Defective Ryanodine Receptor Interdomain Interactions May Contribute to Intracellular Ca^{2+} Leak

A Novel Therapeutic Target in Heart Failure

Stephan E. Lehnart, MD; Xander H.T. Wehrens, MD, PhD; Andrew R. Marks, MD

Heart failure (HF) is a leading cause of morbidity and mortality, and despite optimal medical treatment, patients with NYHA class III–IV symptoms have a 2-year mortality rate approaching 50%.[1,2] About half of the deaths from HF occur suddenly,[3,4] and ventricular arrhythmias account for a large percentage of these sudden cardiac deaths (SCDs).[1] There is a strong correlation between chronic increased activity of the sympathetic nervous system (SNS) and poor prognosis in HF.[5–7] Interestingly, β-adrenergic receptor (βAR) blockers that block the target receptors for catecholamines released during SNS activation reduce HF progression and arrhythmia vulnerability in patients with HF, leading to improved survival.[8,9] Activation of the βAR signaling cascade driven by the SNS caused by HF initially results in a compensatory response aimed at enhancing cardiac contractility, but when SNS activation is sustained, maladaptive changes occur.[10] Chronic SNS activation desensitizes cardiac βAR signaling,[5] which further contributes to reduced inotropic reserve and worsens remodeling in HF, as part of a maladaptive response that ultimately fails to protect the heart or preserve cardiac function.[11]

See p 3400

Defective intracellular Ca^{2+} homeostasis has been consistently reported in HF.[12,13] Because intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) directly regulate contractility of cardiomyocytes, a reduced $[Ca^{2+}]_i$ transient amplitude in HF results in decreased force development. A prolonged decay of the $[Ca^{2+}]_i$ transient and increased diastolic $[Ca^{2+}]_i$ may contribute to slowed relaxation of the failing heart.[13–15] Several defects in the intracellular $[Ca^{2+}]_i$ metabolism have been reported, including depressed Ca^{2+} uptake, storage, and/or release of Ca^{2+} from the sarcoplasmic reticulum (SR) storage organelle.[15–17] Depressed SR Ca^{2+} release may result from decreased SR Ca^{2+} uptake via the SR Ca^{2+} ATPase (SERCA2a), as well as a competitive increase of Ca^{2+} extrusion to the extracellular space via upregulated function of the plasma membrane Na^+/Ca^{2+} exchanger.[14,18] Increased diastolic SR Ca^{2+} leak may also result in decreased SR Ca^{2+} loading and depressed contractility in HF.[19,20] Diastolic SR Ca^{2+} leak is caused by "leaky" ryanodine receptor (RyR2)/SR Ca^{2+} release channels, which display an increased sensitivity to Ca^{2+}-induced Ca^{2+} release and incomplete channel closure during diastole in HF.[21] We[21–23] and others[24–26] have previously demonstrated that chronic SNS hyperactivity in HF leads to increased protein kinase A (PKA) phosphorylation of RyR2 in the heart, which results in decreased binding of the channel-stabilizing subunit calstabin2 (FKBP12.6).[21] These findings highlight the important role of defects in RyR2 in HF as previously discussed in a *Circulation* editorial.[10] The role of leaky RyR2 and SNS activation in triggering ventricular arrhythmias is supported by the observation that inherited mutations in the *RyR2* gene cause sudden cardiac death.[27,28] Patients with catecholaminergic polymorphic ventricular tachycardia (CPVT) are heterozygous for the mutant *RyR2* allele and are highly susceptible to exercise-induced cardiac arrhythmias.[29,30] The new study by Oda and colleagues in this issue of *Circulation* expands the insights into the fundamental mechanisms of defective RyR2 function in HF and cardiac arrhythmias and suggests a role for defective interdomain interactions within RyR2 as a contributing factor in the development of an abnormal diastolic SR Ca^{2+} leak.[31]

Concept of Intramolecular Domain Unzipping

Missense mutations in the homologous *RyR1* channel gene that have been linked to diseases of the skeletal muscle, including malignant hyperthermia (MH) and central core disease (CCD), cluster in regions of the RyR1 channel that correspond to the regions of the RyR2 channel that contain the CPVT mutations. We previously noted that similarity between the distribution of CPVT and MH/CCD mutations in RyR2 and RyR1, respectively, raises the possibility that similar biophysical defects in the cardiac and skeletal RyR channels account for CPVT and MH/CCD.[32] Indeed, the majority of MH mutations in *RyR1* are located in well-defined domains of the RyR1 channel, also referred to as MH clusters or hot-spot regions.[32] Under experimental conditions, MH mutant RyR1 channels display a phenotype similar to CPVT mutations in RyR2, hyperactivity under resting conditions, and Ca^{2+} leak from the SR into the cytoplasm. Ikemoto and colleagues have proposed that N-terminal and central domains in RyR1 contribute to the conformational stabilization of the closed state of the channel via domain-zipping interactions.[33,34] More simply put, they predict that

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From the Clyde and Helen Wu Center for Molecular Cardiology, Department of Physiology and Cellular Biophysics (S.E.L., X.H.T.W., A.R.M.) and the Department of Medicine, College of Physicians and Surgeons (A.R.M.), Columbia University, New York, NY.

Correspondence to Dr Andrew R. Marks, Department of Physiology and Cellular Biophysics, College of Physicians and Surgeons, Columbia University, 630 West 168th St, Box 65, Room 9-401, New York, NY 10032. E-mail arm42@columbia.edu

(*Circulation.* 2005;111:3342-3346.)

***Circulation* is available at http://www.circulationaha.org**
DOI: 10.1161/CIRCULATIONAHA.105.551861

sequences of the channel that are not adjacent to one another in the linear peptide sequence can interact once the channel is folded into its 3-dimensional structure. Furthermore, Ikemoto and colleagues have proposed that channel opening during excitation-contraction coupling requires domain unzipping to reduce the conformational energy constraints necessary to induce the open state.[34,35] According to the domain-zipping model, MH-associated mutations occurring in either the N-terminal or central domains of RyR1 reduce the affinity of the zipper domains, resulting in leaky channels.

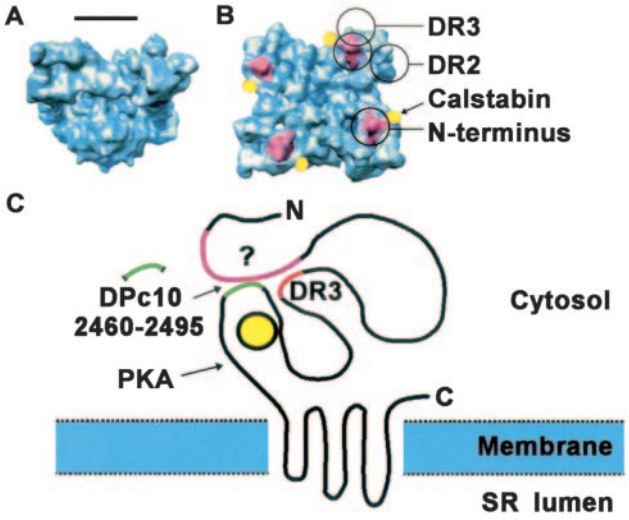
To test this hypothesis, a domain-specific peptide (DP4) corresponding to the putative RyR1 central zipper domain was applied to inhibit binding of the central to the N-terminal zipper domain. The resulting channel defects in RyR1 are similar to those observed in MH-associated RyR1 mutations (eg, increased channel open probability, abnormal SR Ca^{2+} release, and hypersensitivity to activation).[33,36,37] A note of caution should be introduced, however, because experiments with synthetic peptides are susceptible to artifacts from unspecific peptide binding resulting from excess peptide concentrations. It was proposed that defective interdomain interactions within MH-mutant RyR1 channels contribute to leaky RyR1 channels that cause MH.[38]

Defective Intermolecular Domain Interactions in the Cardiac RyR2 Isoform and Correlation With Structural Studies

Because skeletal RyR1 and cardiac RyR2 are 66% homologous at the protein level, it was proposed that domain unzipping may also occur in the cardiac Ca^{2+}-release channel RyR2. Oda and colleagues have designed a synthetic peptide (DPc10), corresponding to Gly2460-Pro2495 of RyR2. Binding of the peptide DPc10 to the hypothetical N-terminal zipper domain of RyR2 was found to increase sensitivity to Ca^{2+}-dependent channel activation and diastolic SR Ca^{2+} leak to levels observed in failing hearts. In contrast, introduction of the CPVT-associated RyR2 mutation Arg2474Ser into DPc10 (DPc10-mut) abolished these effects indirectly, suggesting that the DPc10-mut peptide, which was not able to interfere with the interdomain interaction or to produce domain unzipping effects, may mimic effects of the identical CPVT mutation in the RyR2 channel, as we have shown earlier.[28]

To further test the hypothesis that DPc10 produced unzipping of interacting N-terminal and central domains in RyR2, the fluorescent label MCA was cross-linked to RyR2 with DPc10 peptide targeting. Site-specific antibody labeling of calpain-digested RyR2 fragments revealed that MCA bound to the N-terminal 120 kDa of RyR2. These data support the idea that the N-terminal and central domains of RyR2 interact with each other. Interestingly, data from cryo-electron microscopy studies of RyR2 support this observation as well.[39]

Serysheva and coworkers recently published the 3-dimensional RyR1 structure at 14 Å resolution, and projected residues 216 to 572 of the N-terminus within a group of structural surface domains that form the 4 corners of the cytoplasmic aspect of the channel tetramer, also referred to as the clamp regions (Figure, A and B). These findings are consistent with previous localization studies of the RyR1 and



Three-dimensional RyR1 channel surface representation, with approximate localization of domains and extrapolation to RyR2 monomeric subunit topology. A, B, Square mushroom structure of the RyR1 closed channel conformation (side view) with the cytoplasmic scaffold lying on top and the transmembrane ion conducting pore region on the bottom (A) and top view from the cytoplasmic side (B). Bar indicates 100 Å.[56] Calstabin1 (yellow) binds to a central domain in the channel complex including Val^{2461}.[43,44] Homology modeling projects the N-terminal region 216 to 572 (pink) to the cytoplasmic clamp region[56] in proximity of the calstabin binding region, indicating that the N-terminal and central domains associate, as proposed by Ikemoto and colleagues.[32] Similarly, divergent region 3 (DR3) corresponding to the central channel domain was localized to clamp domain 9 near the projected N-terminus and near the calstabin binding region. C, Schematic representation of regulatory domains in the RyR2 monomeric subunit as viewed from the side. The RyR2 channel is a homotetrameric complex composed of 4 RyR2 monomers, each containing a C-terminal pore region and a larger cytoplasmic scaffold domain. PKA phosphorylation of RyR2 at Ser^{2809} decreases binding of calstabin2 (yellow circle) to the central domain including Ile^{2427}.[44] The central zipper domain corresponding to DPc10 is indicated in green and is proposed to interact with an as-yet undefined N-terminal zipper domain possibly corresponding to the projected clamp region sequence (pink). DR3 (orange) is shown in close proximity to the zipper and the calstabin2 binding domains. Alterations of N-terminal with central domain interactions by PKA RyR2 hyperphosphorylation, CPVT mutations, and/or calstabin2 depletion may destabilize the channel closed state and contribute to a diastolic SR Ca^{2+} leak that promotes heart failure progression and/or triggers fatal cardiac arrhythmias.[21,49] The figure is not drawn to scale. Reprinted with permission from *Journal of Molecular Biology*.[57] Copyright 2005, Elsevier.

RyR3 N-termini at significantly lower resolutions.[40,41] Interestingly, the clamp structure containing the N-terminus projection was shown to undergo conformational changes corresponding to channel opening and closing[42,43] and appears to be localized near the calstabin1 (FKBP12) binding region.[44] Because we and others have shown that calstabin1 (FKBP12) binds to the central domain of the full-length RyR1 involving the critical amino acid Val^{2461} corresponding to Ile^{2427} in the cardiac RyR2,[45,46] the structural and experimental data provide further support for the concept of N-terminal and central domain interaction. Moreover, Wagenknecht and colleagues have demonstrated that Thr^{1366} within divergent region 2 (DR2) localizes to clamp domain 6 in RyR1,[47] whereas

Thr[1874] within DR3 localizes to clamp domain 9 near the calstabin binding region in RyR2 (Figure, B).[48] Because of the high degree of sequence identity, it is likely that RyR1 intradomain mechanisms and interactions with calstabin1 similarly apply to the cardiac RyR2 and calstabin2, as summarized in part C of the Figure. It should be noted, however, that no direct structural evidence of interdomain interaction is available, although a casual observer of the RyR channel 3-dimensional structures will readily appreciate extensive domain interactions in the huge cytoplasmic domain of this largest of all ion channels (Figure, B).

Further support for the hypothesis of interacting N-terminal and central domains within RyR2 was obtained with the bulky fluorescence quencher QSY-BSA. The rationale of peptide targeting of the MCA fluorescent probe to the N-terminus of RyR2 was used in combination with the large fluorescence quencher to monitor accessibility of unzipped domain configurations. Measurement of QSY-BSA fluorescence quenching was considerably increased after treatment with DPc10 or cAMP (which increases PKA phosphorylation of RyR2 at Ser[2809]).[49] Confirming previous results, accessibility of the QSY-BSA quencher was also significantly increased in SR preparations from failing hearts, which is consistent with domain unzipping resulting from PKA hyperphosphorylation of RyR2.[21]

Under physiological conditions, stress or exercise result in transiently increased PKA phosphorylation of RyR2 at Ser[2809], which transiently decreases the binding affinity of calstabin2 to RyR2 and increases Ca^{2+}-dependent activation of the channel.[28,49,50] Thus, after PKA phosphorylation of RyR2, there is increased SR Ca^{2+} release for any given trigger $[Ca^{2+}]_i$.[28,50] On the other hand, chronic PKA hyperphosphorylation of RyR2 in HF results in incomplete channel closure during diastole, which causes depletion of the SR Ca^{2+} store and reduced systolic Ca^{2+} release.[19,21,51] Experiments with the QSY-BSA fluorescence quencher revealed that DPc10-induced domain unzipping does not induce calstabin2 dissociation by itself. Rather, the observations by Oda et al suggest that unzipping may (1) facilitate PKA phosphorylation of RyR2 and (2) enhance calstabin2 dissociation in response to PKA phosphorylation of RyR2. These data suggest that peptide-induced domain unzipping would increase the accessibility of the PKA catalytic subunit (which is bound to RyR2 via the targeting protein mAKAP) to the PKA phosphorylation site Ser[2809] on RyR2.[52] Alternatively, under physiological conditions PKA phosphorylation may be expected to induce conformational changes in RyR2 consistent with domain unzipping. Determination of the exact role of domain unzipping in the gating mechanism and regulation of RyR2 will require further studies. Such studies may also reveal how conformational changes in RyR2 resulting from PKA phosphorylation and domain unzipping reduce calstabin2 (FKBP12.6) binding affinity to the channel complex.

Leaky RyR2 Channels as a Therapeutic Target for Heart Failure

The study by Oda and colleagues provides additional confirmation that abnormal RyR2 channel function in failing hearts is caused by chronically increased levels of PKA phosphorylation of the channel resulting in dissociation of the calstabin2 (FKBP12.6) subunit, which is consistent with previous studies.[20,21,23–26,53–55] Moreover, it suggests that domain unzipping may contribute to RyR2 channel dysfunction in HF. Together, these findings provide further support for the concept that normalizing RyR2 channel function may represent a novel therapeutic approach for treating HF.

Initial studies have associated normalization of RyR2 function with therapeutic benefits in a canine model of HF and in patients with HF treated with β-blockers.[22–24] It was shown that blockade of βAR reduced intracellular cAMP levels and decreased PKA hyperphosphorylation of RyR2, leading to normalization of RyR2 macromolecular complex stoichiometry (eg, increasing binding of calstabin2 to RyR2) and inhibition of the diastolic SR Ca^{2+} leak via RyR2 in failing hearts. It is well known, however, that β-blocker treatment offers only partial protection from HF progression and SCD and may come with unacceptable side effects, including lethargy, impotence, depression, and failure to improve exercise tolerance.[56]

In proof-of-principle studies we have shown that a mutant calstabin2 with a neutralized charge that is able to bind to PKA phosphorylated RyR2 rescues channel function in CPVT mutant and PKA hyperphosphorylated RyR2, providing direct evidence for the ability of enhanced calstabin2 binding to normalize defective RyR2 function.[28] Therefore, we have postulated that interventions that increase calstabin2 binding to RyR2 may fix the diastolic SR Ca^{2+} leak and prevent HF progression and triggered cardiac arrhythmias.[21] On the basis of our initial studies that demonstrated leaky RyR2 in HF, Matsuzaki and colleagues showed that JTV-519, a 1,4-benzothiazepine derivative, slows the progression of canine HF by inhibiting RyR2 hyperactivity and intracellular Ca^{2+} leak.[55] We have demonstrated that JTV-519 can effectively suppress ventricular arrhythmias in a genetic mouse model by rebinding calstabin2 to RyR2[50] and normalize CPVT mutant RyR2 channel function,[27] indicating that JTV-519 and its derivatives could constitute a novel class of drugs known as "calcium release channel stabilizers" for the treatment of heart failure and cardiac arrhythmias.

In the study by Oda et al, the DPc10 peptide, cAMP, or calstabin2 dissociation from RyR2 by FK506 increased QSY-BSA quenching, which could be inhibited by JTV-519. Moreover, SR Ca^{2+} store depletion in DPc10-treated or failing cardiomyocytes could be reversed by JTV-519 treatment. These data clearly establish the beneficial effects of JTV-519 at the cellular level, which extends previous in vivo findings. The experiments suggest that JTV-519 may stabilize domain interactions in the zipped state, which could be used as a therapeutic strategy against HF and ventricular arrhythmias. Caution must be applied to proposals to use JTV-519 for patients because it is known to have potent human K^+ channel–blocking activity, which can cause QT prolongation as well as L-type Ca^{2+} channel-blocking activity (shown to be detrimental in HF patients). However, recently we have synthesized novel derivatives of JTV-519 that have reduced human ether-a-go-go– and L-type channel-blocking activity. These new compounds, known as calcium release channel stabilizers, have enhanced efficacy in terms of fixing the leak

in RyR2 channel in HF and preventing exercise-induced arrhythmias in mice, raising the possibility that such compounds could be developed into therapeutics for patients.

References

1. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. The SOLVD Investigators. *N Engl J Med.* 1992;327:685–691.
2. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, Palensky J, Wittes J. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med.* 1999;341:709–717.
3. Pratt CM, Gardner M, Pepine C, Kohn R, Young JB, Greenberg B, Capone R, Kostis J, Henzlova M, Gosselin G, Weiss M, Francis M, Stewart D, Davis E, Yusuf S; SOLVD Investigators. Lack of long-term ventricular arrhythmia reduction by enalapril in heart failure. SOLVD Investigators. *Am J Cardiol*. 1995;75:1244–1249.
4. Stevenson WG, Stevenson LW, Middlekauff HR, Saxon LA. Sudden death prevention in patients with advanced ventricular dysfunction. *Circulation*. 1993;88:2953–2961.
5. Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K, Billingham ME, Harrison DC, Stinson EB. Decreased catecholamine sensitivity and beta-adrenergic-receptor density in failing human hearts. *N Engl J Med*. 1982;307:205–211.
6. Cohn JN, Levine TB, Olivari MT, Garberg V, Lura D, Francis GS, Simon AB, Rector T. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. *N Engl J Med*. 1984;311:819–823.
7. Packer M, Lee WH, Kessler PD, Gottlieb SS, Bernstein JL, Kukin ML. Role of neurohormonal mechanisms in determining survival in patients with severe chronic heart failure. *Circulation*. 1987;75:IV80–IV92.
8. The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II): a randomised trial. *Lancet*. 1999;353:9–13.
9. Packer M, Coats AJ, Fowler MB, Katus HA, Krum H, Mohacsi P, Rouleau JL, Tendera M, Castaigne A, Roecker EB, Schultz MK, DeMets DL. Effect of carvedilol on survival in severe chronic heart failure. *N Engl J Med*. 2001;344:1651–1658.
10. Marks AR, Reiken S, Marx SO. Progression of heart failure: is protein kinase a hyperphosphorylation of the ryanodine receptor a contributing factor? *Circulation*. 2002;105:272–275.
11. Nienaber JJ, Tachibana H, Naga Prasad SV, Esposito G, Wu D, Mao L, Rockman HA. Inhibition of receptor-localized PI3K preserves cardiac beta-adrenergic receptor function and ameliorates pressure overload heart failure. *J Clin Invest*. 2003;112:1067–1079.
12. Morgan JP, Erny RE, Allen PD, Grossman W, Gwathmey JK. Abnormal intracellular calcium handling, a major cause of systolic and diastolic dysfunction in ventricular myocardium from patients with heart failure. *Circulation*. 1990;81:III21–III32.
13. Beuckelmann DJ, Nabauer M, Erdmann E. Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. *Circulation*. 1992;85:1046–1055.
14. Hasenfuss G, Schillinger W, Lehnart SE, Preuss M, Pieske B, Maier LS, Prestle J, Minami K, Just H. Relationship between Na^+-$Ca2^+$-exchanger protein levels and diastolic function of failing human myocardium. *Circulation*. 1999;99:641–648.
15. Pieske B, Sutterlin M, Schmidt-Schweda S, Minami K, Meyer M, Olschewski M, Holubarsch C, Just H, Hasenfuss G. Diminished post-rest potentiation of contractile force in human dilated cardiomyopathy. Functional evidence for alterations in intracellular $Ca2^+$ handling. *J Clin Invest*. 1996;98:764–776.
16. Gomez AM, Valdivia HH, Cheng H, Lederer MR, Santana LF, Cannell MB, McCune SA, Altschuld RA, Lederer WJ. Defective excitation-contraction coupling in experimental cardiac hypertrophy and heart failure. *Science*. 1997;276:800–806.
17. Hobai IA, O'Rourke B. Decreased sarcoplasmic reticulum calcium content is responsible for defective excitation-contraction coupling in canine heart failure. *Circulation*. 2001;103:1577–1584.
18. Hasenfuss G, Reinecke H, Studer R, Meyer M, Pieske B, Holtz J, Holubarsch C, Posival H, Just H, Drexler H. Relation between myocardial function and expression of sarcoplasmic reticulum $Ca(2^+)$-ATPase in failing and nonfailing human myocardium. *Circ Res*. 1994;75:434–442.
19. Shannon TR, Pogwizd SM, Bers DM. Elevated sarcoplasmic reticulum $Ca2^+$ leak in intact ventricular myocytes from rabbits in heart failure. *Circ Res*. 2003;93:592–594.
20. Yamamoto T, Yano M, Kohno M, Hisaoka T, Ono K, Tanigawa T, Saiki Y, Hisamatsu Y, Ohkusa T, Matsuzaki M. Abnormal $Ca2^+$ release from cardiac sarcoplasmic reticulum in tachycardia-induced heart failure. *Cardiovasc Res*. 1999;44:146–155.
21. Marx SO, Reiken S, Hisamatsu Y, Jayaraman T, Burkhoff D, Rosemblit N, Marks AR. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell*. 2000;101:365–376.
22. Reiken S, Gaburjakova M, Guatimosim S, Gomez AM, D'Armiento J, Burkhoff D, Wang J, Vassort G, Lederer WJ, Marks AR. Protein kinase A phosphorylation of the cardiac calcium release channel (ryanodine receptor) in normal and failing hearts. Role of phosphatases and response to isoproterenol. *J Biol Chem*. 2003;278:444–453.
23. Reiken S, Gaburjakova M, Gaburjakova J, He Kl KL, Prieto A, Becker E, Yi Gh GH, Wang J, Burkhoff D, Marks AR. beta-adrenergic receptor blockers restore cardiac calcium release channel (ryanodine receptor) structure and function in heart failure. *Circulation*. 2001;104:2843–2848.
24. Doi M, Yano M, Kobayashi S, Kohno M, Tokuhisa T, Okuda S, Suetsugu M, Hisamatsu Y, Ohkusa T, Matsuzaki M. Propranolol prevents the development of heart failure by restoring FKBP12.6-mediated stabilization of ryanodine receptor. *Circulation*. 2002;105:1374–1379.
25. Ono K, Yano M, Ohkusa T, Kohno M, Hisaoka T, Tanigawa T, Kobayashi S, Matsuzaki M. Altered interaction of FKBP12.6 with ryanodine receptor as a cause of abnormal $Ca(2^+)$ release in heart failure. *Cardiovasc Res*. 2000;48:323–331.
26. Yano M, Ono K, Ohkusa T, Suetsugu M, Kohno M, Hisaoka T, Kobayashi S, Hisamatsu Y, Yamamoto T, Noguchi N, Takasawa S, Okamoto H, Matsuzaki M. Altered stoichiometry of FKBP12.6 versus ryanodine receptor as a cause of abnormal $Ca(2^+)$ leak through ryanodine receptor in heart failure. *Circulation*. 2000;102:2131–2136.
27. Lehnart SE, Wehrens XH, Laitinen PJ, Reiken SR, Deng SX, Cheng Z, Landry DW, Kontula K, Swan H, Marks AR. Sudden death in familial polymorphic ventricular tachycardia associated with calcium release channel (ryanodine receptor) leak. *Circulation*. 2004;109:3208–3214.
28. Wehrens XH, Lehnart SE, Huang F, Vest JA, Reiken SR, Mohler PJ, Sun J, Guatimosim S, Song LS, Rosemblit N, D'Armiento JM, Napolitano C, Memmi M, Priori SG, Lederer WJ, Marks AR. FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. *Cell*. 2003;113:829–840.
29. Laitinen PJ, Brown KM, Piippo K, Swan H, Devaney JM, Brahmbhatt B, Donarum EA, Marino M, Tiso N, Viitasalo M, Toivonen L, Stephan DA, Kontula K. Mutations of the cardiac ryanodine receptor (RyR2) gene in familial polymorphic ventricular tachycardia. *Circulation*. 2001;103:485–490.
30. Priori SG, Napolitano C, Tiso N, Memmi M, Vignati G, Bloise R, Sorrentino V, Danieli GA. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2001;103:196–200.
31. Oda T, Yano M, Yamamoto T, Tokuhisa T, Okuda S, Doi M, Ohkusa T, Ikeda Y, Kobayashi S, Ikemoto N, Matsuzaki M. Defective regulation of interdomain interactions within the ryanodine receptor plays a key role in the pathogenesis of heart failure. *Circulation*. 2005;111:3400–3410.
32. Marks AR, Priori S, Memmi M, Kontula K, Laitinen PJ. Involvement of the cardiac ryanodine receptor/calcium release channel in catecholaminergic polymorphic ventricular tachycardia. *J Cell Physiol*. 2002;190:1–6.
33. Yamamoto T, El-Hayek R, Ikemoto N. Postulated role of interdomain interaction within the ryanodine receptor in $Ca(2^+)$ channel regulation. *J Biol Chem*. 2000;275:11618–11625.
34. Ikemoto N, Yamamoto T. Postulated role of inter-domain interaction within the ryanodine receptor in $Ca(2^+)$ channel regulation. *Trends Cardiovasc Med*. 2000;10:310–316.
35. Ikemoto N, Yamamoto T. Regulation of calcium release by interdomain interaction within ryanodine receptors. *Front Biosci*. 2002;7:d671–d683.
36. Lamb GD, Posterino GS, Yamamoto T, Ikemoto N. Effects of a domain peptide of the ryanodine receptor on $Ca2^+$ release in skinned skeletal muscle fibers. *Am J Physiol Cell Physiol*. 2001;281:C207–C214.
37. Shtifman A, Ward CW, Yamamoto T, Wang J, Olbinski B, Valdivia HH, Ikemoto N, Schneider MF. Interdomain interactions within ryanodine receptors regulate $Ca2^+$ spark frequency in skeletal muscle. *J Gen Physiol*. 2002;119:15–32.
38. Kobayashi S, Yamamoto T, Parness J, Ikemoto N. Antibody probe study of $Ca2^+$ channel regulation by interdomain interaction within the ryanodine receptor. *Biochem J*. 2004;380:561–569.

39. Masumiya H, Wang R, Zhang J, Xiao B, Chen SR. Localization of the 12.6-kDa FK506-binding protein (FKBP12.6) binding site to the NH2-terminal domain of the cardiac $Ca2^+$ release channel (ryanodine receptor). *J Biol Chem*. 2003;278:3786–3792.
40. Liu Z, Zhang J, Sharma MR, Li P, Chen SR, Wagenknecht T. Three-dimensional reconstruction of the recombinant type 3 ryanodine receptor and localization of its amino terminus. *Proc Natl Acad Sci U S A*. 2001; 98:6104–6109.
41. Baker ML, Serysheva II, Sencer S, Wu Y, Ludtke SJ, Jiang W, Hamilton SL, Chiu W. The skeletal muscle $Ca2^+$ release channel has an oxidoreductase-like domain. *Proc Natl Acad Sci U S A*. 2002;99: 12155–12160.
42. Orlova EV, Serysheva II, van Heel M, Hamilton SL, Chiu W. Two structural configurations of the skeletal muscle calcium release channel. *Nat Struct Biol*. 1996;3:547–552.
43. Serysheva II, Schatz M, van Heel M, Chiu W, Hamilton SL. Structure of the skeletal muscle calcium release channel activated with $Ca2^+$ and AMP-PCP. *Biophys J*. 1999;77:1936–1944.
44. Wagenknecht T, Radermacher M, Grassucci R, Berkowitz J, Xin HB, Fleischer S. Locations of calmodulin and FK506-binding protein on the three-dimensional architecture of the skeletal muscle ryanodine receptor. *J Biol Chem*. 1997;272:32463–32471.
45. Gaburjakova M, Gaburjakova J, Reiken S, Huang F, Marx SO, Rosemblit N, Marks AR. FKBP12 binding modulates ryanodine receptor channel gating. *J Biol Chem*. 2001;276:16931–16935.
46. Bultynck G, Rossi D, Callewaert G, Missiaen L, Sorrentino V, Parys JB, De Smedt H. The conserved sites for the FK506-binding proteins in ryanodine receptors and inositol 1,4,5-trisphosphate receptors are structurally and functionally different. *J Biol Chem*. 2001;276:47715–47724.
47. Liu Z, Zhang J, Wang R, Wayne Chen SR, Wagenknecht T. Location of divergent region 2 on the three-dimensional structure of cardiac muscle ryanodine receptor/calcium release channel. *J Mol Biol*. 2004;338: 533–545.
48. Zhang J, Liu Z, Masumiya H, Wang R, Jiang D, Li F, Wagenknecht T, Chen SR. Three-dimensional localization of divergent region 3 of the ryanodine receptor to the clamp-shaped structures adjacent to the FKBP binding sites. *J Biol Chem*. 2003;278:14211–14218.
49. 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–e70.
50. Wehrens XH, Lehnart SE, Reiken SR, Deng SX, Vest JA, Cervantes D, Coromilas J, Landry DW, Marks AR. Protection from cardiac arrhythmia through ryanodine receptor-stabilizing protein calstabin2. *Science*. 2004; 304:292–296.
51. Pieske B, Maier LS, Bers DM, Hasenfuss G. $Ca2^+$ handling and sarcoplasmic reticulum $Ca2^+$ content in isolated failing and nonfailing human myocardium. *Circ Res*. 1999;85:38–46.
52. Marx SO, Reiken S, Hisamatsu Y, Gaburjakova M, Gaburjakova J, Yang YM, Rosemblit N, Marks AR. Phosphorylation-dependent regulation of ryanodine receptors: a novel role for leucine/isoleucine zippers. *J Cell Biol*. 2001;153:699–708.
53. Reiken S, Wehrens XH, Vest JA, Barbone A, Klotz S, Mancini D, Burkhoff D, Marks AR. Beta-blockers restore calcium release channel function and improve cardiac muscle performance in human heart failure. *Circulation*. 2003;107:2459–2466.
54. Reiken S, Lacampagne A, Zhou H, Kherani A, Lehnart SE, Ward C, Huang F, Gaburjakova M, Gaburjakova J, Rosemblit N, Warren MS, He KL, Yi GH, Wang J, Burkhoff D, Vassort G, Marks AR. PKA phosphorylation activates the calcium release channel (ryanodine receptor) in skeletal muscle: defective regulation in heart failure. *J Cell Biol*. 2003; 160:919–928.
55. Yano M, Kobayashi S, Kohno M, Doi M, Tokuhisa T, Okuda S, Suetsugu M, Hisaoka T, Obayashi M, Ohkusa T, Matsuzaki M. FKBP12.6-mediated stabilization of calcium-release channel (ryanodine receptor) as a novel therapeutic strategy against heart failure. *Circulation*. 2003;107: 477–484.
56. White M, Yanowitz F, Gilbert EM, Larrabee P, O'Connell JB, Anderson JL, Renlund D, Mealey P, Abraham WT, Bristow MR. Role of beta-adrenergic receptor downregulation in the peak exercise response in patients with heart failure due to idiopathic dilated cardiomyopathy. *Am J Cardiol*. 1995;76:1271–1276.
57. Serysheva II, Hamilton SL, Chiu W, Ludtke SJ. Structure of $Ca2^+$ release channel at 14 Å resolution. *J Mol Biol*. 2005;345:427–431.

KEY WORDS: Editorials ■ calcium ■ death, sudden ■ heart failure ■ sarcoplasmic reticulum

Defective Ryanodine Receptor Interdomain Interactions May Contribute to Intracellular Ca^{2+} Leak: A Novel Therapeutic Target in Heart Failure

Stephan E. Lehnart, Xander H.T. Wehrens and Andrew R. Marks

Circulation. 2005;111:3342-3346
doi: 10.1161/CIRCULATIONAHA.105.551861
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539