

## Progression of Heart Failure Is Protein Kinase A Hyperphosphorylation of the Ryanodine Receptor a Contributing Factor?

Andrew R. Marks, MD; Steven Reiken, PhD; Steven O. Marx, MD

The cardiac ryanodine receptor (RyR2)/Ca<sup>2+</sup> release channel on the sarcoplasmic reticulum (SR) is regulated by evolutionarily highly conserved signaling pathways that control excitation-contraction (EC) coupling in the heart. Phosphorylation of RyR2 by cAMP-dependent protein kinase (PKA) plays a key role in regulating the channel in response to stress via activation of the sympathetic nervous system (the “fight-or-flight response”).<sup>1</sup> Maladaptive PKA hyperphosphorylation of RyR2 in failing hearts alters channel function, which may cause depletion of SR Ca<sup>2+</sup> and diastolic release of SR Ca<sup>2+</sup>. This can initiate delayed afterdepolarizations that trigger ventricular arrhythmias.<sup>1</sup> Mutations in RyR2 recently have been identified in patients with catecholaminergic induced sudden cardiac death (SCD).<sup>2-4</sup> There may be a direct link between the PKA hyperphosphorylation of RyR2 that occurs during the progression of heart failure and fatal cardiac arrhythmias.

Regulation of cardiac EC coupling by the release of Ca<sup>2+</sup> from the SR via RyR2 in cardiomyocytes, known as Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release (CICR), has been appreciated for more than a decade.<sup>5,6</sup> Furthermore, it is well known that the amplitude of the Ca<sup>2+</sup> transient generated by SR Ca<sup>2+</sup> release determines contractile force in cardiomyocytes. The systems that regulate SR Ca<sup>2+</sup> release include: (1) the triggers (predominantly Ca<sup>2+</sup> influx through the voltage-gated Ca<sup>2+</sup> channel on the plasma membrane); (2) the SR Ca<sup>2+</sup> release channel or type 2 RyR2; and (3) the SR Ca<sup>2+</sup> reuptake pump (SERCA2a) and its regulator phospholamban. These systems (trigger, release, and reuptake) are modulated by signaling pathways, including the  $\beta$ -adrenergic receptor ( $\beta$ -AR) signaling pathway (ie, phosphorylation by PKA).

Activation of the sympathetic nervous system in response to stress results in elevation of cAMP levels and activation of PKA. Phosphorylation of RyR2 may not correlate directly with cellular cAMP levels, however. Rather, it is likely that local signaling via macromolecular complexes comprised of RyR2, kinases, and phosphatases determine the amount of PKA phosphorylation of the channel. We recently have

shown that the kinase (PKA) and phosphatases (protein phosphatase 1 [PP1] and protein phosphatase 2 [PP2A]) are targeted to RyR2 (Figure, A) with targeting proteins that bind via leucine/isoleucine zipper motifs in the channel.<sup>1,7-9</sup> These leucine/isoleucine zipper motifs are highly conserved throughout evolution, from insects to humans, indicating that the signaling pathways that regulate the channels via phosphorylation/dephosphorylation are extremely primitive and fundamental to survival. Indeed, our ability to evolve as a species is due in part to these highly conserved signaling pathways that convey survival advantages, in the present case by enabling an increase in cardiac output (via increased SR Ca<sup>2+</sup> release) in response to stress (fight-or-flight response).

Stimulation of the sympathetic nervous system results in phosphorylation of RyR2 by PKA and activation of the channel (Figure, B). PKA phosphorylation potentially modulates RyR2 function and is physiologically regulated in vivo.<sup>1,7-17</sup> PKA hyperphosphorylation of RyR2 in failing hearts shifts the sensitivity of RyR2 to CICR to the left,<sup>1</sup> resulting in “leaky” channels (channels with increased sensitivity to CICR) (Figure, C) that may cause diastolic Ca<sup>2+</sup> release, which generates delayed afterdepolarizations and triggers ventricular tachycardia (VT).<sup>7,8</sup>

Another effect of PKA hyperphosphorylation of RyR2s in failing hearts would be to functionally uncouple the channels from one another. RyR2s are arranged on the SR membrane in closely packed arrays such that their large cytoplasmic domains contact one another. We have shown that multiple RyR2s can be isolated under conditions such that they remain physically coupled to one another.<sup>18,19</sup> When these coupled channels are examined in planar lipid bilayers, multiple channels exhibit simultaneous gating, termed “coupled gating.”<sup>18,19</sup> Removal of the regulatory subunit, FKBP12.6, functionally but not physically uncouples multiple RyR2 channels.<sup>19</sup> Coupled gating between RyR2 channels may be an important regulatory mechanism in EC coupling as well as in other signaling pathways involving intracellular Ca<sup>2+</sup> release. This may have important implications for understanding the molecular pathophysiology of heart failure, in which PKA hyperphosphorylation of RyR2, which dissociates FKBP12.6, will inhibit coupled gating, thereby reducing EC coupling gain and promoting diastolic SR Ca<sup>2+</sup> leaks that can trigger fatal cardiac arrhythmias.

Recently, 11 RyR2 missense mutations have been linked to 2 inherited forms of SCD: (1) catecholaminergic polymorphic VT<sup>2</sup> or familial polymorphic VT<sup>3</sup>; and (2) arrhythmogenic right ventricular dysplasia type 2.<sup>4</sup> Interestingly, all 11 RyR2 mutations cluster into 3 regions of the channel that correspond to 3 malignant hyperthermia/central core disease mu-

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

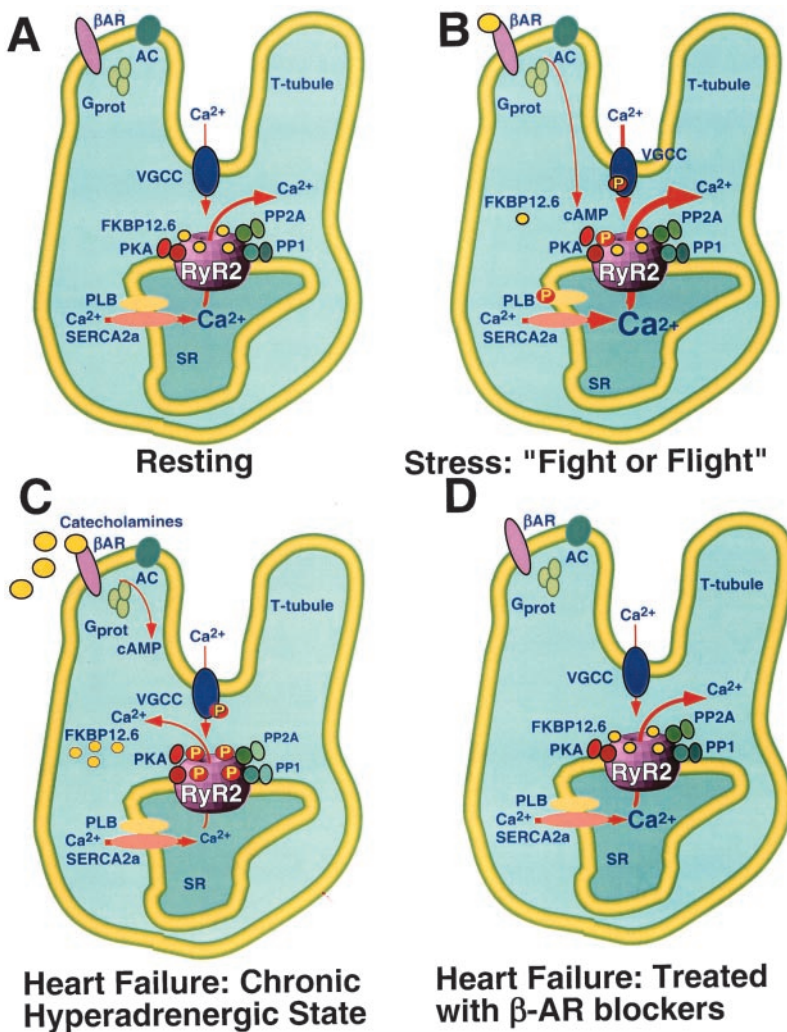
From the Center for Molecular Cardiology, Department of Pharmacology, Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY.

Correspondence to Andrew R. Marks, Center for Molecular Cardiology, Box 65, Columbia University College of Physicians & Surgeons, Rm 9-401, 630 W 168th St, New York, NY 10032. E-mail arm42@columbia.edu

(*Circulation* 2002;105:272-275.)

© 2002 American Heart Association, Inc.

*Circulation* is available at <http://www.circulationaha.org>



Modulation of cardiac EC coupling by PKA phosphorylation. A, Major structures required for cardiac EC coupling are illustrated. Action potential-mediated depolarization of transverse tubule (T-tubule) activates voltage-gated Ca<sup>2+</sup> channel (VGCC). Ca<sup>2+</sup> influx via VGCC activates RyR2 (SR Ca<sup>2+</sup>-release channel), which releases a large amount of Ca<sup>2+</sup> from the SR and raises concentration of Ca<sup>2+</sup> in cytoplasm from  $\approx 100$  nmol/L to  $\approx 1 \mu\text{mol/L}$ . Ca<sup>2+</sup> binds to troponin C, inducing a conformational change that activates muscle contraction. Ca<sup>2+</sup> is then pumped back into SR by Ca<sup>2+</sup>-ATPase (SERCA2a), which is inhibited by phospholamban (PLB). RyR2 macromolecular complex includes 4 RyR2s, and each RyR2 binds 1 FKBP12.6, as well as PKA catalytic and regulatory subunits (RII) and mA<sub>1</sub>KAP, PP2A and its targeting protein PR130, and PP1 and its targeting protein spinophilin (only 1 of the 4 of each of these proteins is shown except in panel C, in which 4 FKBP12.6 are shown).<sup>1,9</sup> Components of  $\beta$ -AR signaling pathway also are shown, including  $\beta$ -AR in plasma membrane, which activates adenylyl cyclase (AC) via G proteins (G<sub>prot</sub>). B, During fight-or-flight response, sympathetic nervous system is activated, releasing catecholamines into circulation that activate  $\beta$ -AR and elevate cAMP levels, which in turn activate PKA by causing release of PKA catalytic subunit from regulatory subunit.<sup>1</sup> PKA phosphorylates and activates (1) VGCC, thereby increasing Ca<sup>2+</sup> influx that activates RyR2; (2) RyR2, thereby increasing Ca<sup>2+</sup>-dependent activation and EC coupling gain; and (3) PLB, thereby releasing inhibition of SERCA2a and increasing SR Ca<sup>2+</sup> uptake. Increasing EC coupling gain increases cardiac output. C, In failing hearts, decreased cardiac function leads to chronic activation of fight-or-flight response (sympathetic nervous system). Because damaged heart cannot respond adequately with increased cardiac output, chronic hyperadrenergic state results, leading to PKA hyperphosphorylation of RyR2.<sup>1</sup> PKA

hyperphosphorylation reduces PP1 and PP2A levels in RyR2 complex and depletes FKBP12.6 from RyR2 complex, pathologically increasing Ca<sup>2+</sup>-dependent activation of RyR2 and resulting in depletion of SR Ca<sup>2+</sup> stores, uncoupling of RyR2 from each other (reducing EC coupling gain), and potentially providing diastolic SR Ca<sup>2+</sup> release that can activate depolarizations and trigger fatal ventricular cardiac arrhythmias. D,  $\beta$ -AR blockade restores FKBP12.6, PP1, and PP2A levels and RyR2 function to normal in failing hearts.<sup>24</sup>

tation regions of the highly homologous skeletal muscle ryanodine receptor/Ca<sup>2+</sup> release channel RyR1. Both RyR2 mutations linked to genetic forms of catecholaminergically induced VT, and PKA hyperphosphorylation of RyR2 in heart failure may alter the regulation of the channel, resulting in increased SR Ca<sup>2+</sup> leak that triggers SCD. RyR2 mutations linked to exercise-induced SCD may share common functional properties with RyR1 mutations linked to malignant hyperthermia and central core disease.

The fact that RyR2 mutations have been discovered in individuals with exercise-induced VT suggest an association between PKA phosphorylation of the channel, which is increased by activation of the sympathetic nervous system, and the defective channel function that predisposes to VT. Another intriguing possibility is that PKA phosphorylation of RyR2 exacerbates the defects in mutant RyR2 that are linked to exercise-induced VT.

There have been apparently contradictory findings showing that short-term treatment with caffeine, which activates

RyR2, fails to alter cardiac EC coupling<sup>20</sup> and that short-term administration of isoproterenol decreases Ca<sup>2+</sup> spark heterogeneity<sup>21</sup> or improves EC coupling in failing cardiomyocytes.<sup>22</sup> Despite the apparent contradictions, these findings from other groups are not at odds with our data showing PKA hyperphosphorylation, FKBP12.6 depletion, and increased Ca<sup>2+</sup> sensitivity of RyR2 in failing cardiomyocytes.<sup>1</sup> Because heart failure is a chronic disease, alterations in RyR2 structure and function in failing hearts persist for months or years.<sup>1,7</sup> The consequences of such long-term structural remodeling on RyR2 function are quite distinct from the effects of short-term administration of stimulatory compounds such as caffeine or isoproterenol. The short-term administration of drugs can transiently modulate RyR2 function, allowing other Ca<sup>2+</sup> handling molecules in the cell to restore homeostasis when RyR2 function returns to normal. In contrast, the chronic alteration of RyR2 structure and function that occurs in failing hearts can contribute to resetting SR Ca<sup>2+</sup> content at a lower level, in part because of increased leak through PKA-

hyperphosphorylated RyR2. This reduction in SR Ca<sup>2+</sup> content can contribute to reduced EC coupling gain.<sup>1,7</sup> Other alterations that occur in failing hearts, such as a decrease in SERCA2a expression and function or an increase in Na<sup>+</sup>/Ca<sup>2+</sup> exchanger, compound these changes as well (ie, by reducing the amount of Ca<sup>2+</sup> reuptake into the SR).

It is important to emphasize the distinction between short-term administration of isoproterenol versus the chronic hyperadrenergic state of heart failure. We have shown that in heart failure there is an alteration in the stoichiometry of the RyR2 macromolecular complex such that there is a reduction in the amount of phosphatases (PP1 and PP2A) and FKBP12.6 in the complex.<sup>1</sup> Moreover, the altered stoichiometry of the RyR2 macromolecular complex is associated with PKA hyperphosphorylation of RyR2 (an increase in the stoichiometry of PKA phosphorylation of the channel from ≈1 [normal] to ≈3.5 [heart failure] moles of phosphate per mole of RyR2).<sup>1</sup> It is unlikely that short-term administration of isoproterenol would have the same effects on Ca<sup>2+</sup> handling as would long-term exposure to the hyperadrenergic state of heart failure. For example, there might be PKA hyperphosphorylation of RyR2 (possibly only if phosphatase inhibitors are included) and some dissociation of FKBP12.6 from the channel, but probably not the decrease in phosphatases in the RyR2 macromolecular complex.

In a recent study, Litwin and colleagues<sup>21</sup> showed a slight decrease in EC coupling gain and demonstrated that isoproterenol (100 nmol/L) decreased the heterogeneity in Ca<sup>2+</sup> transients observed in a rabbit infarct model. The decrease in EC coupling gain in the infarcted heart is consistent with our data showing PKA hyperphosphorylation of RyR2, which we predict would lead to a reduction in SR Ca<sup>2+</sup> content (not documented by Litwin and colleagues,<sup>21</sup> but shown by others<sup>23</sup> in heart failure models, as opposed to infarct models), as well as uncoupling of coupled RyR2 channels.<sup>19</sup>

Critically important in these types of studies is that during isolation of the cardiomyocytes, there can be a restoration of normal function because of the ongoing activity of phosphatases in the heart in the absence of phosphatase inhibitors. Our data showing direct targeting of both PP1 and PP2A to RyR2<sup>1,9</sup> indicate that the phosphatases could be active during cardiomyocyte isolation and could dephosphorylate the channel once the cells were removed from the hyperadrenergic heart failure state in vivo. If this were the case, it would explain at least in part why the cells are responsive to isoproterenol and why there is only a modest decrease in EC coupling gain and no decrease in SR Ca<sup>2+</sup> content. In short, there may be a partial restoration of normal function in cardiomyocytes once they are removed from the heart failure milieu in the animal. Moreover, PKA phosphorylation of specific targets within the cardiomyocyte is compartmentalized such that some proteins (eg, RyR2) are PKA hyperphosphorylated in failing hearts, whereas other Ca<sup>2+</sup> handling proteins (eg, phospholamban) are hypophosphorylated in the same hearts (Marks et al, unpublished observation). Our recent data showing that PKA, PP1, and PP2A are specifically targeted to RyR2 via targeting proteins that bind to highly conserved leucine/isoleucine zippers<sup>1,9</sup> on the channel

provide strong support for the concept of compartmentalization of PKA signaling.

β-AR blockade is one of the most effective treatments for heart failure. However, the use of β-AR blockers in patients with heart failure is counterintuitive, inasmuch as they are known to decrease contractility in normal hearts. We have recently shown that systemic oral administration of a β-AR blocker reverses PKA hyperphosphorylation of RyR2, restores the stoichiometry of the RyR2 macromolecular complex, and normalizes single-channel function (Figure, D) in a canine model of heart failure.<sup>24</sup> These results may explain in part the improved cardiac function observed in heart failure patients treated with β-AR blockers.

In the past decade, elucidation of the molecular basis of cardiac EC coupling has led to a dramatic increase in our understanding of basic mechanisms that regulate cardiac function. Application of new knowledge to the problems of heart failure and cardiac arrhythmogenesis has yielded further insights that have important therapeutic implications. Complicating this new understanding have been the challenging problems of studying integrative physiology with reductionist models. Model systems are required because of the complexity of the cellular and organ physiology, which defy currently available experimental and theoretical tools. A better understanding of the potential role of PKA hyperphosphorylation of RyR2 in heart failure and its role in the generation of fatal cardiac arrhythmias may emerge from studying the biophysical properties of RyR2 mutations linked to catecholamine-induced ventricular arrhythmias. Integrating single-channel data with cellular and animal physiology and emerging with a unifying theory for a mechanism that causes heart failure and SCD will be a challenge, however. Nevertheless, elucidating the molecular pathogenesis of heart failure and VT will be the basis for strategies that lead to novel therapeutics.

## References

- 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.
- 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.
- Laitinen PJ, Brown KM, Piippo K, et al. Mutations of the cardiac ryanodine receptor (RyR2) gene in familial polymorphic ventricular tachycardia. *Circulation*. 2001;103:485–490.
- 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;10:189–194.
- Fabiato A. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *Am J Physiol*. 1983;245:C1–C14.
- Nabauer M, Callewart G, Cleeman L, et al. Regulation of calcium release is gated by calcium current, not gating charge, in cardiac myocytes. *Science*. 1989;244:800–803.
- Marks AR. Cardiac intracellular calcium release channels: role in heart failure. *Circ Res*. 2000;87:8–11.
- Marks AR. Ryanodine receptors/calcium release channels in heart failure and sudden cardiac death. *J Mol Cell Cardiol*. 2001;33:615–624.
- 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.
- Hain J, Onoue H, Mayrleitner M, et al. Phosphorylation modulates the function of the calcium release channel of sarcoplasmic reticulum from cardiac muscle. *J Biol Chem*. 1995;270:2074–2081.



11. Hohenegger M, Suko J. Phosphorylation of the purified cardiac ryanodine receptor by exogenous and endogenous protein kinases. *Biochem J*. 1993; 296:303–308.
12. Islam MS, Leibiger I, Leibiger B, et al. In situ activation of the type 2 ryanodine receptor in pancreatic beta cells requires cAMP-dependent phosphorylation. *Proc Natl Acad Sci U S A*. 1998;95:6145–6150.
13. Lokuta AJ, Rogers TB, Lederer WJ, et al. Modulation of cardiac ryanodine receptors of swine and rabbit by a phosphorylation-dephosphorylation mechanism. *J Phys*. 1995;487:609–622.
14. Takasago T, Imagawa T, Shigekawa M. Phosphorylation of the cardiac ryanodine receptor by cAMP-dependent protein kinase. *J Biochem*. 1989; 106:872–877.
15. Takasago T, Imagawa T, Furukawa K, et al. Regulation of the cardiac ryanodine receptor by protein kinase-dependent phosphorylation. *J Biochem*. 1991;109:163–170.
16. Valdivia HH, Kaplan JH, Ellis-Davies GC, et al. Rapid adaptation of cardiac ryanodine receptors: modulation by  $Mg^{2+}$  and phosphorylation. *Science*. 1995;267:1997–2000.
17. Yoshida A, Takahashi M, Imagawa T, et al. Phosphorylation of ryanodine receptors in rat myocytes during beta-adrenergic stimulation. *J Biochem*. 1992;111:186–190.
18. Marx SO, Ondrias K, Marks AR. Coupled gating between individual skeletal muscle  $Ca^{2+}$  release channels (ryanodine receptors). *Science*. 1998;281:818–821.
19. Marx SO, Gaburjakova J, Gaburjakova M, et al. Coupled gating between cardiac calcium release channels (ryanodine receptors). *Circ Res*. 2001; 88:1151–1158.
20. Trafford AW, Diaz ME, Sibbring GC, et al. Modulation of CICR has no maintained effect on systolic  $Ca^{2+}$ : simultaneous measurements of sarcoplasmic reticulum and sarcolemmal  $Ca^{2+}$  fluxes in rat ventricular myocytes. *J Physiol*. 2000;522(pt 2):259–270.
21. Litwin SE, Zhang D, Bridge JH. Dyssynchronous  $Ca^{2+}$  sparks in myocytes from infarcted hearts. *Circ Res*. 2000;87:1040–1047.
22. Gomez AM, Valdivia HH, Cheng H, et al. Defective excitation-contraction coupling in experimental cardiac hypertrophy and heart failure. *Science*. 1997;276:800–806.
23. 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.
24. Reiken S, Gaburjakova M, Gaburjakova J, et al. Beta-adrenergic receptor blockers restore cardiac calcium release channel (ryanodine receptor) structure and function in heart failure. *Circulation*. 2001;104:2843–2848.

---

KEY WORDS: Editorials ■ sarcoplasmic reticulum ■ calcium ■ excitation ■ contractility

## Progression of Heart Failure: Is Protein Kinase A Hyperphosphorylation of the Ryanodine Receptor a Contributing Factor?

Andrew R. Marks, Steven Reiken and Steven O. Marx

*Circulation*. 2002;105:272-275

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 2002 American Heart Association, Inc. All rights reserved.

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

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://circ.ahajournals.org/content/105/3/272>

**Permissions:** Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

**Reprints:** Information about reprints can be found online at:  
<http://www.lww.com/reprints>

**Subscriptions:** Information about subscribing to *Circulation* is online at:  
<http://circ.ahajournals.org/subscriptions/>