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Ca²⁺ Release Channels (Ryanodine Receptors) and Arrhythmogenesis

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Abstract

Intracellular calcium (Ca²⁺) release channels (ryanodine receptors type 2, RyR2) are present on the sarcoplasmic reticulum (SR) in cardiomyocytes and are required for excitation-contraction coupling in cardiac muscle. RyR2s are macromolecular channel complexes associated with regulatory proteins that modulate RyR2 function in response to extracellular signals. Recent studies have provided new mechanistic insight into the role of diastolic SR Ca²⁺ leak through RyR2 as a trigger for a wide range of cardiac arrhythmias. RyR2 has been implicated to play a central role in arrhythmias associated with catecholaminergic polymorphic ventricular tachycardia (CPVT), heart failure (HF) and drug-induced arrhythmias. At present, there is no FDA approved medication that selectively targets RyR2. However, novel therapeutic approaches are being evaluated to correct defective RyR2 Ca²⁺ release based on recent advances in the understanding of the cellular mechanisms underlying arrhythmias in HF and CPVT.

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Introduction

Intracellular calcium (Ca²⁺) release channels (ryanodine receptors, RyR) are present on the sarcoplasmic reticulum (SR) in cardiomyocytes and are required for excitation-contraction (EC) coupling in cardiac muscle. Each RyR channel contains four pore-forming subunits that contain large cytoplasmic domains, which serve as scaffolds for proteins that regulate the activity of the channel. An important regulatory protein is calstabin2 (FKBP12.6), a subunit that stabilizes the closed state of the channel to prevent aberrant Ca²⁺ leak from the SR [1]. Direct targeting of several protein kinases and phosphatases

to the type 2 cardiac RyR channel (RyR2) allows for rapid and localized modulation of SR Ca^{2+} release in response to extracellular signals [2].

Recent studies have provided new mechanistic insight into the role of diastolic SR Ca^{2+} leak through RyR2 as a trigger of cardiac arrhythmias. For example, inherited mutations in the RyR2 gene have been linked to cardiac arrhythmia syndromes including catecholaminergic polymorphic ventricular tachycardia (see also chap. 31) [3]. Moreover, defective regulation of RyR2 may contribute to arrhythmogenesis in heart failure (HF) [4–9]. These findings have initiated the development of a new class of drugs called 'RyR stabilizers' that specifically target these molecular defects, improve cardiac function and prevent arrhythmias in relevant animal models [10–19].

Excitation-Contraction Coupling in the Heart

The process of EC coupling comprises a sequence of events that translates electrical membrane depolarization into contraction of the cardiomyocyte [20]. During cardiac muscle contraction (systole), Ca^{2+} is released from a network of intracellular Ca^{2+} stores referred to as the SR (Fig. 17.1). When the heart subsequently relaxes to fill with blood (diastole), the cytoplasmic Ca^{2+} is actively pumped back into the SR. Cyclic release of Ca^{2+} from the SR Ca^{2+} store and Ca^{2+} uptake from the cytosol are thus critical for rhythmic contraction and relaxation of the heart [22].

Depolarization of the cardiomyocyte membrane leads to activation of voltage-gated L-type Ca^{2+} channels (LTCC) located in plasma membrane invaginations called transverse or T-tubules (see Fig. 17.1). Entry of Ca^{2+} through LTCCs triggers a much greater release of Ca^{2+} from the SR via the RyR2 intracellular Ca^{2+} release channels, which is known as Ca^{2+}-induced Ca^{2+} release (CICR) [23]. For relaxation to occur during diastole, Ca^{2+} is extruded from the cytoplasm via a specialized Ca^{2+} pump known as the SR Ca^{2+} ATPase (SERCA2a). SERCA2a activity is regulated by phospholamban, which inhibits SR Ca^{2+} uptake in its non-phosphorylated form. Finally, cytosolic Ca^{2+} is also extruded from the cardiomyocyte via the electrogenic plasma membrane Na^+/Ca^{2+} exchanger (NCX) [22, 24].
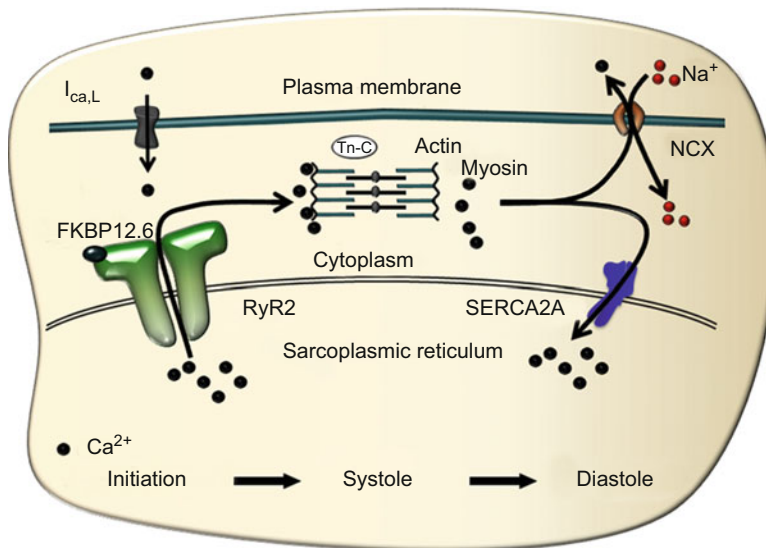

Figure 17–1. Excitation-contraction coupling in the heart. With the advent of an action potential, L-ype Ca^{2+} channels are activated resulting in the influx of Ca^{2+}. This influx of Ca^{2+} activates the adjacent RyR2 resulting in outpouring of Ca^{2+} stored in the sarcoplasmic reticulum (SR) into the cytosol. The release of Ca^{2+} activates the contractile proteins initiating systole. The increased cytosolic Ca^{2+} inactivates L-type Ca^{2+} channels, activates NCX (causing efflux of Ca^{2+}) and activates SERCA2a (causing reuptake of Ca^{2+} into SR). This results in the termination of systole and the onset of diastolic phase. $I_{Ca,L}$ L-type Ca^{2+} current, *NCX* Na^+/Ca^{2+}-exchanger, *PLN* Phospholamban, *RyR2* Ryanodine receptor type 2, *SERCA2a* Sarco/endoplasmic reticulum Ca^{2+}-ATPase, *TnC* Troponin C (Modified from Wehrens et al. [21]. With kind permission from Future Medicine Ltd)

Advanced imaging techniques have revealed that cytosolic Ca^{2+} transients in cardiomyocytes represent the summation of 10^4 up to 10^6 localized, subcellular Ca^{2+} release events called Ca^{2+} sparks [25]. The CICR process amplifies and scales the intracellular Ca^{2+} signal via summation of functionally independent Ca^{2+} sparks [26]. Moreover, Ca^{2+} sparks are characterized by local refractoriness, suggesting that local SR Ca^{2+} release is independent of the duration of the LTCC-mediated Ca^{2+} influx [27]. It is estimated that about 100 or more RyR2 channels may become functionally coupled in a Ca^{2+} release unit in order to open and close simultaneously by the process of coupled gating [28]. On the other hand, Ca^{2+} release from the SR also results in local Ca^{2+} depletion in subcompartments of the SR Ca^{2+} stores [29]. Therefore, the intrinsic (e.g. channel composition) and extrinsic (e.g. regulation by extracellular signals) properties of RyR2 channel gating are an important determinant of the net Ca^{2+} concentration in the cytosol and the SR at any given time point during the cardiac contraction-relaxation cycle.

Cardiac Ryanodine Receptors

RyRs are comprised of four 560-kDa pore-forming subunits, which form tetrameric ion channels located on the SR membranes [30]. RyR2 is the predominant isoform in the heart and functions as the principal intracellular Ca^{2+} release channel during EC coupling [31]. Apart from a relatively small C-terminal pore-forming domain, approximately 90 % of the RyR2 N-terminal sequence forms the electron-dense cytosolic foot structure in the junctional space between the T-tubule and the SR (Fig. 17.2). This
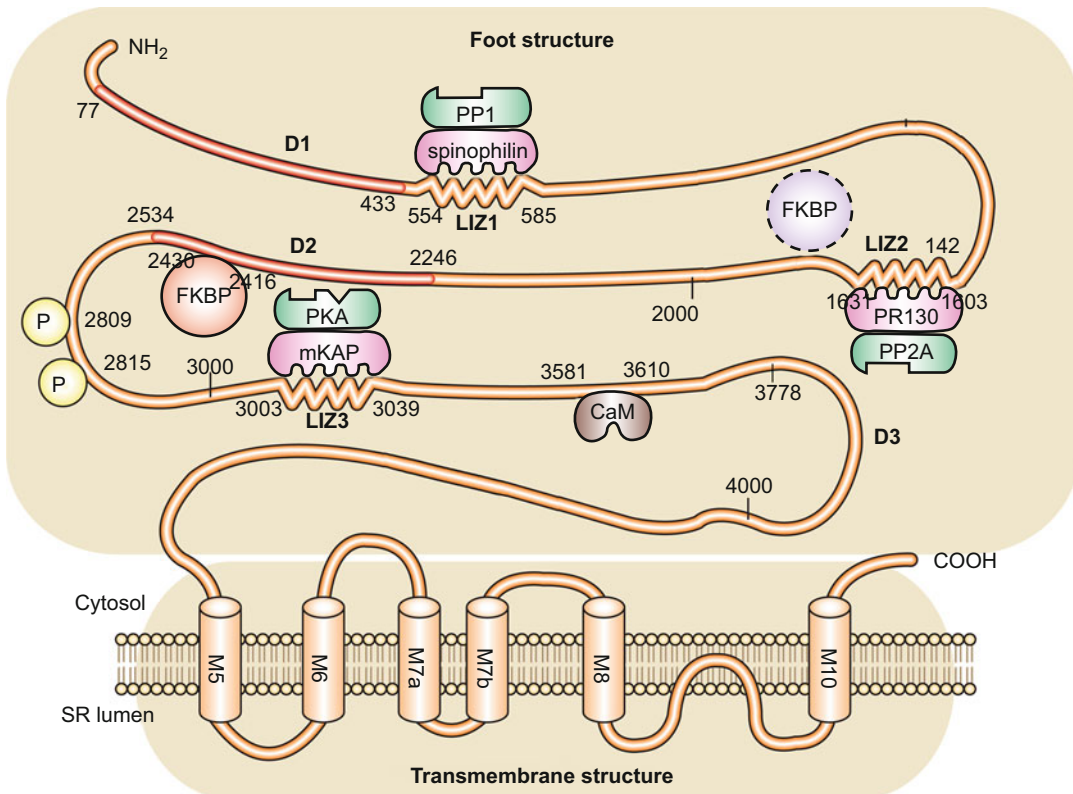

Figure 17–2. Primary structure of the cardiac ryanodine receptor macromolecular complex. The primary structure of a cardiac ryanodine receptor subunit, with the binding domains of protein kinase A (*PKA*), protein phosphatases 1 and 2A (*PP1*, *PP2A*), calmodulin and FKBP12.6 indicated. PKA and PP1/PP2A are bound to RyR2 via their specific adaptor proteins. The domains highlighted in red (77–433, 1724–2534) as well as the transmembrane domains correspond to the three CPVT mutation hotspot regions (*D1*, *D2* and *D3*). *CaM* calmodulin, *FKBP* FKBP12.6, *LIZ* leucine-isoleucine zipper, *PKA* protein kinase A, *PP* protein phosphatases, *SR* sarcoplasmic reticulum (From Macmillan Publishers Ltd: Yano et al. [32]. Reproduced with kind permission from Nature Publishing Group)

cytoplasmic RyR2 domain functions as a scaffold for modulating proteins, which regulate gating of the transmembrane Ca^{2+}-conducting pore [24, 33, 34].

The Ryanodine Receptor Macromolecular Complex

A major regulatory subunit interacting with RyR2 Ca^{2+} release channels is the 12.6 kDa cytosolic FK506-binding protein (FKBP12.6), also known as calstabin2 [35, 36]. FKBP12.6, a peptidyl-prolyl *cis*–*trans* isomerase that tightly associates with RyR2 monomers, stabilizes the closed conformational state of RyR2 enabling the channel to close completely [37, 38]. Therefore, in the heart, an important physiologic role of FKBP12.6 appears to be inhibiting the RyR2 channel at low intracellular Ca^{2+} concentrations to ensure diastolic muscle relaxation and preventing detrimental effects of intracellular Ca^{2+} leak during diastole [13, 38]. In addition, FKBP12.6 plays a role in the orchestration of simultaneous RyR2 gating in Ca^{2+} release units comprised of functionally linked RyR2 [28, 39].

Other modulators associated with the large cytosolic RyR2 domain include calmodulin (CaM) [40], sorcin [41], the protein kinases A (PKA) [5], Ca^{2+}/CaM-dependent protein kinase II (CaMKII) [42, 43], and the phosphatases, PP1 and PPA2A [2]. These kinases and phosphatases are believed to bind to RyR2 via specific anchoring proteins mAKAP, spinophilin and PR130, respectively (see Fig. 17.2). Conserved leucine/isoleucine zipper (LIZ) motifs in the targeting proteins correspond to complementary LIZ domains in RyR2, allowing for highly localized RyR2 regulation.

RyRs also associate with proteins at the luminal surface within the SR membrane. Junctin and triadin are most likely involved in anchoring RyR2 to the SR membrane [44, 45]. Calsequestrin (CSQ) is the major Ca^{2+} binding protein in the SR and provides a high capacity intra-SR Ca^{2+} buffer [46]. Recent studies suggest that Ca^{2+}-dependent conformational changes in CSQ modulate RyR2 channel activity and that dysfunctional luminal regulation of RyR2 may lead to cardiac arrhythmias (see below) [47]. Finally, we recently demonstrated that junctophilin-2 (JPH2) binds to RyR2 and mediates both physical and functional coupling between RyR2 and voltage-gated LTCC on the plasmalemma [48].

Regulation of RyR2

Phosphorylation

The maximal cardiac force development during systole (inotropy) can be enhanced by increasing the amplitude of the intracellular Ca^{2+} transient, which is dependent on the amount of intracellular Ca^{2+} released via RyR2 [49]. Upregulation of SR Ca^{2+} cycling can be achieved by phosphorylation of proteins involved in SR Ca^{2+} release and Ca^{2+} uptake pathways by cAMP-dependent PKA [5], CaMKII [43] and protein kinase C (PKC) [50]. A potent mechanism to increase cardiac contractility rapidly is mediated by stress-mediated activation of β(Beta)-adrenergic receptors by catecholamines resulting in increased intracellular cAMP synthesis and activation of PKA [51]. PKA phosphorylates both RyR2 to increase SR Ca^{2+} release,[2] as well as phospholamban to enhance the activity of SERCA2A [52]. The net effect of β(Beta)-adrenergic receptor activation is amplification of the EC coupling gain such that for any given LTCC Ca^{2+} current more SR Ca^{2+} release is triggered [53].

Some studies have shown that phosphorylation by PKA increases RyR2 channel open probability by increasing the sensitivity to Ca^{2+}-dependent activation [5,38,54,55], although some studies indicate that PKA phosphorylation might not directly alter RyR2 open probability [56–58]. Although some studies suggest PKA might phosphorylate an additional residue on RyR2 *in vitro* [59], PKA phosphorylation of RyR2 is completely prevented in RyR2-S2808A knockin mice, in which serine 2808 is replaced by an alanine [60]. The transient nature of RyR2 activation by PKA phosphorylation in cells suggests powerful negative feedback mechanisms that prevent uncontrolled intracellular Ca^{2+} release. Indeed, RyR2 phosphorylation is locally regulated by protein phosphatases, which are targeted to the channel complex [2, 61]. Moreover, it was

shown that the phosphodiesterase PDE4D3 associates with RyR2 limiting excessive phosphorylation of the channel, which has been shown to detrimentally affect the cardiac function in disease states of the heart [62].

RyR2 channels can also be phosphorylated by CaMKII, which phosphorylates a nearby site (serine 2814) on RyR2 and does not decrease the binding affinity of FKBP12.6 [43]. We demonstrated that genetic inhibition of S2814 in RyR2-S2814A mice inhibits the increase in RyR2 open probability following CaMKII phosphorylation of the channel [48]. CaMKII phosphorylation of RyR2 is believed to play an important role in the positive force-frequency relationship, as S2814A knock-in mice did not develop increased cardiac output at higher heart rates [63].

Redox Modulation

The open probability of RyR2 is not only modulated by phosphorylation but also by redox signaling [64–66]. RyR2 contains around 90 cysteine residues per monomer of which approximately 20 are in a reduced state and are potential targets for various redox modifications, including S-nitrosylation, S-glutathionylation, and disulfide crosslinking [67]. Experimental evidence suggests that redox modulation of RyR2 by NO, H_2O_2, nitroxyl, and hydroxyl radicals alter its activity *in vitro* [68, 69]. In general, oxidizing conditions increase the open probability of RyR2, whereas reducing reagents such as dithiothreitol reverse these effects [70–72]. However, prolonged exposure to high doses of oxidizing agents may cause irreversible and detrimental RyR activation [72, 73].

Although the effect of redox modulation of RyR2 is well established, the physiological role of endogenously produced oxidants is not. S-nitrosylation is an important reversible biological reaction involving nitric oxide in which nitrosyl groups are added to thiol residues. Out of the three known nitric oxide synthase (NOS) isoforms, cardiomyocytes express two constitutive NOS enzymes, neuronal NOS (nNOS) and endothelial NOS (eNOS). eNOS localizes to caveolae [74–76], where compartmentalization with β-adrenergic receptors and L-type Ca²⁺ channels [77] allows NO to inhibit β (beta)-adrenergic-induced inotropy

[76, 78]. nNOS, however, is targeted to cardiac SR [79]. NO stimulation of SR Ca²⁺ release via RyR *in vitro* suggests that nNOS has an opposite, facilitative effect on contractility [67, 80]. This opposite effect is further evidenced by the observation that nNOS deficiency reduces inotropic response in contrast to eNOS deficiency that enhances contractility due to corresponding changes in SR Ca²⁺ release [81]. In addition to redox modulation by nitrosylation, cardiac SR is reported to have NADH oxidase as well as NOX2 oxidase (that utilizes NADPH instead of NADH) that regulate the RyR2 complex under physiological conditions [82–84].

Growing evidence suggests that redox modification of RyR2 may contribute to abnormal Ca²⁺ handling in disease states. S-nitrosylation (>3 sites per monomer) leads to *reversible* RyR activation [67]. In contrast, oxidation does not affect channel function up to a certain threshold (5–6 thiols per monomer), beyond which it leads to *irreversible* activation [67]. Moreover, oxidation of reactive thiols induces cross-linking between the subunits of RyR resulting in channel activation, which can be reversed by S-nitrosylation [65]. In addition, nNOS deficiency leads to an increase in reactive oxygen species (ROS) that may lead to oxidation of reactive thiols on RyR2 [85]. Thus, whereas S-nitrosylation seems to control the basal redox state of the channel, oxidation is a pathological process found in disease/stress states like HF [64].

Role of RyR2 in Arrhythmogenesis

Arrhythmias have a complex pathogenesis that include disorders of impulse formation or conduction or combinations thereof. Among the disorders of impulse formation, triggered activity in the form of early or delayed afterdepolarizations (EAD and DAD, respectively) is the underlying mechanisms for most ventricular arrhythmias. It is believed that diastolic opening of RyR2 may lead to the release of Ca²⁺ from the SR, which in turn activates the Na⁺/Ca²⁺-exchanger (NCX). Activation of NCX removes Ca²⁺ from the cytosol but also concurrently results in an inward Na⁺ current, which – if large enough – can trigger a DAD, extrasystole or even ventricular arrhythmia (Fig. 17.3) [90]. Such
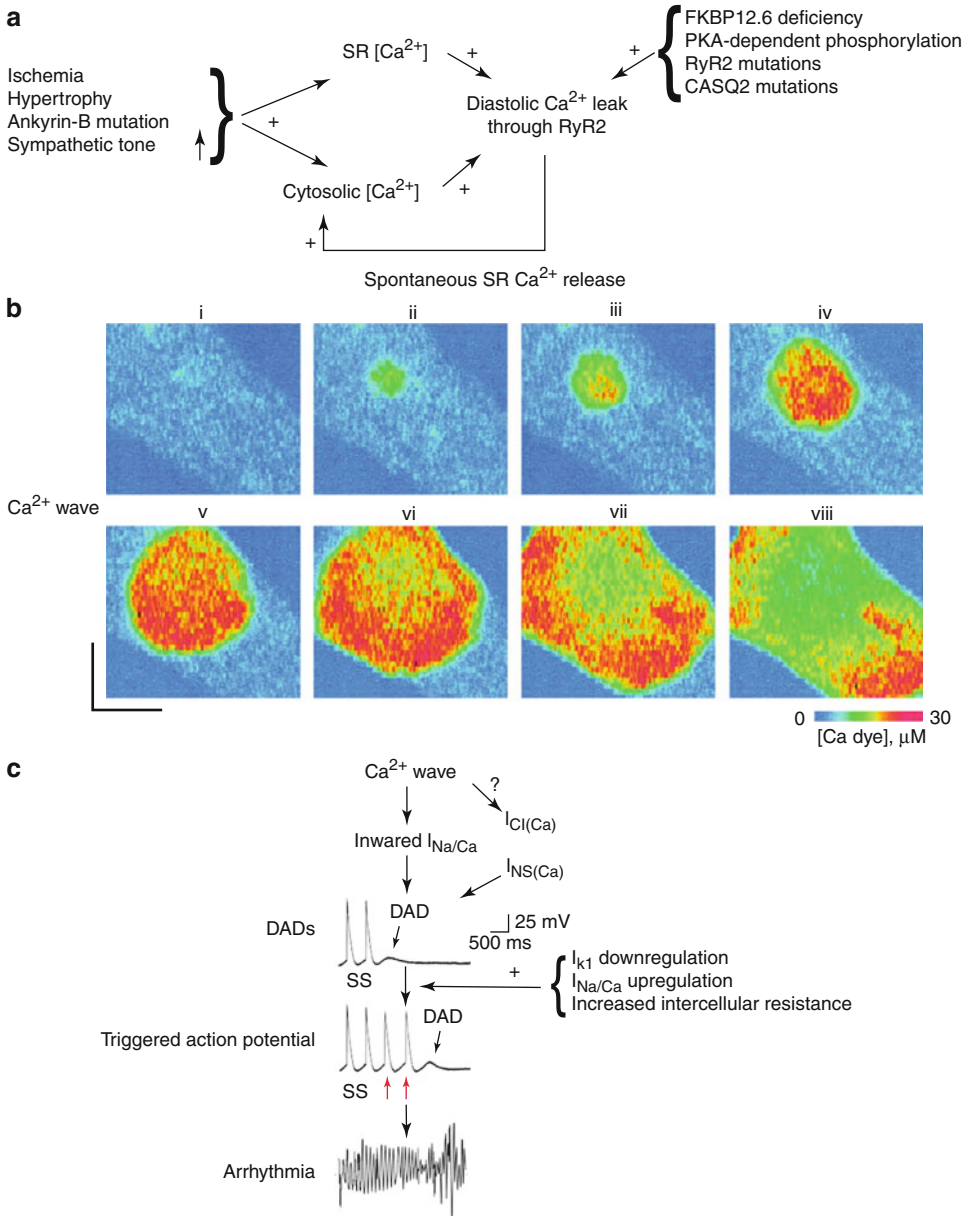
a

b

Ca²⁺ wave

c

FIGURE 17–3. Proposed scheme of events leading to delayed after-depolarizations (DAD) and triggered tachyarrhythmia. (**a**) Congenital (e.g., ankyrin-B mutation) and/or acquired factors (e.g., ischemia, hypertrophy, increased sympathetic tone) may cause a diastolic Ca²⁺ leak through ryanodine receptor channels type 2 (*RyR2*), resulting in localized and transient increases in $[Ca^{2+}]_i$ in cardiomyocytes. (**b**) Representative series of images showing changes in $[Ca^{2+}]_i$ during a Ca²⁺ wave in a single cardiomyocyte loaded with a Ca²⁺-sensitive fluorescent dye. Intracytosolic Ca²⁺ (*i*), focally elevated Ca²⁺ (*ii*) diffuses to adjacent junctional sarcoplasmic reticulum (*SR*), where it initiates more Ca²⁺ release events, resulting in a propagating Ca²⁺ wave (*iii–viii*). (**c**) The Ca²⁺ wave, through activation of Ca²⁺-sensitive inward currents, will depolarize the cardiomyocyte (*DAD*). In cardiomyocytes, the inward I(Na⁺/Ca²⁺) is the major candidate for the transient inward current underlying DADs, although the role of the Ca²⁺-activated Cl⁻ current [I(Cl⁻(Ca²⁺))] and a Ca²⁺-sensitive nonspecific cation current [I(NS(Ca²⁺))] cannot be excluded. If of sufficient magnitude, the DAD will depolarize the cardiomyocyte above threshold resulting in a single or repetitive premature heartbeat (*red arrows*), which can trigger an arrhythmia. Downregulation of the inward rectifier potassium current (I_{K1}), upregulation of I(Na⁺/Ca²⁺), or a slight increase in intercellular electrical resistance can promote the generation of DAD-triggered action potentials. S, stimulus (Modified from Rubart and Zipes [86], Mohler et al. [87], Qin et al. [88], Subramanian et al. [89]. With kind permission from American Society for Clinical Investigation)

DAD-induced arrhythmias are found in patients with genetic mutations in RyR2 in the absence of structural heart disease (e.g., catecholaminergic polymorphic ventricular tachycardia, CPVT). In addition, conditions that cause $[Ca^{2+}]_i$ overload such as HF and high digoxin levels, are also associated with DAD mediated ventricular arrhythmias [5, 6, 8, 91–94].

Catecholaminergic Polymorphic Ventricular Tachycardia

CPVT is an inherited disorder characterized by exercise or stress-induced ventricular arrhythmias in patients with structurally normal heart (see also the CPVT chapter) [95]. The most common form is the autosomal-dominant CPVT type 1 (CPVT1), which is caused by mutations in the *RYR2* gene. A less common form is CPVT type 2, which is autosomal-recessive and is caused by mutations in the *CSQ2* gene, which encode CSQ isoform 2 (CSQ2) [3].

Over the past 10 years, >140 CPVT-linked mutations have been identified in the *RYR2* gene (www.fsm.it/cardmoc). It is believed that *RYR2* mutations cluster into three definable regions that are highly conserved across species [3]. These domains are often designated as the N-terminal domain (amino acids 1–600), the central domain (amino acids 2000–2500), and the C-terminal pore-forming domain (mutations clustered between amino acids 3800–4000 and 4500–5000) [3]. The vast majority of *RYR2* mutations identified so far are missense mutations with less than 5 % being caused by small insertions/deletions [3].

Most studies have revealed that the functional consequence of RyR2 mutations is an increased open probability of the Ca^{2+} release channel [34]. The increased open probability of RyR2 is due to increased sensitivity of RyR2 to either luminal or cytosolic Ca^{2+} or both, such that minimal increases in either of the two Ca^{2+} stores lead to diastolic Ca^{2+} leak [38, 47]. Other studies have demonstrated that the gain-of-function channel phenotype can be unmasked following PKA phosphorylation [38]. These experimental findings correspond to the clinical observation that CPVT patients develop ventricular tachycardia in response to exercise and/or adrenergic

stimulation [95–97]. Together, these observations support the view that the arrhythmias in CPVT1 need two substrates, a leaky RyR2 channel and a precipitant in the form of beta-adrenergic stimulation and/or increased luminal/cytosolic overload. This is because an increased diastolic Ca^{2+} leak at baseline will lead to depletion of SR Ca^{2+} stores thus restoring the Ca^{2+} release/leak to near-normal levels [98]. However, under conditions of beta-adrenergic stimulation and Ca^{2+} overload, enhanced SR Ca^{2+} loading will promote diastolic Ca^{2+} releases via mutant RyR2 channels leading to DADs and ventricular tachycardia [14].

Several mechanisms have been proposed to explain the gain-of-function defects found in CPVT mutant RyR2 channels. RyR2 has N-terminal and central domains that interact with each other to act as a switch that opens and closes the channel [99]. Zipping of the interacting domains facilitates channel closing, whereas unzipping promotes RyR2 opening. The presence of a CPVT mutation in either domain might weaken the interdomain interaction leading to domain unzipping and erroneous activation of RyR2 and diastolic Ca^{2+} leak [99]. This was evidenced in a mouse model with central domain CPVT1 mutation R2474S that showed aberrant unzipping of the domain switch regions, lowering of the threshold of luminal $[Ca^{2+}]$ for channel activation, sensitizing the channel to PKA-dependent phosphorylation, and CPVT [100]. In addition to the domain switch, central domain seems to have subdomain zipping too. A knock-in mouse model of mutation S2246L in the central domain revealed that an abnormally tight local subdomain-subdomain interaction might lead to defective interaction between the N-terminal and central domains [101].

Other studies have demonstrated that RyR2 mutations may impair the interaction between RyR2 and FKBP12.6, a channel-stabilizing subunit [38]. Several mutations in the N-terminal and central domain have been shown to lower FKBP12.6-RyR2 binding affinity [38, 90]. However, some studies have challenged this mechanism as some of these mutations were shown not to alter FKBP12.6 binding to RyR2 [47, 102]. On the other hand, a recent study linked domain unzipping and FKBP12.6 unbinding

wherein PKA mediated hyperphosphorylation and dissociation of FKBP12.6 was associated with domain unzipping in failing hearts [103]. Although, the relative importance of the two mechanisms remains uncertain, but both domain unzipping and FKBP12.6 dissociation appear to promote pathogenic RyR2 gain-of-function associated with ventricular arrhythmias.

Although the pathogenic mechanisms underlying CSQ2 dysfunction in patients with CPVT-2 are beyond the scope of this chapter, it is thought that mutations in CSQ2 may lead to destabilization of the CICR mechanism by reducing effective Ca^{2+} buffering inside the SR [46], or causing altered interactions with the RyR2 channel complex leading to impaired RyR2 regulation by luminal Ca^{2+} [104]. Moreover, it has been suggested that RyR2 mutations might cause arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) [105–107]. However, this association is generally doubted by most scientists in the field, and therefore, we will not discuss this condition in this chapter.

Drug-Induced Arrhythmias

It is well recognized that several drugs, in particular, some inotropic agents, can increase $[Ca^{2+}]_i$ either by enhancing RyR2 activity or by inhibiting Na^+/K^+-ATPase [91, 92]. Among these, digoxin is the prototypical drug associated with $[Ca^{2+}]_i$ overload-induced arrhythmias. Digitalis glycosides, including digoxin were the mainstay of HF therapy until the mid-1990s when the 'Digitalis Investigation Group' (DIG) trial showed a lack of reduction in mortality, and post-hoc analyses showing a possible increase in mortality in a subgroup of patients [108]. This increase in mortality is due to the narrow therapeutic range of digoxin. Because digoxin inhibits Na^+/K^+-ATPase, an excessive increase in $[Na^+]_i$ can promote Ca^{2+} entry via the Na^+/Ca^{2+} exchanger, which can eventually lead to SR Ca^{2+} overload [91]. Whereas increased SR Ca^{2+} content leads to enhanced systolic Ca^{2+} release and positive inotropy, diastolic SR Ca^{2+} leak might be detrimental due to the increase risk of ventricular arrhythmias as discussed above [91].

Other inotropes that include β (beta)1 agonist (epinephrine) activate the β (beta)-adrenoceptor/ adenylylcyclase/PKA cascade [92]. This results in production of cAMP and activation of intracellular PKA [92]. PKA in turn phosphorylates many proteins, including RyR2, which may promote diastolic Ca^{2+} leak, an increase in $[Ca^{2+}]_i$, and possibly activation of CaMKII, another important kinase that phosphorylates and activates RyR2 [5, 38, 43, 48, 54, 55]. Thus, most inotropic agents available till date have the potential to promote RyR2-mediated SR Ca^{2+} leak either directly by RyR2 phosphorylation or by increasing $[Ca^{2+}]_i$, setting the stage for increased arrhythmogenesis [91, 92].

Ventricular Arrhythmias in Failing Hearts

HF is a progressive disorder in which the heart is unable to pump blood commensurate to the needs of the body. HF is a leading cause of death with approximately half of the patients eventually dying due to lethal ventricular arrhythmias [109, 110]. HF is characterized by reduced cardiac contractility, which at the cellular level is related to reduced intracellular Ca^{2+} transients [8, 24, 111]. This reduction in Ca^{2+} transients has been attributed to both an increased SR Ca^{2+} leak (via RyR2) and reduction in SR Ca^{2+} loading (due to downregulation of SERCA2a activity) [5, 8, 111]. In early stages of HF, RyR2 phosphorylation due to β(Beta)-adrenergic receptor activation is believed to help maintain the systolic Ca^{2+} transient [5]. At more advanced stages of HF, however, chronic hyperphosphorylation of RyR2 will promote maladaptive remodeling characterized by sustained RyR2 leakiness associated with reduced SR Ca^{2+} stores and decreased systolic Ca^{2+} release [5, 6, 8, 93, 94].

Moreover, enhanced diastolic SR Ca^{2+} leak via RyR2 may induce arrhythmias via EADs or DADs [9, 112, 113]. As SR Ca^{2+} release depends on SR Ca^{2+} store loading, increased SR Ca^{2+} leak and reduced SR Ca^{2+} loading in HF loading has been predicted to reset the SR Ca^{2+} stores to a lower level that might intuitively reduce the diastolic Ca^{2+} leak [114, 115]. However, studies have revealed that in spite of reduced SR Ca^{2+} content, SR Ca^{2+} leak continues unabated in HF ('Ca^{2+} overload paradox') [8]. This increased diastolic Ca^{2+} leak in the presence of reduced SR Ca^{2+}

content has been attributed to increased open probability of RyR2 due to either post-translational modification of the protein or changes in the composition of RyR2 macromolecular complex [8]. One of the most important modifiers of RyR2 function in HF is its increased phosphorylation by PKA and CaMKII, and reduced PP1 and PP2a activity [5, 6]. Activation of PKA and CaMKII can occur by many pathways, including chronic hyperadrenergic state found in patients with HF (PKA activation) [116], increased cytosolic Ca^{2+} (CaMKII activation) [6], and increased oxidative stress [8, 117, 118]. Increased oxidative stress can lead to oxidation of thiol residues on RyR2 or increase RyR2 phosphorylation by oxidative activation of CaMKII [64, 85]. Our recent studies have shown that inhibition of CaMKII phosphorylation of RyR2 reduces diastolic Ca^{2+} leak and ventricular arrhythmias in animal model of HF [48]. Thus, abnormal RyR2 function may contribute to ventricular arrhythmias in HF and could serve as a potential target for preventing sudden cardiac death in such patients [4–9, 48].

Duchenne Muscular Dystrophy

Duchenne muscular dystrophy (DMD) is the most common muscular dystrophy with an incidence of 1 in 3,500 male births [119]. Overall, a quarter of patients die from cardiac causes, half of which are due to sudden cardiac death [119]. The *dystrophin* gene codes for dystrophin protein and dystrophin-associated glycoproteins provide a structural link between the myocytes cytoskeleton and extracellular matrix [120]. In DMD, due to the absence of dystrophin, there is fragility of cell membrane, which results in abnormal stress-induced entry of Ca^{2+} into the cells leading to diastolic SR Ca^{2+} leak via RyR2 [121]. This was further confirmed in animal studies wherein diastolic Ca^{2+} release events and VT could be suppressed by pharmacological inhibition of RyR2 [16, 121]. DMD is one of many cardiac conditions that do not have a primary RyR2 disorder but have a higher RyR2-mediated SR Ca^{2+} leak. This highlights the central role of RyR2 in arrhythmogenesis not only in DMD but in other types of cardiomyopathies.

Ryanodine Receptors as a Therapeutic Target

Based on the discussion above, RyR2 plays an important role in the pathogenesis of atrial and ventricular arrhythmias arising from both genetically and acquired cardiac conditions [48, 122–126]. At present, there is no FDA approved medication that selectively target RyR2. Class II anti-arrhythmic agents (β (beta)-blockers) affect RyR2 function indirectly by reducing the sympathetic drive that drives RyR2 phosphorylation [116]. This class of drugs is also modestly effective in patients with CPVT, who typically still require ICD implantation once they have experienced syncope [95, 96]. There are efforts ongoing to develop drugs that block RyR2 specifically for the treatment of cardiac arrhythmias, some of which will be discussed below.

JTV519 and Related Compounds

JTV519 (also referred to as K201) is a 1,4-benzothiazepine derivative, that in addition to being a non-specific multiple channel blocker, also reduces diastolic SR Ca^{2+} leak by stabilizing RyR2 (Fig. 17.4) [128, 129]. Although, JTV519 has been shown to reduce diastolic SR Ca^{2+} leak, the exact mechanisms remain contentious [12, 130]. As per the current school of thought, JTV519 stabilizes the closed state of RyR2 by increasing its affinity for FKBP12.6 [12]. This is supported by studies showing that HF and CPVT are characterized by diastolic Ca^{2+} leak via RyR2 and depletion of FKBP12.6 and vice versa FKBP12.6 deficiency leads to Ca^{2+} leak and ventricular arrhythmias indicating the importance of FKBP12.6 in arrhythmogenesis [5, 10, 38]. JTV519 has been shown to inhibit both exercise and epinephrine-induced arrhythmias only in mice that have lower FKBP12.6 levels but not in mice that are completely deficient in FKBP12.6 providing indirect evidence that the effect of JTV519 on RyR2 requires rebinding of FKBP12.6 [12]. This is supported by another study that showed that JTV519 prevents pacing induced HF and restores the stoichiometry of FKBP12.6-RyR2 to normal levels [10]. On the other hand, few studies have shown that FKBP12.6 is not
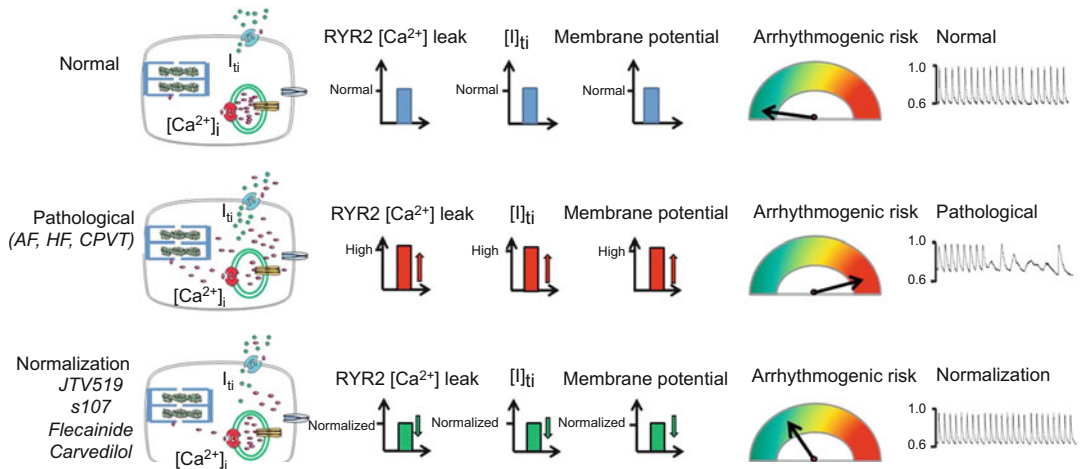
Figure 17–4. Blockers and stabilizers of ryanodine receptors type 2 (*RyR2*) prevent arrhythmia. Under normal conditions, RyR2 rarely opens in diastole. Spontaneous opening leads to leaky RyR2 in various pathophysiological situations, generating aberrant Ca^{2+} sparks and Ca^{2+} waves that activate inward depolarizing I_{ti} currents via Na^+/Ca^{2+} Exchanger (NCX), that in turn generate delayed after-depolarizations (DADs) and arrhythmia. Compounds that block or stabilize RyR2 prevent Ca^{2+} leakage and arrhythmogenic risk (Modified from Thiereau et al. [127] with kind permission from Elsevier)

required for an effect of JTV519 on RyR2 [130, 131] and that the effect of JTV519 has been attributed to reversal of domain unzipping to produce a zipped state which reduces SR-mediated Ca^{2+} leak in failing hearts [103]. Still, even in CPVT animal models that do not have apparent dissociation of FKBP12.6 from RyR2, JTV519 reduces ventricular arrhythmias once challenged with increased Ca^{2+} load [14, 15]. Thus, JTV519 remains an important investigational drug that has a potential to prevent and/or treat arrhythmias with the added advantage of improvement in cardiac function if used in patients with HF induced arrhythmias [10, 13]. However, its off-target effects on other ion channels, including I_{Kr} can lead to prolongation of QTc interval that requires further studies to exclude its proarrhythmic potential [132].

S107 is a derivative of JTV519 that was developed because of its specificity for RyR2 and its favorable drug properties, including drug stability and oral bioavailability [133]. Like JTV519, S107 has been shown to increase the binding of FKBP12.6 to RyR2 and reduce the diastolic SR Ca^{2+} leak and ventricular arrhythmias in an animal model of CPVT (RyR2-R2474S knock-in mice) [90]. Duchenne muscular dystrophy is also characterized by enhanced diastolic SR Ca^{2+} leak and ventricular arrhythmias that can be reversed by S107 in animal model [16]. Although S107 seems to be a better drug than JTV519 [133], its applicability might be limited to arrhythmias that are caused by diastolic SR Ca^{2+} leak.

Flecainide

Flecainide is a Class Ic anti-arrhythmic agent that is approved by FDA for the treatment of ventricular and supraventricular arrhythmias [134–136]. Its predominant mechanism of action is inhibition of fast Na^+ channels resulting in slowing of atrial and ventricular conduction [134]. However, recent work has shown that flecainide also acts as an open state RyR2 blocker [17, 137]. Studies have shown that flecainide reduces diastolic SR Ca^{2+} leak and ventricular arrhythmias in an animal model of CPVT and also in three patients with this disease [17, 18]. Despite the promising results, there are several limitations in the use of this drug. Flecainide causes significant electrical heterogeneity that can both suppress and provoke arrhythmias [138]. The drug also depresses cardiac function and is contraindicated in patients with moderate to severe cardiac failure [139]. Lastly, it suppresses sinus node function and cardiac

conduction and is contraindicated in patients with sinus node dysfunction and conduction blocks [134]. The Cardiac Arrhythmia Suppression Trial (CAST) showed that flecainide increases all-cause and arrhythmic deaths in patients with recent myocardial infarction [140]. Despite all the limitations, flecainide provides an exciting opportunity to treat CPVT patients with ventricular arrhythmias due to abnormal SR diastolic Ca²⁺ leak in the absence of structural heart disease and is currently being evaluated in clinical trials with promising results [141]. In addition, further modification of this drug to increase its specificity of action on RyR2 may eliminate its proarrhythmic properties making it a powerful tool in the treatment of RyR2 leak mediated arrhythmias.

Carvedilol Derivatives

Carvedilol is a non-selective β (beta)-blocker that has additional antioxidant and α (alpha)-adrenergic blocking properties [142]. It is currently indicated for patients with HF and has shown significant survival benefit in such patients owing partly to reduction in sudden cardiac death [143, 144]. Although β (beta)-blockers in general reduce sudden cardiac death in HF patients, carvedilol use leads to a higher reduction in sudden cardiac death, in comparison with β (beta)1-specific blockers like metoprolol [144]. This additional protection by carvedilol has previously been attributed to stabilization of RyR2 channels via its antioxidant properties or stabilization of FKBP12.6 [11, 145]. Recent work has shown that carvedilol can block RyR2 in its open state [19]. Further, the RyR2 blocking properties of carvedilol have been shown to be separate from its β (beta)-blocking properties evidenced in a study wherein a carvedilol analog could block RyR2 and prevent ventricular arrhythmias in a susceptible animal model without significant β (beta)-blockade [19]. This discovery provides exciting new opportunities to stabilize RyR2 without the blood pressure and heart rate lowering effects of carvedilol, which limit its titration in many patients. Future studies will be required to evaluate whether such derivatives have any significant side effects including reduction of

cardiac contractility. This is less of a concern with carvedilol and flecainide as both are open state blockers (compared to closed state blockers like tetracaine) [17, 137]. Together, the discovery of the antiarrhythmic effects of JTV519, flecainide and carvedilol derivatives herald a new approach in treating arrhythmias by normalizing Ca²⁺ handling in cardiomyocytes via stabilization of RyR2 (see Fig. 17.4).

Summary and Conclusions

RyR2 Ca²⁺ channels on the SR are required for EC coupling in cardiac muscle. RyRs are macromolecular channel complexes associated with regulatory proteins that modulate RyR2 function in response to extracellular signals. Cardiac arrhythmia is an important cause of death in patients with HF and inherited arrhythmia syndromes, such as CPVT. Alterations in RyR2 function in CPVT cause diastolic Ca²⁺ leak from the SR, which may lead to DADs and triggered cardiac arrhythmias. Novel therapeutic approaches, based on recent advances in the understanding of the cellular mechanisms underlying arrhythmias in HF and CPVT, are currently being evaluated that correct defective RyR2 Ca²⁺ release specifically in these lethal syndromes.

References

1. Jayaraman T, Brillantes AM, Timerman AP, et al. FK506 binding protein associated with the calcium release channel (ryanodine receptor). J Biol Chem. 1992;267:9474–7.
2. Marx SO, Reiken S, Hisamatsu Y, et al. Phosphorylation-dependent regulation of ryanodine receptors. A novel role for leucine/isoleucine zippers. J Cell Biol. 2001;153:699–708.

3. Priori SG, Chen SR. Inherited dysfunction of sarcoplasmic reticulum Ca2+ handling and arrhythmogenesis. Circ Res. 2011;108:871–83.

4. Kubalova Z, Terentyev D, Viatchenko-Karpinski S, et al. Abnormal intrastore calcium signaling in chronic heart failure. Proc Natl Acad Sci USA. 2005;102:14104–9.

5. Marx SO, Reiken S, Hisamatsu Y, et al. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. Cell. 2000; 101:365–76.

6. Ai X, Curran JW, Shannon TR, Bers DM, Pogwizd SM. Ca2+/calmodulin-dependent protein kinase modulates cardiac ryanodine receptor phosphorylation and sarcoplasmic reticulum Ca2+ leak in heart failure. Circ Res. 2005;97:1314–22.

7. Shannon TR, Pogwizd SM, Bers DM. Elevated sarcoplasmic reticulum Ca2+ leak in intact ventricular myocytes from rabbits in heart failure. Circ Res. 2003;93:592–4.

8. Belevych AE, Terentyev D, Terentyeva R, et al. The relationship between arrhythmogenesis and impaired contractility in heart failure: role of altered ryanodine receptor function. Cardiovasc Res. 2011;90:493–502.

9. Pogwizd SM, Bers DM. Cellular basis of triggered arrhythmias in heart failure. Trends Cardiovasc Med. 2004;14:61–6.

10. Yano M, Kobayashi S, Kohno M, et al. FKBP12.6-mediated stabilization of calcium-release channel (ryanodine receptor) as a novel therapeutic strategy against heart failure. Circulation. 2003;107: 477–84.

11. Kohno M, Yano M, Kobayashi S, et al. A new cardioprotective agent, JTV519, improves defective channel gating of ryanodine receptor in heart failure. Am J Physiol Heart Circ Physiol. 2003;284:H1035–42.

12. Wehrens XH, Lehnart SE, Reiken SR, et al. Protection from cardiac arrhythmia through ryanodine receptor-stabilizing protein calstabin2. Science. 2004;304:292–6.

13. Wehrens XH, Lehnart SE, Reiken S, et al. Enhancing calstabin binding to ryanodine receptors improves cardiac and skeletal muscle function in heart failure. Proc Natl Acad Sci USA. 2005;102:9607–12.

14. Liu N, Colombi B, Memmi M, et al. Arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia: insights from a RyR2 R4496C knock-in mouse model. Circ Res. 2006;99:292–8.

15. Sedej S, Heinzel FR, Walther S, et al. Na+−dependent SR Ca2+ overload induces arrhythmogenic events in mouse cardiomyocytes with a human CPVT mutation. Cardiovasc Res. 2010;87:50–9.

16. Fauconnier J, Thireau J, Reiken S, et al. Leaky RyR2 trigger ventricular arrhythmias in Duchenne muscular dystrophy. Proc Natl Acad Sci USA. 2010;107:1559–64.

17. Watanabe H, Chopra N, Laver D, et al. Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. Nat Med. 2009;15:380–3.

18. Biernacka EK, Hoffman P. Efficacy of flecainide in a patient with catecholaminergic polymorphic ventricular tachycardia. Europace. 2011;13:129–30.

19. Zhou Q, Xiao J, Jiang D, et al. Carvedilol and its new analogs suppress arrhythmogenic store overload-induced Ca2+ release. Nat Med. 2011;17:1003–9.

20. Nabauer M, Callewart G, Cleeman L, Morad M. Regulation of calcium release is gated by calcium current, not gating charge, in cardiac, myocytes. Science. 1989;244:800–3.

21. Wehrens XH, Ather S, Dobrev D. Abnormal sarcoplasmic function in atrial fibrillation. Therapy. 2010;7:147–58.

22. Bers DM, Guo T. Calcium signaling in cardiac ventricular myocytes. Ann N Y Acad Sci. 2005;1047:86–98.

23. Fabiato A. Time and calcium dependence of activation and inactivation of calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac purkinje cell. J Gen Physiol. 1985;85:247–89.

24. Wehrens XHT, Lehnart SE, Marks AR. Intracellular calcium release channels and cardiac disease. Annu Rev Physiol. 2005;67:69–98.

25. Cheng H, Lederer WJ, Cannell MB. Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. Science. 1993;262:740–4.

26. Lindegger N, Niggli E. Paradoxical SR Ca2+ release in guinea-pig cardiac myocytes after b-adrenergic stimulation revealed by two-photon photolysis of caged Ca2+. J Physiol. 2005;565: 801–13.

27. Cannell MB, Cheng H, Lederer WJ. The control of calcium release in heart muscle. Science. 1995;268:1045–9.

28. Marx SO, Gaburjakova J, Gaburjakova M, Henrikson C, Ondrias K, Marks AR. Coupled gating between cardiac calcium release channels (ryanodine receptors). Circ Res. 2001;88:1151–8.

29. Brochet DX, Yang D, Di Maio A, Lederer WJ, Franzini-Armstrong C, Cheng H. Ca2+ blinks:

rapid nanoscopic store calcium signaling. Proc Natl Acad Sci USA. 2005;102:3099–104.

30. Otsu K, Willard HF, Khanna VK, Zorzato F, Green NM, MacLennan DH. Molecular cloning of cDNA encoding the Ca2+ release channel (ryanodine receptor) of rabbit cardiac muscle sarcoplasmic reticulum. J Biol Chem. 1990;265:13472–83.

31. Tunwell RE, Wickenden C, Bertrand BM, et al. The human cardiac muscle ryanodine receptor-calcium release channel: identification, primary structure and topological analysis. Biochem J. 1996;318:477–87.

32. Yano M, Yamamoto T, Ikeda Y, Matsuzaki M. Mechanisms of disease: ryanodine receptor defects in heart failure and fatal arrhythmia. Nat Clin Pract Cardiovasc Med. 2006;3:43–52.

33. Kushnir A, Betzenhauser MJ, Marks AR. Ryanodine receptor studies using genetically engineered mice. FEBS Lett. 2010;584:1956–65.

34. Wehrens XH. CaMKII regulation of the cardiac ryanodine receptor and sarcoplasmic reticulum calcium release. Heart Rhythm. 2011;8:323–5.

35. Marks AR, Tempst P, Hwang KS, et al. Molecular cloning and characterization of the ryanodine receptor/junctional channel complex cDNA from skeletal muscle sarcoplasmic reticulum. Proc Natl Acad Sci USA. 1989;86:8683–7.

36. Lam E, Martin MM, Timerman AP, et al. A novel FK506 binding protein can mediate the immunosuppressive effects of FK506 and is associated with the cardiac ryanodine receptor. J Biol Chem. 1995;270:26511–22.

37. Brillantes AB, Ondrias K, Scott A, et al. Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. Cell. 1994;77:513–23.

38. Wehrens XH, Lehnart SE, Huang F, et al. FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. Cell. 2003;113:829–40.

39. Lehnart SE, Huang F, Marx SO, Marks AR. Immunophilins and coupled gating of ryanodine receptors. Curr Top Med Chem. 2003;3:1383–91.

40. Chu A, Sumbilla C, Inesi G, Jay SD, Campbell KP. Specific association of calmodulin-dependent protein kinase and related substrates with the junctional sarcoplasmic reticulum of skeletal muscle. Biochemistry. 1990;29:5899–905.

41. Meyers MB, Pickel VM, Sheu SS, Sharma VK, Scotto KW, Fishman GI. Association of sorcin with the cardiac ryanodine receptor. J Biol Chem. 1995;270:26411–8.

42. Currie S, Loughrey CM, Craig MA, Smith GL. Calcium/calmodulin-dependent protein kinase IIdelta associates with the ryanodine receptor complex and regulates channel function in rabbit heart. Biochem J. 2004;377:357–66.

43. Wehrens XH, Lehnart SE, Reiken SR, Marks AR. Ca2+/calmodulin-dependent protein kinase II phosphorylation regulates the cardiac ryanodine receptor. Circ Res. 2004;94:e61–70.

44. Zhang L, Kelley J, Schmeisser G, Kobayashi YM, Jones LR. Complex formation between junctin, triadin, calsequestrin, and the ryanodine receptor. Proteins of the cardiac junctional sarcoplasmic reticulum membrane. J Biol Chem. 1997;272:23389–97.

45. Gyorke I, Hester N, Jones LR, Gyorke S. The role of calsequestrin, triadin, and junctin in conferring cardiac ryanodine receptor responsiveness to luminal calcium. Biophys J. 2004;86:2121–8.

46. Terentyev D, Viatchenko-Karpinski S, Gyorke I, Volpe P, Williams SC, Gyorke S. Calsequestrin determines the functional size and stability of cardiac intracellular calcium stores: mechanism for hereditary arrhythmia. Proc Natl Acad Sci USA. 2003;100:11759–64.

47. Jiang D, Wang R, Xiao B, et al. Enhanced store overload-induced Ca2+ release and channel sensitivity to luminal Ca2+ activation are common defects of RyR2 mutations linked to ventricular tachycardia and sudden death. Circ Res. 2005;97:1173–81.

48. van Oort RJ, McCauley MD, Dixit SS, et al. Ryanodine receptor phosphorylation by calcium/calmodulin-dependent protein kinase II promotes life-threatening ventricular arrhythmias in mice with heart failure. Circulation. 2010;122:2669–79.

49. Stull LB, Leppo MK, Marban E, Janssen PM. Physiological determinants of contractile force generation and calcium handling in mouse myocardium. J Mol Cell Cardiol. 2002;34:1367–76.

50. Braz JC, Gregory K, Pathak A, et al. PKC-alpha regulates cardiac contractility and propensity toward heart failure. Nat Med. 2004;10:248–54.

51. Lefkowitz RJ, Rockman HA, Koch WJ. Catecholamines, cardiac beta-adrenergic receptors, and heart failure. Circulation. 2000;101:1634–7.

52. Jones LR, Simmerman HK, Wilson WW, Gurd FR, Wegener AD. Purification and characterization of phospholamban from canine cardiac sarcoplasmic reticulum. J Biol Chem. 1985;260:7721–30.

53. Gomez AM, Valdivia HH, Cheng H, et al. Defective excitation-contraction coupling in experimental

cardiac hypertrophy and heart failure. Science. 1997;276:800–6.

54. Hain J, Onoue H, Mayrleitner M, Fleischer S, Schindler H. Phosphorylation modulates the function of the calcium release channel of sarcoplasmic reticulum from cardiac muscle. J Biol Chem. 1995;270:2074–81.

55. Valdivia HH, Kaplan JH, Ellis-Davies GC, Lederer WJ. Rapid adaptation of cardiac ryanodine receptors: modulation by Mg2+ and phosphorylation. Science. 1995;267:1997–2000.

56. Xiao J, Tian X, Jones PP, et al. Removal of FKBP12.6 does not alter the conductance and activation of the cardiac ryanodine receptor or the susceptibility to stress-induced ventricular arrhythmias. J Biol Chem. 2007;282:34828–38.

57. Danila CI, Hamilton SL. Phosphorylation of ryanodine receptors. Biol Res. 2004;37:521–5.

58. Xiao B, Sutherland C, Walsh MP, Chen SR. Protein kinase A phosphorylation at serine-2808 of the cardiac Ca2+−release channel (ryanodine receptor) does not dissociate 12.6-kDa FK506-binding protein (FKBP12.6). Circ Res. 2004;94:487–95.

59. Xiao B, Jiang MT, Zhao M, et al. Characterization of a novel PKA phosphorylation site, serine-2030, reveals no PKA hyperphosphorylation of the cardiac ryanodine receptor in canine heart failure. Circ Res. 2005;96:847–55.

60. Wehrens XH, Lehnart SE, Reiken S, Vest JA, Wronska A, Marks AR. Ryanodine receptor/calcium release channel PKA phosphorylation: a critical mediator of heart failure progression. Proc Natl Acad Sci USA. 2006;103:511–8.

61. Lokuta AJ, Rogers TB, Lederer WJ, Valdivia HH. Modulation of cardiac ryanodine receptors of swine and rabbit by a phosphorylation-dephosphorylation mechanism. J Physiol. 1995; 487:609–22.

62. Lehnart SE, Wehrens XH, Reiken S, et al. Phosphodiesterase 4D deficiency in the ryanodine-receptor complex promotes heart failure and arrhythmias. Cell. 2005;123:25–35.

63. Kushnir A, Shan J, Betzenhauser MJ, Reiken S, Marks AR. Role of CaMKIIdelta phosphorylation of the cardiac ryanodine receptor in the force frequency relationship and heart failure. Proc Natl Acad Sci USA. 2010;107:10274–9.

64. Yano M, Okuda S, Oda T, et al. Correction of defective interdomain interaction within ryanodine receptor by antioxidant is a new therapeutic strategy against heart failure. Circulation. 2005;112: 3633–43.

65. Aghdasi B, Reid MB, Hamilton SL. Nitric oxide protects the skeletal muscle Ca2+ release channel from oxidation induced activation. J Biol Chem. 1997;272:25462–7.

66. Santos CX, Anilkumar N, Zhang M, Brewer AC, Shah AM. Redox signaling in cardiac myocytes. Free Radic Biol Med. 2011;50:777–93.

67. Xu L, Eu JP, Meissner G, Stamler JS. Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. Science. 1998;279:234–7.

68. Gonzalez DR, Treuer A, Sun QA, Stamler JS, Hare JM. S-Nitrosylation of cardiac ion channels. J Cardiovasc Pharmacol. 2009;54:188–95.

69. Hidalgo C, Donoso P. Crosstalk between calcium and redox signaling: from molecular mechanisms to health implications. Antioxid Redox Signal. 2008;10:1275–312.

70. Zima AV, Blatter LA. Redox regulation of cardiac calcium channels and transporters. Cardiovasc Res. 2006;71:310–21.

71. Abramson JJ, Salama G. Sulfhydryl oxidation and Ca2+ release from sarcoplasmic reticulum. Mol Cell Biochem. 1988;82:81–4.

72. Eager KR, Roden LD, Dulhunty AF. Actions of sulfhydryl reagents on single ryanodine receptor Ca(2+)-release channels from sheep myocardium. Am J Physiol. 1997;272:C1908–18.

73. Marengo JJ, Hidalgo C, Bull R. Sulfhydryl oxidation modifies the calcium dependence of ryanodine-sensitive calcium channels of excitable cells. Biophys J. 1998;74:1263–77.

74. Feron O, Saldana F, Michel JB, Michel T. The endothelial nitric-oxide synthase-caveolin regulatory cycle. J Biol Chem. 1998;273:3125–8.

75. Garcia-Cardena G, Martasek P, Masters BS, et al. Dissecting the interaction between nitric oxide synthase (NOS) and caveolin. Functional significance of the nos caveolin binding domain in vivo. J Biol Chem. 1997;272:25437–40.

76. Hare JM, Lofthouse RA, Juang GJ, et al. Contribution of caveolin protein abundance to augmented nitric oxide signaling in conscious dogs with pacing-induced heart failure. Circ Res. 2000;86:1085–92.

77. Schwencke C, Yamamoto M, Okumura S, Toya Y, Kim SJ, Ishikawa Y. Compartmentation of cyclic adenosine 3',5'-monophosphate signaling in caveolae. Mol Endocrinol. 1999;13:1061–70.

78. Hare JM, Givertz MM, Creager MA, Colucci WS. Increased sensitivity to nitric oxide synthase inhibition in patients with heart failure: potentiation of beta-adrenergic inotropic responsiveness. Circulation. 1998;97:161–6.

79. Xu KY, Huso DL, Dawson TM, Bredt DS, Becker LC. Nitric oxide synthase in cardiac sarcoplasmic

reticulum. Proc Natl Acad Sci USA. 1999; 96:657–62.

80. Eu JP, Sun J, Xu L, Stamler JS, Meissner G. The skeletal muscle calcium release channel: coupled O2 sensor and NO signaling functions. Cell. 2000;102:499–509.

81. Barouch LA, Harrison RW, Skaf MW, et al. Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. Nature. 2002; 416:337–9.

82. Cherednichenko G, Zima AV, Feng W, Schaefer S, Blatter LA, Pessah IN. NADH oxidase activity of rat cardiac sarcoplasmic reticulum regulates calcium-induced calcium release. Circ Res. 2004; 94:478–86.

83. Sanchez G, Pedrozo Z, Domenech RJ, Hidalgo C, Donoso P. Tachycardia increases NADPH oxidase activity and RyR2 S-glutathionylation in ventricular muscle. J Mol Cell Cardiol. 2005;39:982–91.

84. Hidalgo C, Sanchez G, Barrientos G, Aracena-Parks P. A transverse tubule NADPH oxidase activity stimulates calcium release from isolated triads via ryanodine receptor type 1 S -glutathionylation. J Biol Chem. 2006;281:26473–82.

85. Khan SA, Lee K, Minhas KM, et al. Neuronal nitric oxide synthase negatively regulates xanthine oxidoreductase inhibition of cardiac excitation-contraction coupling. Proc Natl Acad Sci USA. 2004;101:15944–8.

86. Rubart M, Zipes DP. Mechanisms of sudden cardiac death. J Clin Invest. 2005;115:2305–15.

87. Mohler PJ, Schott JJ, Gramolini AO, et al. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. Nature. 2003;421:634–9.

88. Qin D, Zhang ZH, Caref EB, Boutjdir M, Jain P, el-Sherif N. Cellular and ionic basis of arrhythmias in postinfarction remodeled ventricular myocardium. Circ Res. 1996;79:461–73.

89. Subramanian S, Viatchenko-Karpinski S, Lukyanenko V, Gyorke S, Wiesner TF. Underlying mechanisms of symmetric calcium wave propagation in rat ventricular myocytes. Biophys J. 2001;80:1–11.

90. Lehnart SE, Mongillo M, Bellinger A, et al. Leaky Ca2+ release channel/ryanodine receptor 2 causes seizures and sudden cardiac death in mice. J Clin Invest. 2008;118:2230–45.

91. McGarry SJ, Williams AJ. Digoxin activates sarcoplasmic reticulum Ca(2+)-release channels: a possible role in cardiac inotropy. Br J Pharmacol. 1993;108:1043–50.

92. Wallukat G. The beta-adrenergic receptors. Herz. 2002;27:683–90.

93. Belevych A, Kubalova Z, Terentyev D, Hamlin RL, Carnes CA, Gyorke S. Enhanced ryanodine receptor-mediated calcium leak determines reduced sarcoplasmic reticulum calcium content in chronic canine heart failure. Biophys J. 2007;93:4083–92.

94. Sag CM, Wadsack DP, Khabbazzadeh S, et al. Calcium/calmodulin-dependent protein kinase II contributes to cardiac arrhythmogenesis in heart failure. Circ Heart Fail. 2009;2:664–75.

95. Priori SG, Napolitano C, Memmi M, et al. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. Circulation. 2002;106:69–74.

96. Sumitomo N, Harada K, Nagashima M, et al. Catecholaminergic polymorphic ventricular tachycardia: electrocardiographic characteristics and optimal therapeutic strategies to prevent sudden death. Heart. 2003;89:66–70.

97. Mohamed U, Napolitano C, Priori SG. Molecular and electrophysiological bases of catecholaminergic polymorphic ventricular tachycardia. J Cardiovasc Electrophysiol. 2007;18:791–7.

98. Trafford AW, Diaz ME, Sibbring GC, Eisner DA. Modulation of CICR has no maintained effect on systolic Ca2+: simultaneous measurements of sarcoplasmic reticulum and sarcolemmal Ca2+ fluxes in rat ventricular myocytes. J Physiol. 2000;522(Pt 2):259–70.

99. Tateishi H, Yano M, Mochizuki M, et al. Defective domain-domain interactions within the ryanodine receptor as a critical cause of diastolic Ca2+ leak in failing hearts. Cardiovasc Res. 2009;81: 536–45.

100. Uchinoumi H, Yano M, Suetomi T, et al. Catecholaminergic polymorphic ventricular tachycardia is caused by mutation-linked defective conformational regulation of the ryanodine receptor. Circ Res. 2010;106:1413–24.

101. Suetomi T, Yano M, Uchinoumi H, et al. Mutation-linked defective interdomain interactions within ryanodine receptor cause aberrant Ca(2)release leading to catecholaminergic polymorphic ventricular tachycardia. Circulation. 2011;124:682–94.

102. George CH, Higgs GV, Lai FA. Ryanodine receptor mutations associated with stress-induced ventricular tachycardia mediate increased calcium release in stimulated cardiomyocytes. Circ Res. 2003;93:531–40.

103. Oda T, Yano M, Yamamoto T, et al. Defective regulation of interdomain interactions within the ryanodine receptor plays a key role in the pathogenesis of heart failure. Circulation. 2005;111: 3400–10.

104. Terentyev D, Nori A, Santoro M, et al. Abnormal interactions of calsequestrin with the ryanodine receptor calcium release channel complex linked to exercise-induced sudden cardiac death. Circ Res. 2006;98:1151–8.

105. Tiso N, Stephan DA, Nava A, et al. Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). Hum Mol Genet. 2001;10:189–94.

106. Bauce B, Nava A, Rampazzo A, et al. Familial effort polymorphic ventricular arrhythmias in arrhythmogenic right ventricular cardiomyopathy map to chromosome 1q42-43. Am J Cardiol. 2000;85:573–9.

107. Koop A, Goldmann P, Chen SR, Thieleczek R, Varsanyi M. ARVC-related mutations in divergent region 3 alter functional properties of the cardiac ryanodine receptor. Biophys J. 2008;94: 4668–77.

108. Ather S, Peterson LE, Divakaran VG, et al. Digoxin treatment in heart failure – unveiling risk by cluster analysis of DIG data. Int J Cardiol. 2011;150:264–9.

109. Mozaffarian D, Anker SD, Anand I, et al. Prediction of mode of death in heart failure: the Seattle Heart Failure Model. Circulation. 2007;116:392–8.

110. Roger VL, Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics – 2011 update: a report from the American Heart Association. Circulation. 2011;123:e18–209.

111. Bers DM, Eisner DA, Valdivia HH. Sarcoplasmic reticulum Ca2+ and heart failure: roles of diastolic leak and Ca2+ transport. Circ Res. 2003;93:487–90.

112. Ter Keurs HE, Boyden PA. Calcium and arrhythmogenesis. Physiol Rev. 2007;87:457–506.

113. Xie LH, Weiss JN. Arrhythmogenic consequences of intracellular calcium waves. Am J Physiol Heart Circ Physiol. 2009;297:H997–1002.

114. Gyorke S, Gyorke I, Lukyanenko V, Terentyev D, Viatchenko-Karpinski S, Wiesner TF. Regulation of sarcoplasmic reticulum calcium release by luminal calcium in cardiac muscle. Front Biosci. 2002;7:d1454–63.

115. Trafford AW, Diaz ME, O'Neill SC, Eisner DA. Integrative analysis of calcium signalling in cardiac muscle. Front Biosci. 2002;7:d843–52.

116. Reiken S, Wehrens XH, Vest JA, et al. Betablockers restore calcium release channel function and improve cardiac muscle performance in human heart failure. Circulation. 2003;107: 2459–66.

117. Swaminathan PD, Purohit A, Soni S, et al. Oxidized CaMKII causes cardiac sinus node dysfunction in mice. J Clin Invest. 2011;121: 3277–88.

118. Terentyev D, Gyorke I, Belevych AE, et al. Redox modification of ryanodine receptors contributes to sarcoplasmic reticulum Ca2+ leak in chronic heart failure. Circ Res. 2008;103:1466–72.

119. Groh WJ, Zipes DL. Neurological disorders and cardiovascular disease. In: Bonow RO, Mann DL, Zipes DP, Libby P, editors. Braunwald's heart disease: a textbook of cardiovascular medicine. 9th ed. Philadelphia: Saunders; 2011. p. 1916–9.

120. Lapidos KA, Kakkar R, McNally EM. The dystrophin glycoprotein complex: signaling strength and integrity for the sarcolemma. Circ Res. 2004;94:1023–31.

121. Jung C, Martins AS, Niggli E, Shirokova N. Dystrophic cardiomyopathy: amplification of cellular damage by Ca2+ signalling and reactive oxygen species-generating pathways. Cardiovasc Res. 2008;77:766–73.

122. Chelu MG, Sarma S, Sood S, et al. Calmodulin kinase II-mediated sarcoplasmic reticulum Ca2+ leak promotes atrial fibrillation in mice. J Clin Invest. 2009;119:1940–51.

123. Neef S, Dybkova N, Sossalla S, et al. CaMKII-dependent diastolic SR Ca2+ leak and elevated diastolic Ca2+ levels in right atrial myocardium of patients with atrial fibrillation. Circ Res. 2010;106:1134–44.

124. Vest JA, Wehrens XH, Reiken SR, et al. Defective cardiac ryanodine receptor regulation during atrial fibrillation. Circulation. 2005;111:2025–32.

125. Mathur N, Sood S, Wang S, et al. Sudden infant death syndrome in mice with an inherited mutation in RyR2. Circ Arrhythm Electrophysiol. 2009;2:677–85.

126. Priori SG, Napolitano C, Tiso N, et al. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. Circulation. 2001;103: 196–200.

127. Thireau J, Pasquie JL, Martel E, Le Guennec JY, Richard S. New drugs vs. old concepts: a fresh look at antiarrhythmics. Pharmacol Ther. 2011;132:125–45.

128. Kaneko N, Matsuda R, Hata Y, Shimamoto K. Pharmacological characteristics and clinical applications of K201. Curr Clin Pharmacol. 2009;4:126–31.

129. Kaneko N, Matsuda R, Toda M, Shimamoto K. Inhibition of annexin V-dependent Ca2+ movement in large unilamellar vesicles by K201, a new

1,4-benzothiazepine derivative. Biochim Biophys Acta. 1997;1330:1–7.

130. Hunt DJ, Jones PP, Wang R, et al. K201 (JTV519) suppresses spontaneous Ca2+ release and [3H] ryanodine binding to RyR2 irrespective of FKBP12.6 association. Biochem J. 2007;404: 431–8.

131. Yamamoto T, Yano M, Xu X, et al. Identification of target domains of the cardiac ryanodine receptor to correct channel disorder in failing hearts. Circulation. 2008;117:762–72.

132. Hasumi H, Matsuda R, Shimamoto K, Hata Y, Kaneko N. K201, a multi-channel blocker, inhibits clofilium-induced torsades de pointes and attenuates an increase in repolarization. Eur J Pharmacol. 2007;555:54–60.

133. Bellinger AM, Reiken S, Dura M, et al. Remodeling of ryanodine receptor complex causes "leaky" channels: a molecular mechanism for decreased exercise capacity. Proc Natl Acad Sci USA. 2008;105:2198–202.

134. Miller JM, Zipes DP. Therapy for cardiac arrhythmias. In: Bonow RO, Mann DL, Zipes DP, Libby P, editors. Braunwald's heart disease: a textbook of cardiovascular medicine, vol. 11. Philadelphia: Elsevier Saunders; 2011. p. 710–44.

135. Anderson JL, Stewart JR, Perry BA, et al. Oral flecainide acetate for the treatment of ventricular arrhythmias. N Engl J Med. 1981;305:473–7.

136. Anderson JL, Gilbert EM, Alpert BL, et al. Prevention of symptomatic recurrences of paroxysmal atrial fibrillation in patients initially tolerating antiarrhythmic therapy. A multicenter, double-blind, crossover study of flecainide and placebo with transtelephonic monitoring. Flecainide Supraventricular Tachycardia Study Group. Circulation. 1989;80:1557–70.

137. Hilliard FA, Steele DS, Laver D, et al. Flecainide inhibits arrhythmogenic Ca2+ waves by open state block of ryanodine receptor Ca2+ release channels and reduction of Ca2+ spark mass. J Mol Cell Cardiol. 2010;48:293–301.

138. Muhiddin K, Nathan AW, Hellestrand KJ, Banim SO, Camm AJ. Ventricular tachycardia associated with flecainide. Lancet. 1982;2:1220–1.

139. Brembilla-Perrot B, Amor M, Auque F, et al. Effect of flecainide on left ventricular ejection fraction. Eur Heart J. 1987;8:754–61.

140. Preliminary report: effect of encainide and flecainide on mortality in a randomized trial of arrhythmia suppression after myocardial infarction. The Cardiac Arrhythmia Suppression Trial (CAST) Investigators. N Engl J Med. 1989;321: 406–12.

141. van der Werf C, Kannankeril PJ, Sacher F, et al. Flecainide therapy reduces exercise-induced ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia. J Am Coll Cardiol. 2011;57:2244–54.

142. Frishman WH. Carvedilol. N Engl J Med. 1998;339:1759–65.

143. Hunt SA, Abraham WT, Chin MH, et al. 2009 focused update incorporated into the ACC/AHA 2005 Guidelines for the Diagnosis and Management of Heart Failure in Adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines: developed in collaboration with the International Society for Heart and Lung Transplantation. Circulation. 2009;119:e391–479.

144. Remme WJ, Cleland JG, Erhardt L, et al. Effect of carvedilol and metoprolol on the mode of death in patients with heart failure. Eur J Heart Fail. 2007;9:1128–35.

145. Mochizuki M, Yano M, Oda T, et al. Scavenging free radicals by low-dose carvedilol prevents redox-dependent Ca2+ leak via stabilization of ryanodine receptor in heart failure. J Am Coll Cardiol. 2007;49:1722–32.