cell to threshold resulting in an AP (Capogrossi et al., 1987). In intact muscle, currents can spread from cell to cell through the gap junctions, summating over a region covering a group of myocytes (area comparable in size to the electrical

space constant). When spontaneous Ca^{2+} release occurs asynchronously among cells, the likelihood that this summation could reach threshold to trigger an ectopic AP is low. However, spontaneous Ca^{2+} releases can be relatively synchronized following an AP. Such temporal concurrence of spontaneous release events could be due to similarly timed recovery of RyR2s from luminal-dependent refractoriness and simultaneous attainment of the $[Ca^{2+}]_{SR}$ threshold for spontaneous Ca^{2+} release.

Afterdepolarizations of sufficient amplitude to reach the threshold voltage for a myocyte can initiate ectopic (triggered) beats. Arrhythmias can be induced by either isolated or sustained triggered beats. Notably, conduction of either EADs and DADs can give rise to ectopic (premature) beats which can initiate reentrant arrhythmias (see Fig. 5) resulting in sustained cardiac arrhythmias. Re-entry is thought to be the most common cause of sustained cardiac arrhythmias. Re-entrant arrhythmias require an underlying substrate; the substrate (predisposition) for re-entry is dispersion of both conduction velocities and refractory periods within a region of the heart. An illustration of the substrate and initiation of re-entry is shown in Fig. 5.

6.5. Catecholaminergic polymorphic ventricular tachycardia

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmia characterized by exercise or stress-induced syncope and sudden death (Eldar et al., 2002; Laitinen et al., 2004; Napolitano & Priori, 2007). CPVT accounts for approximately 20% of all sudden deaths that occur in the absence of structural heart disease. Two genetic forms of CPVT have been described: a prevailing dominant form linked to mutations in RyR2s and a rare recessive form associated with mutations in CASQ2 (Postma et al., 2002; Lahat et al., 2004). Currently about 60 mutations in RyR2 and 7 mutations in CASQ2 have been linked to CPVT. Mutations in these proteins account only for about 50% of all cases of CPVT suggesting that genetic defects in other proteins can also lead to this syndrome. Notably, these different defects result in the same arrhythmic phenotype; and experimental work has reconciled the mechanisms of CPVT from these varying mutations.

Adenoviral expression studies of CPVT-associated CASQ2 mutants in cardiac myocytes have demonstrated that various mutations act by inducing irregular Ca^{2+} transients and arrhythmogenic DADs (Terentyev et al., 2003b; Gyorke & Terentyev, 2008). The mechanisms by which CASQ2 mutations alter regulation of SR Ca^{2+} release have been examined (Terentyev et al., 2006; Gyorke & Terentyev, 2008). Although various CASQ2 mutations affect different aspects of CASQ2 function, the results converge in a single common pathway to shorten RyR2 refractoriness and promote spontaneous Ca^{2+} release in the diastolic phase. See Fig. 3C. First, as described above, CASQ2 polymers are required for high-capacity Ca^{2+} binding, and certain mutations (Del.339–354) appear to disrupt CASQ2 polymerization (Terentyev et al., 2008). Reduced Ca^{2+} binding capacity of the SR results in accelerated recovery of $[Ca]_{SR}$ by the SERCA and thus premature functional recovery of RyR2 from luminal Ca^{2+}-dependent deactivation. Secondly, interactions of CASQ2 with the RyR2 complex modulate the sensitivity of the RyR2 channel to luminal Ca^{2+}. CASQ2 mutations [R33Q; (Terentyev et al., 2006)] alter the modulation of RyR2s by luminal Ca^{2+} without apparently altering the Ca^{2+} binding capacity of CASQ2. The resulting altered modulation of RyR2s by luminal Ca^{2+} (i.e. compromised ability of CASQ2 to deactivate the RyR2) with these mutations leads again to premature recovery of Ca^{2+} release from refractoriness.

The mechanisms by which CPVT-associated mutations in RyR2 alter RyR2 channel function have been investigated (George et al., 2007; Liu & Priori, 2008). Based on studies using a genetic mouse model, Marx and coworkers have suggested that RyR2s mutations lead to dissociation of the regulatory protein FKBP12.6 from RyR2 causing increased diastolic Ca^{2+} leak, DADs and arrhythmias (Wehrens et al., 2003). However, several aspects of this

hypothesis have been disputed by other laboratories (George et al., 2007; Xiao et al., 2007; Liu & Priori, 2008). Alternatively, based on studies using a heterologous expression system, Chen and coworkers demonstrated that CPVT mutations increase the sensitivity of RyR2 to activation by luminal Ca^{2+} (Jiang et al., 2005). Similar to the effects of arrhythmogenic CASQ2 mutants, sensitization to luminal Ca^{2+} would reduce the $[Ca^{2+}]_{SR}$ threshold for the generation of spontaneous Ca^{2+} release events and DADs. Thus, abnormal luminal modulation may be a common mechanism for genetically-distinct forms of CPVT.

Based on accumulated data it appears that CPVT can arise as a result of a genetic defect in any component of the Ca-storage-release machinery involved in controlling, sensing and responding to changes in luminal Ca^{2+}. The defects affect the ability of RyRs to stay refractory, thus resulting in abnormal diastolic Ca^{2+} release and DADs. In the context of this mechanism, the role of adrenergic stimulation in CPVT can be explained by PKA-dependent stimulation of SERCA, which would accelerate SR Ca^{2+} store reuptake. This would further enhance the rate and extent of recovery of release sites from luminal Ca^{2+}-dependent refractoriness exacerbating the arrhythmogenic effects of CASQ2/RyR2 mutations. As to the sites of origin within the heart, a recent study using epicardial mapping and a genetic mouse model (RyR2/RyR2.R4496C) of CPVT reported that focal arrhythmias arise predominantly in the His-Purkinje system (Cerrone et al., 2007).

6.6. Ca^{2+} alternans

Altered intracellular Ca^{2+} homeostasis is not only involved in triggered activity but also may be part of pathogenesis of reentrant arrhythmias. Instabilities in Ca^{2+} handling have been directly linked to heterogeneities in conduction and repolarization providing a substrate for reentrant excitation. These instabilities often manifest themselves as beat-to-beat variations in contractility and AP duration referred to as cardiac alternans and its cellular equivalent Ca^{2+} alternans (Weiss et al., 2006; Eisner et al., 2006; Laurita&Rosenbaum, 2008).When alternans is initiated it usually occurs in an identical phase, i.e. long–short–long APs, in all regions of the tissue (concordant alternans).When the heart rate is faster than the critical rate, a shift of phase occurs such that some regions continue to alternate in the long–short–long pattern while other regions alternate in the reverse pattern (discordant alternans). Spatially discordant alternans are highly arrhythmogenic and greatly amplify the heterogeneity of repolarization and electrical refractoriness producing conduction block and a substrate for reentrant excitation (Pastore et al., 1999; Qu et al., 2000). Cardiac alternans occurs in various pathologic settings including ischemia, electrolyte disbalances and HF (Eisner et al., 2006; Laurita & Rosenbaum, 2008). Moreover, T-wave alternans (electrocardiographic manifestation of cardiac alternans), is recognized as a reliable predictor of VF and sudden cardiac death in patients with HF (Rosenbaum et al., 1994).

Initially, it was thought that cardiac alternans has its origin in a non-linear relationship between the diastolic interval and electrical repolarization, resulting in periodic changes in AP duration. However, more recent studies have suggested that Ca^{2+} alternans might be a cause rather than a consequence of AP alternans (Chudin et al.,1999; Huser et al., 2000; Diaz et al., 2004;Weiss et al., 2006; Eisner et al., 2006; Laurita & Rosenbaum, 2008). The influence of the Ca^{2+} transient on AP is attributable to effects on Ca^{2+} dependent currents, such as the NCX current and I_{Ca}, during the AP plateau. The coupling between Ca^{2+} and membrane potential (Vm) can be either positive or negative. When a large Ca^{2+} transient increases the duration of the AP via stimulating the inward NCX current (concordant electrical and mechanical activity), the Ca^{2+}–Vm coupling is referred to as positive. When the large Ca^{2+} transient decreases the duration of AP (discordant electrical and mechanical activity), the Ca–Vm coupling is said to be negative.

In general, Ca^{2+} alternans is thought to arise when the rate of myocyte Ca^{2+} cycling cannot keep up with heart rate. In various pathological settings, slowed Ca^{2+} cycling pushes the heart into alternans even at normal heart rates. Thus, when SERCA Ca^{2+} transport activity is decreased the SR Ca^{2+} store can be refilled only on an alternating basis. Increasing SR Ca^{2+} buffering capacity by overexpressing CASQ2 or introducing low affinity exogenous buffers into the SR should slow functional recharging of the Ca^{2+} store and has also been shown to cause alternans. The steepness of the relationship between SR Ca^{2+} content and SR Ca^{2+} release is another important factor in the development of alternans (Weiss et al., 2006; Eisner et al., 2006). As discussed above, the steepness arises as a result of the combination of stimulatory effects of luminal Ca^{2+} on RyR function, and the presence of the positive feedback loop inherent to CICR. With a steep Ca^{2+} release–$[Ca^{2+}]_{SR}$ relationship, the SR will produce a large Ca^{2+} transient when the SR Ca^{2+} content is relatively high and a disproportionately small Ca^{2+} transient when the SR Ca^{2+} content is relatively low. An especially large Ca^{2+} transient will cause extrusion from the cell of an extra amount of Ca^{2+} resulting in an under-filled SR Ca^{2+} store and hence a smaller Ca^{2+} transient in the next cycle. This will decrease Ca^{2+} efflux and increase SR Ca^{2+} content to a larger extent, such that the Ca^{2+} transient will again be larger in the third cycle. In most disease conditions including acute ischemia and HF, alternans are thought to be caused by slowed Ca^{2+} cycling due to inhibition of SERCA (Weiss et al., 2006). However, we recently showed that in a post-infarction model of sudden cardiac death alternans is attributable to increased steepness of the Ca^{2+} release–$[Ca^{2+}]_{SR}$ relationship (Belevych et al., 2008). In this model abnormal SR Ca^{2+} release regulation is caused by redox modification of RyRs and can be attenuated by reducing agents.

Given the non-linear dependence of Ca^{2+} release on $[Ca]_{SR}$ in the development of alternans, beat-to-beat variations in Ca^{2+} transients are expected to correlate with beat-to-beat variations in SR Ca^{2+} content. Indeed, such a correlation has been demonstrated experimentally by measuring both total SR Ca^{2+} content and free $[Ca]_{SR}$ in myocytes exhibiting Ca^{2+} alternans (Diaz et al., 2004). However, other investigators failed to observe consistent changes in $[Ca]_{SR}$ during alternans (Picht et al., 2006). These studies suggested that processes such as refractoriness control the number of available RyRs during each heart beat, and can produce alternans irrespective of changes in SR Ca^{2+} content. It is to be noted however, that RyR refractoriness itself appears to be controlled by $[Ca]_{SR}$ (Terentyev et al., 2002; Sobie et al., 2005; Gyorke &; Terentyev, 2008). Thus alterations in the number of available RyRs still would be expected to correlate with alterations in diastolic Ca^{2+} transients. One potential explanation as to why this is not observed in some studies is that changes are too small to be detected. Alternatively, recovery occurs with a time lag following $[Ca]_{SR}$ recovery, thus providing a window in which alternans can develop without significant alterations in diastolic $[Ca]_{SR}$.

6.7. Heart failure

Systolic heart failure (HF) is an increasingly prevalent disease and occurs when the cardiac muscle is too weak to pump sufficient blood through the body. Patients with HF typically die either due to ventricular arrhythmias or progressive failure of cardiac mechanical function (pump failure). A recent analysis of the mode of death in patients with heart failure (arrhythmias vs. pump failure) suggests that during earlier (less severe) stages of heart failure, death is seven times more likely to occur from cardiac arrhythmias, while in end-stage HF, death is twice is likely to occur from pump failure (Mozaffarian et al., 2007). Although the pathophysiologic mechanisms of HF are diverse, highly complex and not fully defined, reduced Ca^{2+} transient amplitude and RyR2 dysfunction with elevated SR Ca^{2+} leak are increasingly recognized features of the disease process (Marx et al., 2000; Shannon et al., 2003b; Kubalova et al., 2005). Uncontrolled RyR2 gating and increased diastolic SR Ca^{2+} leak have been shown to cause a reduction in the SR Ca^{2+} content, thus limiting the ability of cardiac muscle to contract

in myocytes from failing hearts (Belevych et al., 2007). Additionally, abnormal RyR2 activity could contribute to the pathogenesis of arrhythmia in patients with HF.

Similar to CPVT, elevated RyR2-mediated SR Ca^{2+} leak in HF is associated with altered responsiveness of RyRs to luminal Ca^{2+} (Kubalova et al., 2005). See Fig. 3D. In particular, luminal Ca^{2+} dependence is markedly left-shifted in RyR2s from failing hearts. As discussed for CPVT, this specific defect may explain why Ca^{2+} leak remains high even at reduced SR Ca^{2+} load despite the well established positive relationship between RyR2 activity and SR Ca^{2+} content. Thus, acquired and genetic alterations in RyR seem to affect RyR behavior in a similar manner. While it is apparent that HF-associated RyR2-mediated SR Ca^{2+} leak could contribute to 1) depleted SR Ca^{2+} stores and reduced contractility and/or 2) Ca^{2+}-dependent arrhythmias, the underlying mechanisms of RyR2 dysfunction remains a subject of intense debate.

One proposed mechanism for abnormal RyR behavior involves hyperphosphorylation of RyR2 by PKA (Marx et al., 2000; Wehrens et al., 2004). RyR2 phosphorylation at Ser-2809 reportedly causes dissociation of FKBP12.6 followed by increased RYR activity (see above). Another possibility is that increased RyR activity is caused by CAMKII phosphorylation at Ser-2815 (Ai et al., 2005). However, both the functional effects of phosphorylation on RyR function and the role of abnormal RyR phosphorylation in HF require further clarification. An alternative mechanism for enhanced RyR2 activity is modification of the channel protein by reactive oxygen and/or nitrogen species generated during HF. ROS levels are markedly elevated in failing hearts (Belch et al.,1991; Sawyer et al., 2002; Giordano, 2005) and redox modification is known to stimulate RyR activity (see above). Preliminary results obtained in our laboratory suggest that sulfhydryl oxidation of RyRs contributes to enhanced SR Ca^{2+} leak in chronic heart failure (Gyorke et al., 2007). Importantly, oxidation impairs the luminal Ca^{2+} dependence of RyRs in amanner characteristic of HF. At the same time, reducing agents normalize SR Ca^{2+} leak, RyR activity and luminal Ca^{2+} sensitivity. Additional studies are required to establish the relative contribution of this mechanism to HF-induced abnormalities. In summary, evidence obtained using variousmodels of HF and in human HF suggests that RyR2s become excessively active i.e., “leaky” in HF. This increase in RyR2 activity has been linked to defective modulation of the channel by SR luminal Ca^{2+}, a mechanism that normally operates to terminate SR Ca^{2+} release and keep the RyR2 channels closed during diastole (see above). Modest alterations in this control mechanism destabilize CICR causing arrhythmogenic dispersion of Ca^{2+} signaling, whereas more severe defects result in a massive diastolic leak and consequently the depleted SR Ca^{2+} stores characteristic of heart failure.

6.8. Atrial fibrillation

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia. It has been estimated that the lifetime risk for the development of AF is 1 in 4 after reaching the age of 40 (Lloyd-Jones et al., 2004). Occasionally, AF occurs in the absence of detectable structural heart disease or any other recognizable risk factor. However, most commonly, AF occurs in the presence of known risk factors or diseases, including valvular heart disease, advanced age, hypertension, coronary artery disease, diabetes, hyperthyroidism, chronic obstructive pulmonary disease, and heart failure. AF is known to generally increase the risk of death, and specifically to increase the risk of death during heart failure.(Benjamin et al., 1998; Kammersgaard & Olsen, 2006) Recently, abnormal electrical activity originating in the sleeves of the pulmonary veins (PVs) has been implicated in the initiation of AF, particularly paroxysmal AF. However, non-PV atrial foci have also been clearly identified as a common cause of atrial arrhythmias in those with persistent AF(Haissaguerre et al., 2005a; Haissaguerre et al., 2005b). Thus, the initiation of AF is likely to be a multi-factorial process. Abnormalities in calcium handling have recently been implicated in the pathogenesis of AF. In atrial tissues from patients with chronic AF there

are reported reductions in message for L-type Ca^{2+} channels and SERCA, most commonly without changes in message for phospholamban, CASQ, NCX or RyR2 (VanWagoner et al., 1999; Van Gelder et al.,1999; Brundel et al.,1999; Lai et al.,1999). However, RyR messagewas reportedly reduced in one study, and accompanied by reduced binding of ^{3}H-ryanodine (Ohkusa et al., 1999). In vitro studies in normal tissues have found that activation of RyR2s with ryanodine (0.5–2 μM) results in increased spontaneous electrical activity in the PVs. As has been reported in the ventricle during HF, it has been suggested that hyperphosphorylation of RyR2s contributes to diastolic SR Ca^{2+} leak and may contribute to the initiation or maintenance of AF (Vest et al., 2005). In this study of atrial tissue from either dogs or humans with chronic AF, there was increased PKA-dependent phosphorylation of RyR2 at serine 2809, reduced FKBP12.6 bound to the RyR2 complex, and increased RyR2 channel open probability, compared to controls. In addition, treatment with JTV519 was reported to normalize AF-induced abnormalities in RyR2 function. More recently, reduced activity of SR-associated PP1 has been reported in atrial tissues from patients with chronic AF (Dobrev, 2006), which could contribute to increased phosphorylation of RyR2s. However in light of recent negative results on the role of FKBP in other disease states these findings should be viewed with caution.

7. Therapeutic strategies

7.1. Arrhythmia

Current antiarrhythmic treatments are often empiric and ineffective. One significant limitation to the effective prevention and treatment of arrhythmias is that the initiating and/or perpetuating event(s) are often unknown in a given patient. For arrhythmias of the atria or ventricles, pharmacologic antiarrhythmic therapies act by blocking fast inward sodium channels (slowing conduction) or blocking repolarizing potassium currents (prolonging refractoriness). Examples of clinically useful sodium channel blockers would include procainamide, lidocaine, mexiletine, flecainide or propafenone. Within these are drugs with pharmacology which varies from pure sodium channel block, (e.g. lidocaine), to procainamide which also prolongs refractoriness through effects on potassium channels, to propafenone which is also a non-selective beta-adrenergic blocker. Examples of clinically useful potassium current blockers include dofetilide, sotalol (also a non-selective beta-adrenergic blocker) and amiodarone (also a blocker of fast inward sodium current, slow inward calcium current, a non-selective beta-adrenergic blocker, and an antioxidant). Non-pharmacologic treatment with implantable cardioverter defibrillators has been shown to be more effective that optimal pharmacologic therapy in multiple clinical trials; although many patients receive a combination of pharmacologic and non-pharmacologic therapy for ventricular tachyarrhythmias (Carnes et al., 1998). Interestingly, the drug with the highest rate of efficacy is amiodarone which is the least pharmacologically selective.

However, for those diagnosed with specific forms of arrhythmic diseases, there may be targeted therapy. For example, catecholaminergic polymorphic ventricular tachycardia (CPVT) is typically treated initially with beta-adrenergic blockers (e.g. nadolol), with implantable cardioverter defibrillators also used for either primary or secondary prevention of cardiac arrest (Liu et al., 2007). Ideally, as arrhythmic mechanisms are better understood, additional targeted and specific therapies can be developed for the treatment and prevention of cardiac arrhythmias.

The antiarrhythmic effects of ryanodine have been investigated in multiple animal models. In canine models, ryanodine has been shown to reduce ouabain-induced (Ca^{2+} overload) arrhythmias (Lappi & Billman, 1993), but not ischemia-induced arrhythmias (Lappi & Billman, 1993). In addition, ryanodine has demonstrated efficacy in reducing early afterdepolarizations and Torsades de Pointes in a canine model of chronic AV nodal block (Verduyn et al., 1995). Similar to the findings in canines, ryanodine has been shown to reduce ouabain-induced arrhythmias in guinea pigs (Golovina et al., 1988; Zakharov et al., 1991).

There is also evidence suggesting that ryanodine may reduce reperfusion-induced arrhythmias (Thandroyen et al., 1988; Hayashi et al., 1996). Thus, ryanodine, by reducing pathologic leak of Ca^{2+} from the SR, appears to have antiarrhythmic efficacy in multiple models of arrhythmogenesis, in particular when then there is increased SR Ca^{2+} load (e.g. ouabain toxicity). However, as might be expected, ryanodine is a profound negative inotrope (Thandroyen et al., 1988), and thus optimal therapy should aim to normalize, rather than simply inhibiting RyR2 function in order to maintain systolic function.

7.2. Heart failure

Heart failure is a constellation of symptoms (circulatory congestion, dyspnea, fatigue, and/or weakness) which results when cardiac output is not sufficient to meet the body's metabolic demands. One of the most common defects causing heart failure is impaired contractility of the ventricle (cardiomyopathy or ventricular systolic dysfunction). With respect to calcium cycling, hallmark characteristics of heart failure include impaired contractility, and low amplitude, broad calcium transients in isolated myocytes. Current therapies for heart failure are intended to reduce circulatory congestion, reduce cardiac hypertrophy and structural remodeling, reduce cardiac afterload and preload, and modify adrenergic signaling. Current therapeutic strategies for patients with current or previous symptoms of chronic systolic HF include a diuretic, beta-adrenergic blocker, an angiotensin converting enzyme inhibitor or angiotensin receptor blocker, and in selected patients an aldosterone antagonist (Hunt et al., 2005). Other therapeutic strategies which may be appropriate for specific patient populations include an implantable cardioverter defibrillator, cardiac resynchronization therapy, digoxin and/or combined hydralazine and nitrate therapy. The ideal therapy for HF would be one that increases contractility while preserving relaxation (lusitropy) and avoiding proarrhythmia (the development of new arrhythmias or worsening of existing arrhythmias). Given the Ca^{2+}-dependence of both arrhythmia initiation and contractility this remains a difficult and challenging goal.

7.3. Normalizing ryanodine receptor function as a treatment strategy for cardiac disease

Since RyR Ca^{2+} leak contributes to various disease phenotypes ranging from uncontrolled Ca^{2+} release and SR depletion, simply blocking RyRs is not a viable strategy for treating arrythmia or HF in general. Selectivity for RyR2s (relative to the RyR1 complexes in skeletal muscle) would be critical for any clinically viable therapy targeting RyR2 modulation. Additionally, implementing a successful therapy based on RyR2 targeting could be challenging due to the complex nature of store-mediated Ca^{2+} signaling. Indeed, inhibition of RyR2s may fix the leak, but has the potential to impair systolic function. Moreover, inhibition of RyR2s can also result in inhomogeneities of Ca^{2+} release that can be arrythmogenic (Diaz et al., 2002). Still RyR inhibitors, such as an RyR2-selective derivative of tetracaine, might prove to be useful for treating certain conditions when the SR Ca^{2+} leak is excessively large such as end-stage HF. Indeed, Eisner and coworkers (Venetucci et al., 2006) showed that tetracaine increased Ca^{2+} transients by increasing the SR Ca^{2+} load in myocytes exposed to isoproterenol. A caveat is that the therapeutic action of such an agent would be highly concentration-dependent such that at high concentrations it could inhibit systolic Ca^{2+} release.

RyR2 inhibitors could be potentially used in combination with other agents to enhance SERCA-mediated SR Ca^{2+} uptake thus restoring further SR Ca^{2+} content. Although potentially useful for treating end-stage HF where the SR Ca^{2+} content is greatly diminished, both strategies could be detrimental by increasing the risk of arrhythmia. For example, tetracaine can cause homogeneities in release, while adrenergic stimulation (which occurs in response to HF) in isolation can promote arrhythmias. Given these problems with using RyR2 antagonists, targeting the underlying causes of altered RyR2 function appears to be a more promising approach and developing such therapies might be the ultimate goal in treating RyR2-linked

cardiac diseases. Currently several such strategies are emerging based on specific mechanisms proposed to underpin dysregulation of RyR2s in cardiac disease, including abnormal PKA and/or CAMKII phosphorylation and/or altered redox modification.

Much attention has been devoted to a Ca^{2+} channel blocker derivative with reported beneficial effects in HF and a range of reported intracellular targets such as PKC, mitochondrial K^+ channels, and RyR2s. It has been suggested that JTV519 improves Ca^{2+} handling in both HF and CPVT by stabilizing FKBP12.6 binding to RyR2s thereby reducing the RyR-mediated SR Ca^{2+} leak (Yano et al., 2003; Wehrens et al., 2005b; Lehnart et al., 2006). However this mode of action of JTV519 has been disputed by other investigators, further adding to the uncertainties regarding the role of FKBP12.6 in HF-related alterations in RyRs. While most studies agree that JTV519 improves cardiac function in HF, its beneficial effects in CPVT are not supported by recent investigations (Liu et al., 2006; Hunt et al., 2007; George et al., 2007). This may indicate that the beneficial effects of JTV519 on cardiac function in HF do not involve RyRs, or that the RyR defects in CPVT and HF are caused by different mechanisms.

Given the recent evidence that RyR-mediated alterations in HF and in certain types of arrhythmia are mediated by CAMK2-phosphorylation of RyR (Ai et al., 2005; Grueter et al., 2007) targeting CAMK might be another strategy to normalize RyR function in cardiac disease. Finally, preventing/reversing pathologic RyR modification by reactive oxygen/nitrogen species has been recently proposed as a strategy to treat HF and arrhythmias caused by abnormal Ca^{2+} release. The ability of antioxidants to improve cardiac performance was demonstrated in a number of studies using animal models of HF (e.g. Mochizuki et al., 2007). However, a recent clinical trial examining xanthine oxidase inhibition was not successful (Hare et al., 2006). It is unclear to what extent this lack of success is due to the particular antioxidants used. A better understanding of the specific redox pathway abnormalities and the affected Ca^{2+} signaling mechanisms may enable the development of new rationally targeted therapeutic strategies.

8. Conclusion

In recent years there has been significant progress in understanding Ca^{2+} handling in the heart and how changes in Ca^{2+} homeostasis contribute to cardiac disease. Exploring the functional consequences of naturally occurring arrhythmogenic mutations in Ca^{2+} handling proteins such as RyR2 and CASQ2 has been especially instructive. These studies have led to a better understanding of the control of SR Ca^{2+} release at the molecular level in normal and disease settings. An important question for future studies is how derangement of one aspect of Ca^{2+} handling, i.e. Ca^{2+} release through RyR2s, can result in awide spectrum of disease phenotypes ranging from arrhythmogenic Ca^{2+} alternans and dispersed SR Ca^{2+} release to depleted SR Ca^{2+} stores in HF. A comprehensive understanding of these mechanisms may provide a conceptual framework for the development of individualized, targeted therapy of cardiac diseases.

Abbreviations

AF	atrial fibrillation
AP	action potential
CAMKII	calmodulin-dependent protein kinase II
CASQ	calsequestrin
CN	calcineurin
CPVT	catecholaminergic polymorphic ventricular tachycardia

DAD	delayed afterdepolarization
DTT	dithiothreitol
EAD	early afterdepolarization
FKBP	FK binding protein
fSR	free sarcoplasmic reticulum
HF	heart failure
JN	junctin
jSR	junctional sarcoplasmic reticulum
NCX	sodium–calcium exchanger
PKA	protein kinase A
PLB	phospholamban
PV	pulmonary vein
QTc	rate-corrected QT interval of the electrocardiogram
RyR	ryanodine receptor
SERCA	sarco-endoplasmic reticulum calcium ATPase
TRD	triadin
Vm	membrane voltage

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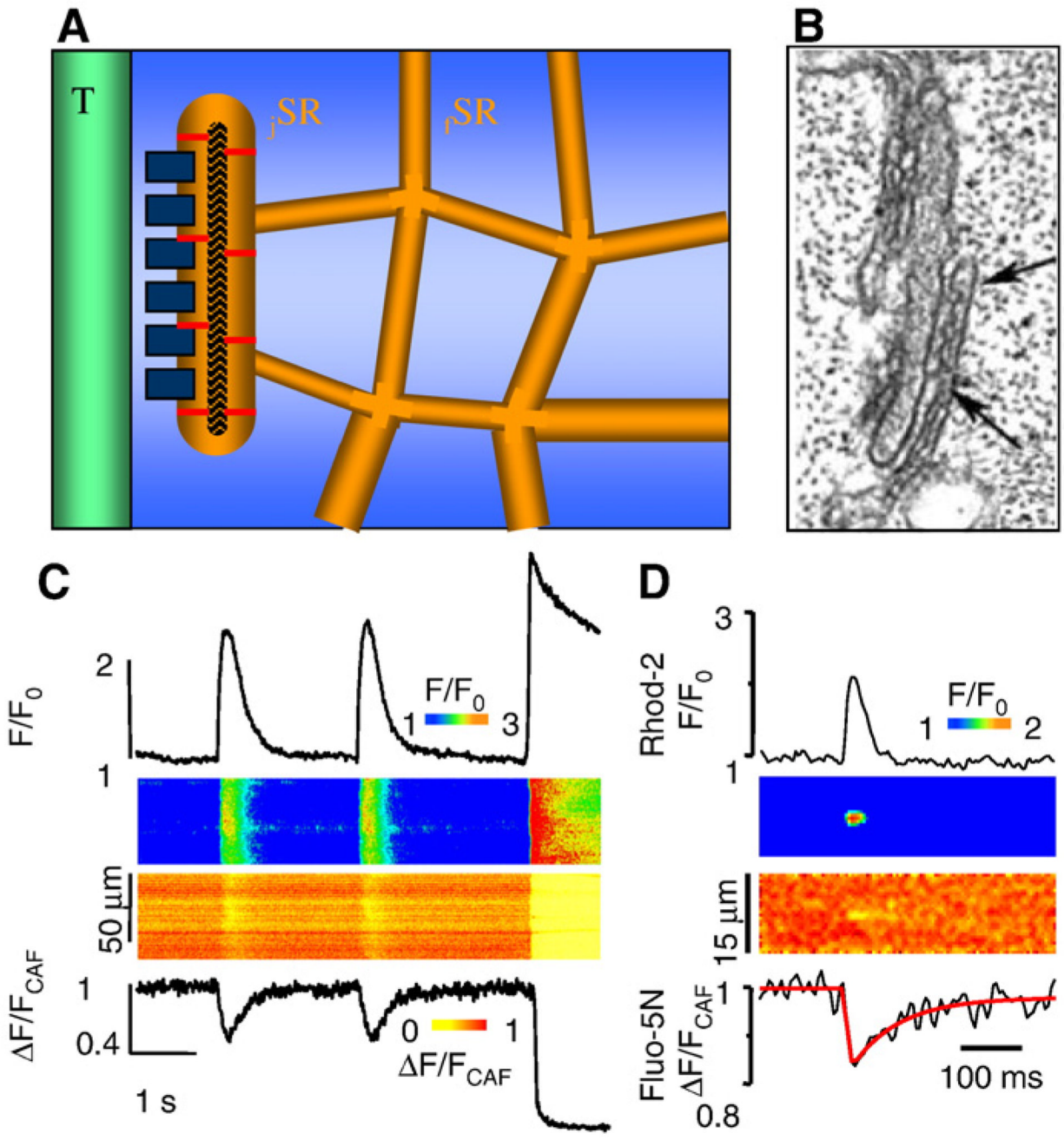

Fig. 1.

SR microanatomy and Ca^{2+} signaling. **A**. Diagram illustrating the relationship between the free SR (fSR) network, the junctional (jSR) cisternae, T tubules, feet and calsequestrin along with its anchoring filaments in a mammalian ventricular myocyte. **B**. Electron micrograph of junctional SR of a rabbit ventricular myocyte. Between the arrows RyR2s are seen as foot structures in the gap between jSR and T tubule membranes. **C**. Action potential-elicited cytosolic and intra-SR Ca^{2+} signals followed by caffeine-induced Ca^{2+} release and SR depletion. Upper: profiles and linescan images of cytosolic Ca^{2+} changes measured with Rhod-2; lower: profiles and linescan images of $[Ca^{2+}]_{SR}$ changes monitored with Fluo 5N. **D**. Simultaneous measurements of local SR Ca^{2+} release (Rhod-2, upper) and depletion signals

(Fluo 5N, lower) (i.e. sparks and blinks). Panel **B** from DXP Brochet, **D** Yong, De Maio **A**, WJ Lederer, **C** Franzini-Armstrong, H Cheng. Ca^{2+} blinks: rapid nanoscopic store calcium signaling. PNAS 2005; 102: 3099–3104. Copyright (2005) National Academy of Sciences, U.S.A.

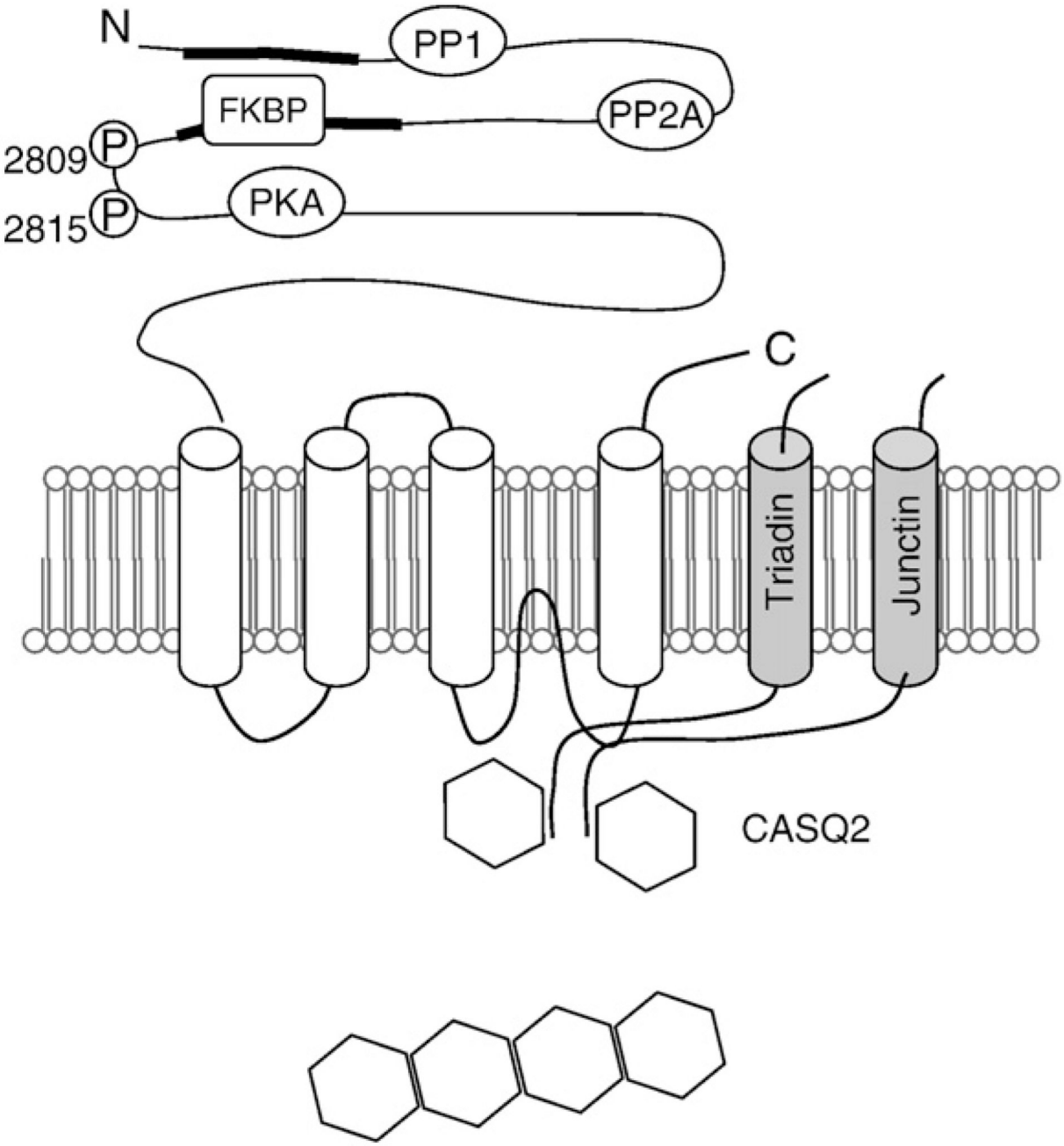

Fig. 2.

Schematic presentation of the RyR2 channel complex. The domain structure of RyR2 with sites of interaction with auxiliary proteins and phosphorylation sites. Two major divergent regions located in the cytosolic portion are indicated. Calsequestrin, junctin, and triadin, proteins interacting with RyR and between themselves and RyR2 in the SR, are shown. PP, protein phosphatase; P, phosphorylation sites; CaM, calmodulin; CaMKII, calmodulin-dependent protein kinase II. Adapted from Gyorke and Terentyev, 2008.

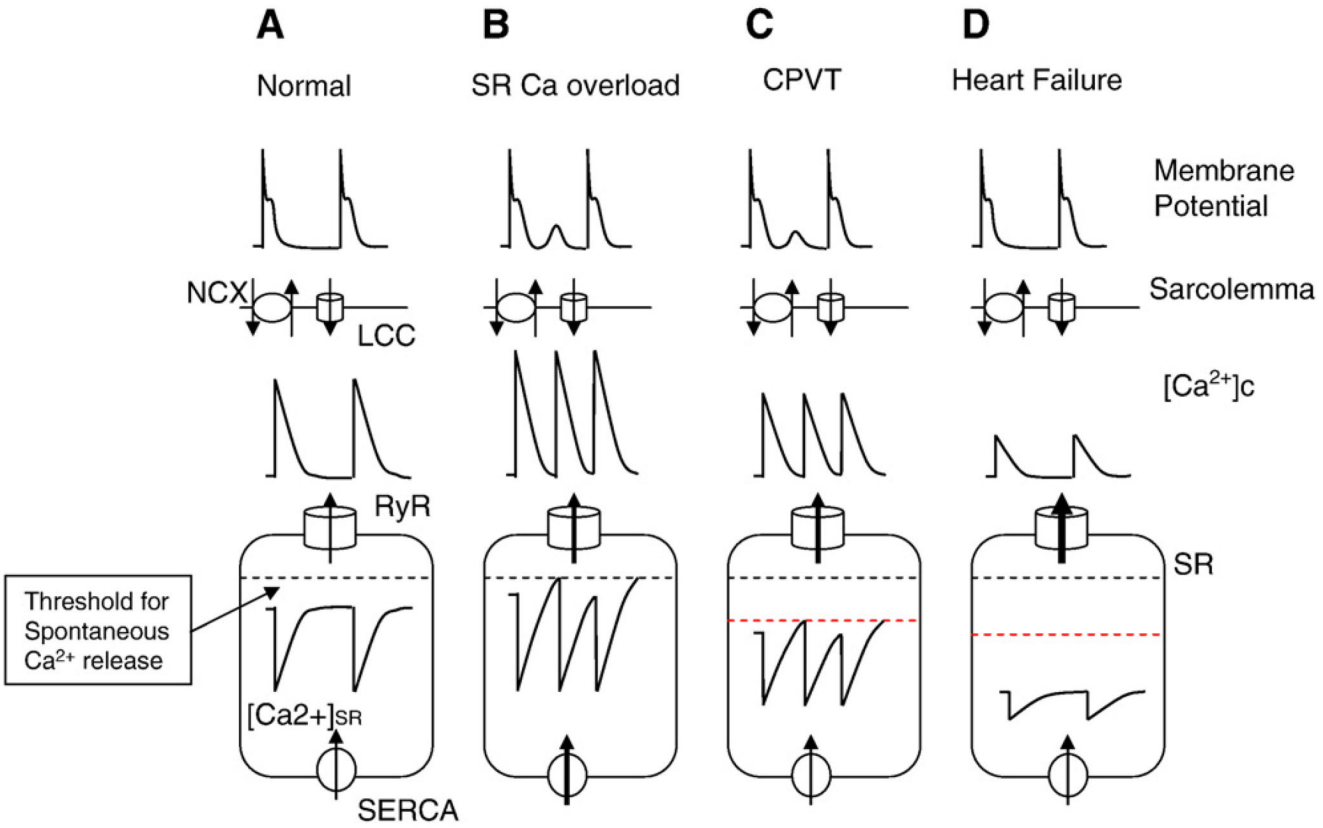

Fig. 3.

Ca^{2+} cycling in normalmyocytes andmyocytes under three different disease conditions: SR Ca overload, CPVT and heart failure. **A**. In normalmyocytes, Ca^{2+} influx through L-type Ca^{2+} channels (LCC) during the action potential activates RyR2s and initiates the release of Ca^{2+} stored in the SR on a beat-to-beat basis. Diastolic SR Ca^{2+} release is prevented due to RyR2 deactivation at reduced $[Ca]_{SR}$; $[Ca]_{SR}$ is always below the threshold for activation of spontaneous Ca release (indicated by dashed line). **B**. Under conditions of Ca^{2+} overload, $[Ca]_{SR}$ reaches the threshold for activation of spontaneous Ca release, diastolic SR Ca^{2+} release occurs, resulting in arrhythmogenic DADs through stimulation of NCX. **C**. Abnormal RyR2 channel function caused by CPVT-linked mutations in RyR2 and CASQ2 result in a reduced threshold (indicated by red dashed line) for spontaneous Ca^{2+} release (i.e. perceived Ca^{2+} overload), diastolic Ca^{2+} release and DADs. **D**. In heart failure, acquired defects in RyR2 result in a more severe RyR2 deregulation ($[Ca^{2+}]_{SR}$ threshold for spontaneous Ca^{2+} release is further reduced); excessive SR Ca^{2+} leak leads to depletion of the $[Ca]_{SR}$ and diminished systolic Ca^{2+} release and contraction.

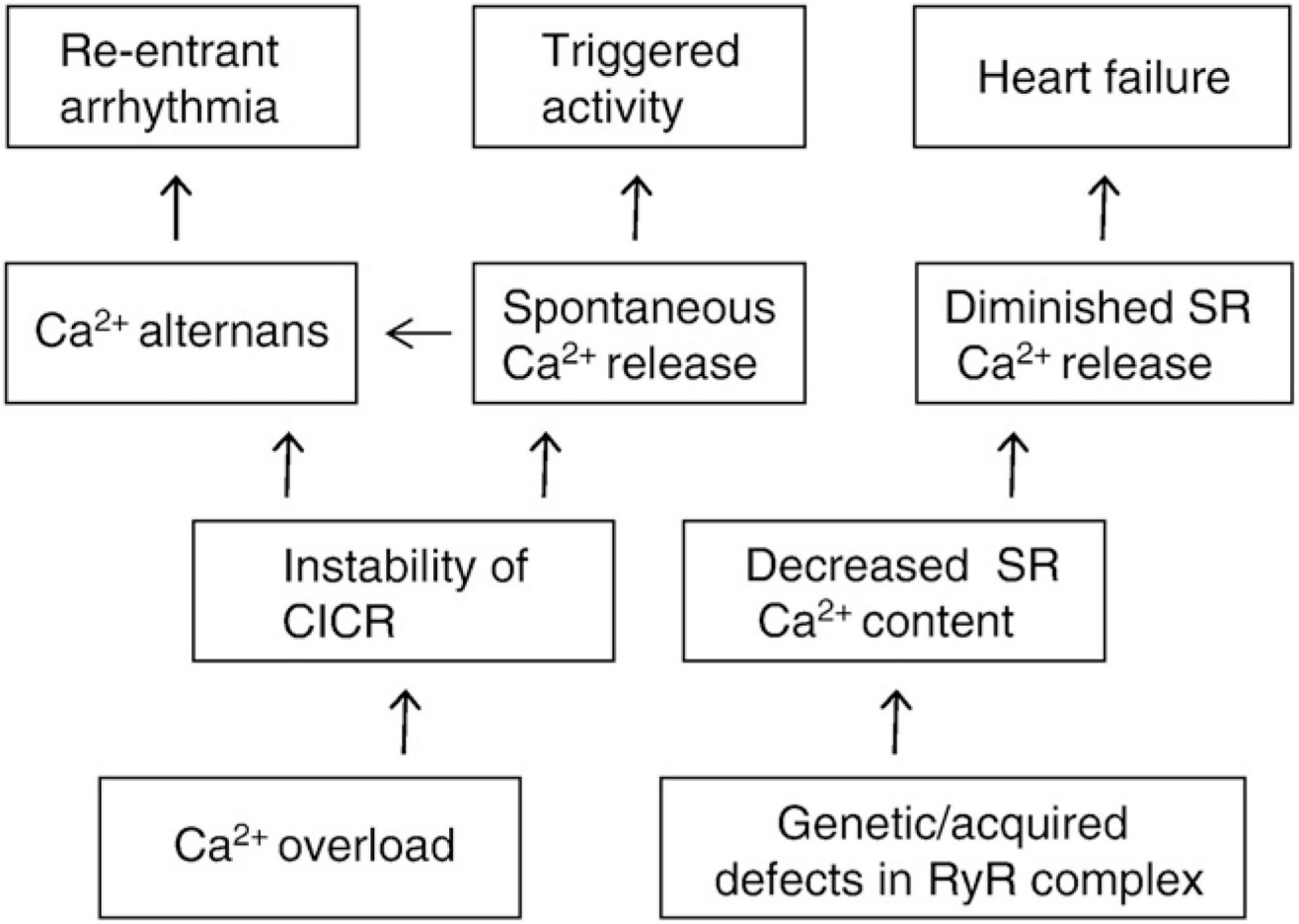

Fig. 4.
Classification of Ca^{2+}-dependent disease states based on mechanisms of alterations of Ca^{2+} handling.

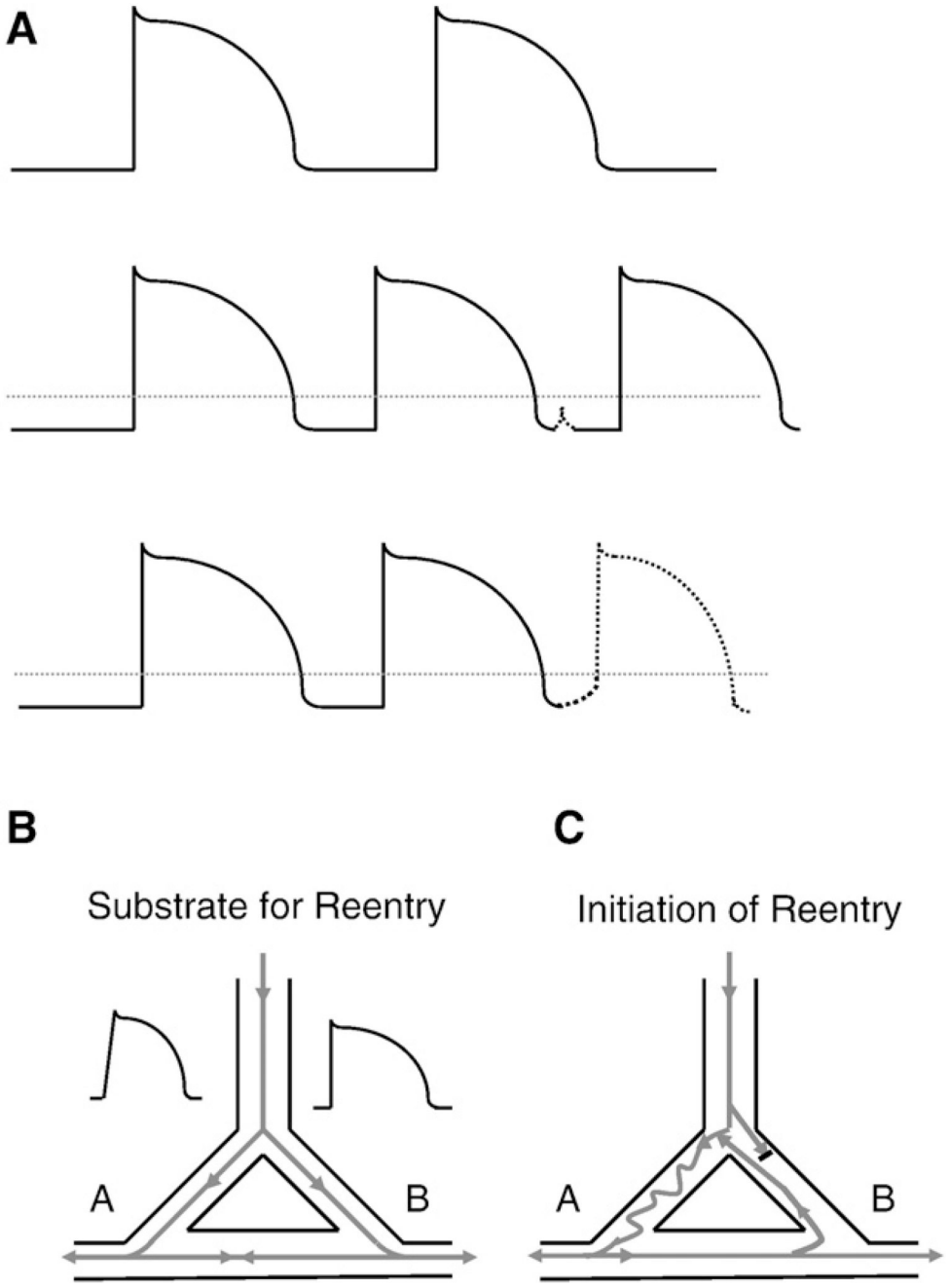

Fig. 5.

Substrate for re-entry and initiation of re-entry. **A**. Top panel: action potentials under control conditions. Middle panel: action potentials at a faster rate, with a delayed afterdepolarization (dashed line). Note that the delayed afterdepolarization does not reach the voltage threshold to trigger an action potential (threshold indicated by dotted line). Bottom panel: the delayed afterdepolarization is of sufficient amplitude to reach the threshold voltage and triggers an early action potential. **B**. The figure depicts an anatomic circuit which has the functional prerequisites to form a substrate for re-entry. Note that in pathway A, there is slowed conduction (represented by the slower upstroke of the action potential), while in pathway B, there is normal conduction but a prolongation of the action potential duration (and therefore prolongation of

the refractory period) in the representative action potential. **C**. A properly timed premature beat excites the anatomic circuit and initiates a reentrant arrhythmia. Note that due to the slower conduction in pathway A, the impulse is very slowly conducted down pathway A. However, due to the longer refractory period which exists in pathway B, the premature beat is too early to excite pathway B, and conduction is blocked. As the premature impulse exits pathway A, the refractory period in pathway B has passed and the impulse excites pathway B in a retrograde manner. Due to the heterogeneities of conduction velocity and refractoriness, this anatomical circuit can maintain reentrant activation and thus, a tachyarrhythmia.