

EXPEDITED REVIEW

The Ryanodine Receptor Leak: Its Role in the Development of Heart Failure

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ABSTRACT

The cardiac ryanodine receptor-calcium (Ca^{2+}) release channel type 2 (RyR2) is an essential sarcoplasmic reticulum (SR) transmembrane protein that plays a central role in excitation-contraction coupling in cardiomyocytes. Aberrant spontaneous, diastolic Ca^{2+} leak from the SR due to dysfunctional RyR2 is the mechanism underlying contractile and relaxation dysfunctions in heart failure. Several potential mechanisms have been proposed to explain the dysfunctional RyR2 in heart failure including over-phosphorylation status of RyR2, altered RyR2 regulation and perturbed RyR2 intra/intermolecular interactions. Novel therapeutic strategies that enhance myocyte Ca^{2+} homeostasis could prevent and reverse adverse cardiac remodeling and improve clinical outcomes in patients with heart failure.

INTRODUCTION

Heart failure (HF) is the leading cause of mortality and morbidity in developed countries. Despite substantial advances in the treatment, the incidence of HF continues to increase after the age of 65, affecting nearly 1 in 100 individuals.¹ The search for novel therapeutics in HF has led investigators to examine the molecular mechanisms underlying HF, to uncover potential therapeutic targets that can slow HF progression, improve quality of life and reduce mortality.

Much attention has been given for understanding the role of defects in Ca^{2+} regulation in HF.² This is due to the role of Ca^{2+} as the signal that regulates cardiac muscle contraction. Alterations in the excitation-contraction coupling (ECC) may lead to the progression of HF and development of lethal arrhythmias. Their common pathophysiologic phenomenon is the “diastolic Ca^{2+} leak” which involves an aberrant release of Ca^{2+} .³ In HF, diastolic Ca^{2+} leak through ryanodine receptor (RyR) 2 results in a decrease in the sarcoplasmic reticulum (SR) Ca^{2+} content, along with a decrease in Ca^{2+} reuptake by SR Ca^{2+} -ATPase 2a (SERCA2a) provoking a mismatch in the ECC.⁴

Defects in every stage of ECC lead to diastolic and systolic abnormalities, as well as an increased propensity for ventricular arrhythmias, all of which have been reported in patients with HF.⁵ These defects are the result of altered expression or function of proteins that are required for the maintenance of Ca^{2+} homeostasis. In this review, we explain the association between abnormalities in Ca^{2+} handling in the SR and HF and discuss the therapeutic potential of targeting key proteins involved in this process.

ABBREVIATIONS

CaMKII = Ca^{2+} /calmodulin-dependent protein kinase II
ECC = excitation-contraction coupling
FKBP12.6 (calstabin 2) = FK506 (tacrolimus) binding protein
HF = heart failure
NCX = $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger
PKA = protein kinase A
PLB = phospholamban
RyR = ryanodine receptor
SERCA = sarcoplasmic reticulum Ca^{2+} -ATPase
SR = sarcoplasmic reticulum

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EXCITATION-CONTRACTION COUPLING

In 1883 Sydney Ringer discovered that Ca^{2+} is required for cardiac contraction.⁶ Twenty-four years later Locke and Roseheim observed that Ca^{2+} is responsible for the link between myocardial excitation and contraction.⁷ Following these seminal discoveries, important advances have been made toward understanding the molecular determinants of cardiac Ca^{2+} regulation and its role in determining cardiac function. In cardiomyocytes, Ca^{2+} is stored in an intracellular vesicular network called sarcoplasmic reticulum (SR).⁸ Cardiac contraction can be divided into electrical (excitation) and contractile phases. The electrical phase begins with depolarization of the sinoatrial node which causes a wave of depolarization to spread via the conduction system through the atria and ventricles. On the cellular level current flows between a depolarized cardiomyocyte and its resting neighbor through the gap junctions causing depolarization of the membrane potential of the resting cell.⁹ Thereupon, Ca^{2+} ions passively drawn by a concentration and electrochemical gradient enter the cytoplasm and bind to the ryanodine receptors (RyR) type 2 that are located on the surface of the SR and trigger the release of even bigger amount of Ca^{2+} out of the SR. This process termed Ca^{2+} induced Ca^{2+} release, raises the cytosolic $[\text{Ca}^{2+}]$ about 10-fold to $1\mu\text{M}$ and initiates the ECC. The Ca^{2+} binds then to troponin C, which causes a conformational change exposing binding sites for myosin on the actin filaments. Myosin forms crossbridges with actin, shortening thus the sarcomere and causing cardiac muscle

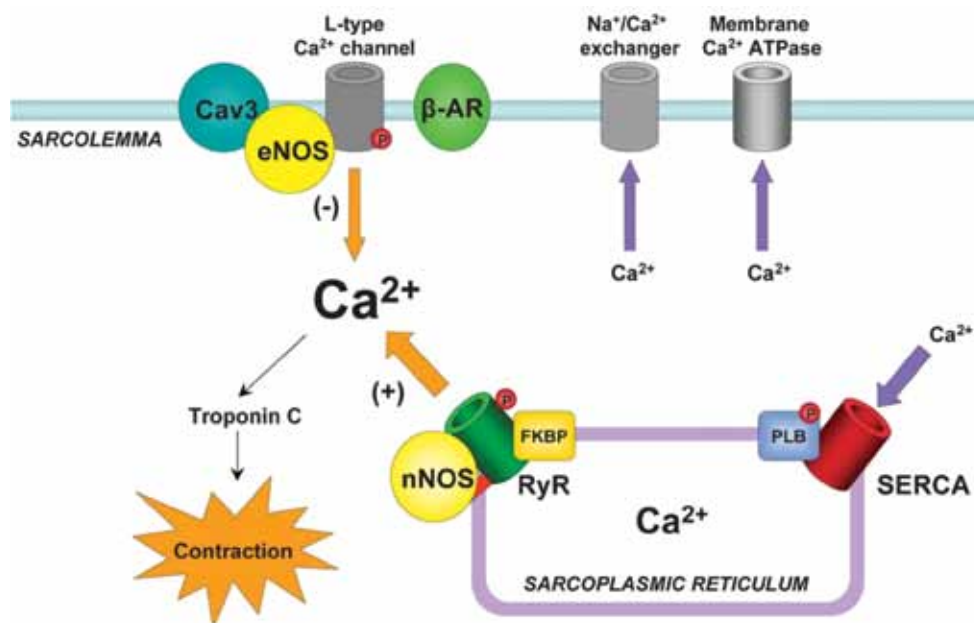
contraction.¹ Calcium is then pumped back into the SR by the sarcoplasmic/endoplasmic reticulum ATPase (SERCA 2a) and exchanged across the plasma membrane for Na^+ by the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, lowering the cytosolic $[\text{Ca}^{2+}]$ to baseline of 100 nM, thereby causing relaxation.¹¹

STRUCTURE AND FUNCTION OF RYANODINE RECEPTORS IN NORMAL HEART

Ryanodine receptors are the largest ion channels known in literature. They are key components of the excitation-contraction coupling.¹² They were originally identified using the plant alkaloid ryanodine, isolated from *Ryania speciosa* found in Central and South America. At that time, ryanodine was being tested as a potential pesticide¹³ due to its paralyzing effect on insects.¹⁴ Ryanodine was subsequently found to induce profound paralysis of cardiac and skeletal muscle (Figure 1).¹⁵

Ryanodine was used as a high affinity ligand to track the purification of its receptor from sarcoplasmic reticulum preparations. The purified ryanodine receptors were shown to be Ca^{2+} release channels in skeletal and cardiac muscles.^{16,17} Ryanodine was shown to lock the channel open in a characteristic sub-conductance state resulting in a SR Ca^{2+} leak that provided a mechanism for the paralytic action of the drug.¹⁸

There are 3 RyR isoforms in mammals. RyR1 is predominant in skeletal muscle.¹⁹ RyR3 was first cloned from rabbit brain.²⁰ It is more widely distributed and is expressed not only

Annex 1

FIGURE 1.

in the brain but also in skeletal muscle, diaphragm and many other organs.²⁰ The cardiac ryanodine receptor isoform RyR2, is a homotetramer comprising four 565KDa monomers. Each monomer contains a transmembrane segment located at the carboxyterminus that is formed by 10% of the linear sequence, whereas the remaining 90% of the protein sequence encodes an enormous cytoplasmic domain that serves as a scaffold for regulatory subunits and enzymes that modulate the function of the channel.²¹

During the action potential, Ca^{2+} entry through the L-type calcium channels triggers synchronized openings of RyR2 allowing a rapid and transient release of Ca^{2+} from the SR.²¹ The subsequent intracellular Ca^{2+} elevation triggers myocytes to contract. Relaxation occurs when RyR2 closes, Ca^{2+} dissociates from C-Troponin and is mainly taken back into the SR by SERCA 2a and partially extruded by the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX).

The amount of Ca^{2+} released by the SR during a cardiac cycle depends on the L-type Ca^{2+} channel, the RyR2 Ca^{2+} sensitivity and the SR Ca^{2+} load. Activation of RyR2 is highly sensitive to Ca^{2+} concentration and intracellular Ca^{2+} is the main modulator of RyR2 function in the heart. To date, the mechanism responsible for local Ca^{2+} release termination remains unclear.²² RyR2 opening probability (P_o) is regulated by other endogenous factors such as Mg^{2+} ions, ATP, pH and redox state.^{23,24} Furthermore, on its large N-terminal cytosolic side, RyR2 is associated and regulated by several proteins including FK-506 (tacrolimus) binding protein 12.6 (FKBP12.6 also called calstabin2), calmodulin, protein kinase A, calmodulin kinase II, phosphatase 1, phosphatase 2A and phosphodiesterase 4D3.²⁵⁻²⁹ These proteins control RyR2 stability, Ca^{2+} sensitivity and P_o .

From the luminal side, RyR2 are directly linked to triadin, junctin and calcineurin.³⁰ These proteins sense SR Ca^{2+} content especially through their interactions with calsequestrin which affects RyR2 channel gating.³¹ Independently of the classical Ca^{2+} -induced Ca^{2+} release mechanism, spontaneous Ca^{2+} release may occur when SR Ca^{2+} load reaches a certain threshold. This process also called store-overload-induced Ca^{2+} release generates cytosolic Ca^{2+} waves and subsequent delayed after depolarization. Mutation of the RyR2 or the associated proteins on the luminal side may affect intra store Ca^{2+} sensitivity and reduced store-overload-induced Ca^{2+} release threshold (Figure 2).

MECHANISMS OF RYR2 DYSREGULATION IN HEART FAILURE

Over the past 10 years, the role of pathological diastolic Ca^{2+} leak through dysfunctional RyR2 has been recognized as an important contributor to the progression of the heart failure.²⁷ The heart failure RyR2 Ca^{2+} leak hypothesis was

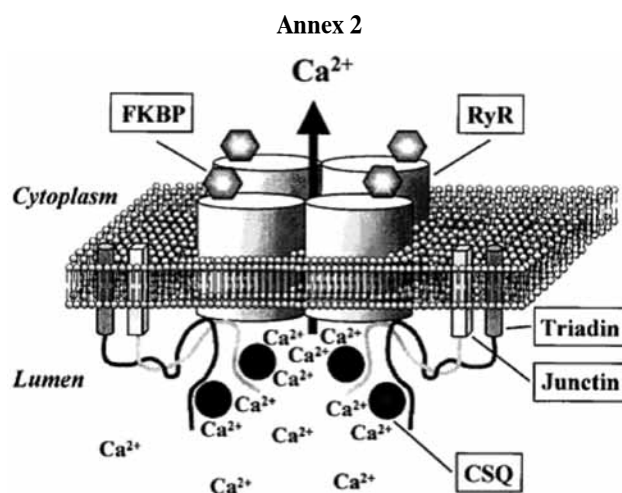


FIGURE 2.

that the chronic hyperadrenergic state observed in HF patients induced chronic protein kinase A (PKA) hyperphosphorylation of RyR2 at Ser2808, causing depletion of calstabin2 from the channel complex.²⁷ The term hyperphosphorylation describes RyR2 in which 3-4 of the four RyR2 monomers are chronically PKA phosphorylated. PKA hyperphosphorylated/calstabin 2 depleted channels are sensitized to cytosolic Ca^{2+} release during diastole, referred to as a diastolic SR Ca^{2+} leak. This latter would reduce SR Ca^{2+} stores and activate transient inward current.²⁹

The HF RyR2 Ca^{2+} leak hypothesis is supported by studies demonstrating that HF patients have PKA hyperphosphorylated and calstabin 2 depleted RyR2. This hypothesis has been challenged on the observation that β adrenergic receptor density on cardiomyocytes is reduced in HF and that this coincides with a global reduction in cytosolic cAMP levels.³² It is unclear how the presence of a chronic hyperadrenergic state in HF can lead to chronic PKA hyperphosphorylation of RyR2. This paradox can be explained by the general remodeling of RyR2 macromolecular complex and depletion of phosphatases³³ that occurs in HF. Depletion of phosphatases can induce discrete microdomains of elevated levels of cAMP in the vicinity of RyR2 and decreased rate of dephosphorylation of a hyperphosphorylated channel.²⁹

Alternative mechanisms have been proposed to explain SR Ca^{2+} leak in HF. Disulfide oxidation of free cysteine residues on RyR2 increases the sensitivity of the channel to luminal Ca^{2+} in lipid bilayer as well as enhances SR Ca^{2+} leak, manifested by reduced SR Ca^{2+} load in cardiomyocytes.³⁴ Also the Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) levels are elevated in human HF samples suggesting that CaMKII phosphorylation of RyR2 may contribute to the pathogenesis of HF.³⁵ This hypothesis is supported by reports that there is an increase in CaMKII-dependent phosphorylation of RyR2 in HF, which enhances SR Ca^{2+} leak.³⁶

ARRHYTHMIAS DUE TO ABNORMAL RYR2 FUNCTION

There is sufficient evidence, particularly from experimental models to suggest that RyR2 function in HF is perturbed and that diastolic leak is a feature of HF even if there is no agreement on the precise mechanism of dysfunction. These changes in RyR2 function in HF take place against a greatly modified cellular landscape which could also contribute to the propensity for arrhythmia. The close spatial relationship between T-tubule and junctional SR-the dyadic cleft- which is also important to the electrophysiological homeostasis of the heart beat can become disrupted in HF.^{37,38} Co-localisation between RyR2 and L type calcium channel is disturbed as T-Tubules are lost from the Z-line positions leaving junctional RyR2 "orphaned".³⁸ This will have serious repercussions for the critical interplay between RyR2 activation of L-type calcium channel (from RyR2 Ca²⁺ release).³⁹

Action potential duration is increased in human HF. Electrical remodeling and down regulation of K⁺ channels occurs early in the development of hypertrophy.^{40,41} However, L-type calcium channel protein expression itself in most studies is unchanged in HF.⁴¹ Many studies show upregulation of NCX expression two or three fold.⁴² The inward rectifying K⁺ current normally stabilizes the resting potential of the membrane but its activity is reduced by Ca²⁺. The increased diastolic leak of Ca²⁺ in HF may contribute to destabilization of the resting potential and together with the increase in NCX could lower the threshold for delayed after-depolarizations so that even a modest diastolic leak from RyR2 may result in an increased propensity to trigger a fatal arrhythmia.⁴³

RYANODINE RECEPTOR AS A NEW THERAPEUTIC TARGET OF HEART FAILURE

In the past few years, a considerable amount of new information has been made available with regard to the molecular mechanism of abnormal Ca²⁺ handling in HF. Because the alteration of RyR2 plays a key role in the defective, intracellular Ca²⁺ handling in HF, it is logical to consider the RyR2 as a promising therapeutic target of HF. In HF, the myocyte, because of the increased levels of circulating catecholamines, has already compensated by reducing β -receptor number.⁴⁴ However, these compensatory mechanisms result in a uncoordinated state of phosphorylation as regards ECC proteins, with hypophosphorylation of phospholamban (PLB)⁴⁵ and hyperphosphorylation of RyR2.⁴⁶ The long term effect of β -blockade has broad spectrum consequences on ECC by increasing phosphorylation of PLB, hence increasing SERCA activity and elevating SR Ca²⁺ store concentrations.⁴⁷

RyR2 hyperphosphorylation is reported to be reduced and FKBP12.6 binding levels restored in a paced dog model treated with propranolol⁴⁸ and in human heart muscle strips treated with carvedilol, metoprolol or atenolol.⁴⁹ The AT1 receptor antagonist, valsartan, which inhibits noradrenaline release from the synaptic pool, also restores SR function by reducing Ca²⁺ leak.⁵⁰ There is evidence that patients prescribed the β -blocker carvedilol had improved clinical outcomes in HF.⁵¹ Animal studies with carvedilol suggest its additional effectiveness might be attributed to its antioxidant properties and stimulation of SERCA gene transcription,⁵² implicating a new protective role of the drug through molecular alterations.

STABILIZING RYANODINE RECEPTOR TYPE 2 ACTIVITY

Improvements in knowledge of Ca²⁺ dynamics have led to increased understanding of the therapeutic potential of targeting Ca²⁺ handling proteins. Thus, a number of pharmacological and gene therapy strategies aimed at restoring the function of the sarcoplasmic reticulum by preventing Ca²⁺ leakage have been investigated.

Pharmacological strategies aimed at reducing Ca²⁺ leakage through RyR2 involve the use of agents that interact with RyR2 and inhibit Ca²⁺ release from the SR, either by altering the gating of RyR2 channel or controlling into translocation. Unfortunately, most of the cloning RyR2-targeting drugs, such as dantrolene and flecainide have unacceptable adverse effects or lack of long term efficacy.⁵³

JTV-519 (K201) is a promising new drug with cardioprotective effects.⁵⁴ It is a 1-4- benzothiazepine drug and a nonspecific blocker of Na⁺, K⁺ and Ca²⁺ channels with antiarrhythmic properties.⁵⁵ Like diltiazem, JTV 519 blocks the L-type calcium current, but it is not classified as a Ca²⁺-channel blocker.⁵⁵ It is a relatively nonselective blocker of cation currents, including the Na⁺ current in a voltage-dependant and frequency-dependant manner.⁵⁶ It also blocks the inward rectifying K⁺ current and rapidly activating component of the delayed rectified K⁺ current but not the slowly activating component.⁵⁷

JTV-519 stabilizes the closed state of RyR2 and increases the binding affinity of FKBP12.6 for RyR2, thereby reducing and preventing Ca²⁺ leak and protecting against ventricular arrhythmia, contractile dysfunction and Ca²⁺ overload.^{55,58} This beneficial combination of activity dramatically ameliorates the progression of HF as a result of myocardial damage from Ca²⁺ overload. The most recent evidence shows that JTV519 protects against Ca²⁺ leak from the SR independent of the interaction between FKBP12.6 and RyR2.⁵⁹ Thus, stabilization of RyR2 reduced detrimental intracellular Ca²⁺ leak and improves both diastolic and systolic contractile function in the human heart with or without FKBP12.6-RyR2 binding interaction.⁵⁹

Rycals (CPU0213, S107 and S44121) are drugs that sta-

bilize RyR2-FKBP12.6 complexes and inhibit Ca²⁺ leakage from the sarcoplasmic reticulum; they could therefore also have beneficial effects in patients with heart failure.⁶⁰ To our knowledge, cardioprotective effects of Rycal drugs have not yet been shown. However, the efficacy of S44121 is currently being evaluated in patients with chronic HF who are at risk for ventricular arrhythmias.⁶¹

Another potential target for HF therapy is the RyR2-stabilizing protein S100A1. Reduced S100A1 expression has been implicated in cardiomyopathies⁶² and animal models have shown the potential of S100A1 gene therapy for treatment of HF.⁶³ The safety and efficacy of coronary venous S100A1 delivery has been shown in a pig model of post-ischemic heart failure; contractile function and cardiac remodeling were significantly improved.⁶⁴ However, this system has not yet been evaluated in human HF.

CONCLUSION

An increasing body of evidence in the literature suggests that alteration of the properties of the Ca²⁺ release complex consisting of RyR2 and its satellite proteins (particularly FKBP12.6 or calstabin2) is the key element to understand the pathogenic mechanism of HF. An abnormally high level of PKA-mediated phosphorylation of RyR2 and the dissociation of one of the satellite proteins of RyR2, FKBP12.6 (calstabin2), are recognized in some cardiac disease models and regarded as important factors related to the mechanism of pathogenesis. This view is supported by the fact that beta-blockers and angiotensin receptor blockers (ARB) (suppressors of PKA phosphorylation) appear to prevent severe disease complications from occurring. However, many researchers have observed no change in phosphorylation and FKBP12.6 (calstabin2) in similar condition; this might suggest that the abnormal properties of phosphorylation and FKBP12.6 (calstabin2) are manifested in particular stages of disease development or under particular conditions.

As various complications linked with pathogenic processes have begun to be resolved at a molecular level, we now have a better chance to hunt for potentially useful therapeutic agents. Of particular interest in this context is the recent finding that JTV519, the drug that has been known to prevent Ca²⁺ overload and related complications, prevents those abnormal changes in the properties of the RyR2, such as interdomain interactions, PKA phosphorylation and the RyR2 bound FKBP12.6 (calstabin2). This new form of therapy targeting the Ca²⁺ regulatory protein should open up a new chapter for the future development of methods for the prevention and treatment of HF.

REFERENCES

- Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med* 2006;3: e442.
- Lompre AM, Hajar RJ, Harding SE, Kranias EF, Lohse MJ, Marks AR. Ca²⁺ cycling and new therapeutic approaches for heart failure. *Circulation* 2010;121:822-830.
- Yano M, Yamamoto T, Ikeda Y. Mechanisms of disease: ryanodine receptor defects in heart failure and fatal arrhythmias. *Nat. Clin. Pract Cardiovasc Med* 2006;3:43-52.
- Yano M, Ikeda Y, Matsuzaki M. Altered intracellular Ca²⁺ handling in heart failure. *J Clin Invest* 2005;115:556-564.
- Priori SG, Chen SR. Inherited dysfunction of sarcoplasmic reticulum Ca²⁺ handling and arrhythmogenesis. *Cir Res* 2011; 108:871-883.
- Ringer S. A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. *J Physiol* 1883; 4:29-42.
- Locke FS, Rosenheim O. Contributions to the physiology of the isolated heart. The consumption of dextrose by mammalian cardiac muscle. *J Physiol* 1907;36:205-220.
- Hasselbach W, Markinose M. On the mechanism of calcium transport across the membrane of sarcoplasmic reticulum. *Biochemische Zeitschrift* 1963;339:94-111.
- Rohr S. Role of gap junctions in the propagation of the cardiac action potential. *Cardiovasc Res* 2004;62:309-322.
- Campbell DL, Giles WR, Hume JR, et al. Inactivation of calcium current in bullfrog atrial myocytes. *J Physiol* 1988;403:287-315.
- Gibbons WR, Zygmunt AC. Excitation-contraction coupling in heart. The heart and cardiovascular system. *New York: Raven Press, Ltd* 1992; p. 1249-1279.
- Marx SO, Gaburjakova J, Gaburjakova M, Henrichs C, Ondrias K, Marks AR. Coupled gating between cardiac calcium release channels (ryanodine receptors). *Circ Res* 88:1151-1158.
- Rogers EF, Koniuszy FR, et al. Plant insecticides: ryanodine, a new alkaloid from *Ryania speciosa*. Vahl. *J Am Chem Soc* 1948;70:3086-3088.
- Edwards GA, Weiant EA, Slocombe AG, et al. The action of ryanodine on the contractile process in striated muscle. *Science* 1948;108:330-332.
- Jenden DJ, Fairhorst AC. The pharmacology of ryanodine. *Pharmacol Rev* 1969;21:1-25.
- Fleischer S, Ogunbunmi EM, Dixon MC, et al. Localization of Ca²⁺ release channels with ryanodine in junctional terminal cisternae of sarcoplasmic reticulum of fast skeletal muscle. *Proceedings of the National Academy of Sciences of the United States of America* 1985;82:7256-7259.
- Meissner G, Henderson JS. Rapid calcium release from cardiac sarcoplasmic reticulum vesicles is dependent on Ca²⁺ and is modulated by Mg²⁺, adenine nucleotide and calmodulin. *J Biol Chem* 1987; 262:3065-3073.
- Fill M, Copello JA. Ryanodine Receptor calcium release channels. *Physiol Rev* 2002;82:893-922.
- Nakai J, Imagana T, Hakamata Y, Shigekawa M, Takeshima H, Numa S. Primary Structure and functional expression from

- cDNA of the cardiac Ryanodine receptor/calcium release channel. *FEBS Lett* 1990;271:169-177.
20. Hakamata Y, Nakai J, Takeshima H, Imoto K. Primary structure and distribution of a novel ryanodine receptor/calcium release channel from rabbit brain *FEBS Lett* 1992;312:229-335.
 21. Zalic R, Lehnart SE, Marks AR. Modulation of the ryanodine receptor and intracellular calcium. *Annu Rev Biochem* 2007; 76:367-385.
 22. Cheng H, Lederer WJ. Calcium sparks. *Physiol Rev* 2008;88: 1491-1545.
 23. Copello JA, Barg S, Sonnleimer A, et al. Differential activation by Ca²⁺, ATP and caffeine of cardiac and skeletal muscle ryanodine receptors after block by Mg²⁺. *J Membr Biol* 2002; 187:51-64.
 24. Marengo JJ, Hidalgo C, Bull R. Sulfhydryl oxidation modifies the calcium dependence of ryanodine-sensitive calcium channels of excitable cells. *Biophys J* 2008;7:1263-1277.
 25. Lam E, Martin MM, Timmerman AP, et al. A novel FK506 binding protein can mediate the immunosuppressive effects of FK506 and associated with the cardiac ryanodine receptor. *J Biol Chem* 1995;270:26511-26522.
 26. Balshaw DM, Yamaguchi N, Meissner G. Modulation of intracellular calcium-release channels by calmodulin. *J Membr Biol* 2002;185:1-8.
 27. 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; 101:365-376.
 28. Rodriguez P, Bhogal MS, Colyer J. Stoichiometric phosphorylation of cardiac ryanodine receptor on serine 2809 by calmodulin-dependent kinase II and protein kinase A. *J Biol Chem* 2003;278:38593-38600.
 29. 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.
 30. Guo W, Campbell KP. Association of triadin with the ryanodine receptor and calsequestrin in the lumen of the sarcoplasmic reticulum. *J Biol Chem* 1995;270:9027-9030.
 31. Kawasaki T, Kasai M. Regulation of calcium channel in sarcoplasmic reticulum by calsequestrin. *Biochem Biophys Res Commun* 1994;199:1120-1127.
 32. Bristow MR, Ginsburg R, Minobe W, et al. Decreased catecholamine sensitivity and beta-adrenergic receptor density in failing human hearts. *N Engl J Med* 1982;307:205-211.
 33. Reiken S, Gaburjakova M, Gaburjakova J, et al. Beta-adrenergic receptor blockers restore cardiac calcium release channel (ryanodine receptor) and function in heart failure. *Circulation* 2001;104:2843-2848.
 34. Terentyev D, Gyorke I, Belevych AE, et al. Redox modification of ryanodine receptors contribute to sarcoplasmic reticulum Ca²⁺ leak in chronic heart failure. *Circ Res* 2008;103:1466-1472.
 35. Hoch B, Meyer R, Hetzer R, et al. Identification and expression of delta-isoform of the multifunctional Ca²⁺/calmodulin-dependent protein kinase in failing and nonfailing human myocardium. *Circ Res* 1999;84:713-721.
 36. Ai W, Curran JW, Shannon TR, et al. Ca²⁺/calmodulin-dependent protein kinase modulates cardiac ryanodine receptor phosphorylation and sarcoplasmic reticulum Ca²⁺ leak in heart failure. *Circ Res* 2005;97:1314-1322.
 37. Izu LT, Means SA, Shadid JN, et al. Interlay of ryanodine receptor distribution and calcium dynamics. *Biophys* 2006;J91:95-112.
 38. Song LS, Sobie EA, Mi Culle S, et al. Orphaned ryanodine receptors in the failing heart. *Proc Natl Acad Sci USA* 2006;103: 4305-4310.
 39. Brette F, Salle L, Orchard CH. Differential modulation of L-type Ca²⁺ current by SR Ca²⁺ release at the T-Tubules and surface membrane of rat ventricular myocytes. *Circ Res* 2004; 95:e1-e7.
 40. Davia K, Bernobich F, Ranu HK, et al. SERCA 2A overexpression decreases the incidence of aftercontractions in adult rabbit ventricular myocytes. *J Mol Cell Cardio* 2001; 33:1005-1015.
 41. Perrier E, Perrier R, Richard S, et al. Ca²⁺ controls functional expression of the cardiac K⁺ transient outward current via the calcineurin pathway. *J Biol Chem* 2004;279:40634-40639.
 42. Hasenfuss G, Resneuce H, Studer R, et al. Relation between myocardial function and expression of sarcoplasmic reticulum Ca²⁺-ATPase in failing and non failing human myocardium. *Circ Res* 1994;75:434-442.
 43. Fauconier J, Lacampagne A, Rauzier JM, Vassort G, Richard S. Ca²⁺ -dependent reduction of I_{K1} in rat ventricular cells: a novel paradigm for arrhythmia in heart failure? *Cardiovasc Res* 2005;68:204-212.
 44. Bristow MR, Ginsburg R, Minobe W, et al. Decreased catecholamine sensitivity and beta-adrenergic receptor density in failing human hearts. *N Engl J Med* 1982;307:205-211.
 45. Dash R, Frank KF, Carr AN, Moravec CS, Kranias EG. Gender influences on sarcoplasmic reticulum Ca²⁺ handling in failing human myocardium. *J Moll Cell Cardiol* 2001;33:1345-1353.
 46. Marks AR. A guide for the perplexed: towards an understanding of the molecular basis of heart failure. *Circulation* 2003;107: 1456-1459.
 47. Mc Lennan DH, Kranias EG. Phospholamban: a crucial regulator of cardiac contractility. *Nat RW Mol Cell Biol* 2003; 566-577.
 48. Doi M, Yano M, Kobayashi S, et al. Propranolol prevents the development of heart failure by restoring FKBP12.6-mediated stabilization of ryanodine receptor. *Circulation* 2002;105:1374-1379.
 49. Reiken S, Wehrens X, Vest JA, et al. Beta-blockers restore calcium release channel function and improve cardiac muscle performance in human heart failure. *Circulation* 2003;107: 2459-2466.
 50. Tokuhisa T, Yano M, Obayashi M, et al. AT1 receptor antagonist restores cardiac ryanodine receptor function rendering isoproterenol-induced failing heart less susceptible to Ca²⁺ leak induced by oxidative stress. *Circ J* 2006;70:777-786.
 51. Poole-Wilson PA, Swedberg K, Cleland JG, et al. Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the Carvedilol or Metoprolol Eu-

- ropean Trial (COMET): randomized controlled trial. *Lancet* 2003;362:7-13.
52. Koitabashi N, Arai M, Tomaru K, et al. Carvedilol effectively blocks oxidative stress-mediated downregulation of sarcoplasmic reticulum Ca²⁺-ATPase 2 gene transcription through modification of Sp1 binding. *Biophys Res Commun* 2005;328:116-124.
 53. Xu L, Tripathy A, Pasck DA, Meissner G. Potential of pharmacology of ryanodine receptor/calcium release channels. *Ann NY Acad Sci* 1998;853:130-148.
 54. Kaneko N. New 1,4-benzothiazepine derivate, K201, demonstrates cardioprotective effects against sudden cardiac cell death and intracellular calcium blocking action. *Drug Dev Res* 1994;33:429-438.
 55. Toischer K, Lehnart SE, Tenderich G, et al. K201 improves aspects of the contractile performance of human failing myocardium via reduction in Ca²⁺ leak from the sarcoplasmic reticulum. *Basic Res Cardiol* 2010;105:279-287.
 56. Kimura J, Kawahara M, Sakai E, Yatabe J, Nakanishi H. Effects of a novel cardioprotective drug, JTV-519, on membrane currents of guinea pig ventricular myocytes. *Jpn J Pharmacol* 1999;79:275-281.
 57. Nakaya H, Furusawa Y, Ogura T, Tamagawa M, Vemura H. Inhibitory effects of JTV-519, a novel cardioprotective drug on potassium currents and experimental atrial fibrillation in guinea-pig hearts. *Br J Pharmacol* 2000;131:1363-1372.
 58. Hasumi H, Matsuda R, Shimamoto K, Hata Y, Kaneko N. K201, a multi-channel blocker inhibits clofilium-induced torsades des pointes and attenuates an increase in repolarization. *Eur J Pharmacol* 2007;555:54-60.
 59. Hunt DJ, Jones PP, Wang R, et al. K201 (JTV-519) suppresses spontaneous Ca²⁺ release and [3H] ryanodine binding to RyR2 irrespective of FKBP12.6 association. *Biochem J* 2007;404:431-438.
 60. Bellinger AM, Reiken S, Carlson C, et al. Hypernitrosylated ryanodine receptor calcium release channels are leaky in dystrophic muscle. *Nat Med* 2009;15:325-330.
 61. International Standard Randomized Controlled Trial Number Register. *Controlled trials.com*; 2012. <http://www.controlled-trials.com/isrctn/>
 62. Remppis A, Greten T, Schäfer BW, et al. Altered expression of the Ca²⁺-binding protein S100A1 in human cardiomyopathy. *Biochem Biophys Acta* 1996;1313:253-257.
 63. Most P. Cardiac S100A1 protein levels determine contractile performance and propensity toward heart failure after myocardial infarction. *Circulation* 2006;114:1258-1261.
 64. Pleger ST, Shan C, Ksienzyk J, et al. Cardiac AAV9-S100A1 gene therapy rescues post-ischemic heart failure in a preclinical large animal model. *Sci Transl Med* 2011;3:92ra64.