The Journal of Physiology

PERSPECTIVES

Complexity, confusion and controversy continue complicating the contribution of RyR2 channel phosphorylation to heart function

Peter H. Backx
Departments of Physiology and Medicine, Division of Cardiology, University Health Network, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Email: p.backx@utoronto.ca

Minute-to-minute fine-tuning of myocardial Ca^{2+} cycling is critical to ensure the appropriate pumping of blood by the working heart (Bers, 2014). To accomplish this task, the heart possesses an exquisitely complex, regulated excitation–Ca^{2+}–contraction system. A key component of this system is the physical proximity of (L-type) voltage-gated Ca^{2+} channel (LTCC) clusters, located in the transverse-tubule (T-tubule) invaginations of the excitable sarcolemma, immediately adjacent to even larger clusters of ryanodine receptors (RyR2) embedded in the cardiac sarcoplasmic reticular (SR) membrane. There are several thousand discrete groupings of these adjacent clusters referred to as 'couplons'. Sarcolemmal depolarization during each cardiac cycle initiates Ca^{2+} entry through open LTCCs which, in turn, catalyses the opening of SR RyR2 Ca^{2+} channels within the couplon thereby allowing the release of Ca^{2+} from the SR store into the cytosol. The localized Ca^{2+}-induced Ca^{2+}-release events within couplons are called Ca^{2+} sparks, which coalesce following membrane depolarization leading to a Ca^{2+} transient (i.e. an increase in free cytosolic $[Ca^{2+}]$ from ~100 nM to ~1 μM. In total the SR releases ~70 μM of Ca^{2+} which, after binding to troponin–C, initiates pressure generation and blood ejection by the heart. The Ca^{2+} released from the SR is, on average, returned to the SR by the SR Ca^{2+}-ATPase (SERCA2a-PLN) while the Ca^{2+} entering the cell via LTTCs is, on average, extruded from the cardiomyocyte via the Na^+/Ca^{2+} exchanger. One of the key feature of this excitation–contraction coupling (ECC) system is the ability to change dramatically the kinetics and amount of Ca^{2+} released from the SR in response to changes in demand for blood flow (as typically occurs with exercise) via sympathetic autonomic nerve activation. Early studies established that two key mechanisms for modulating ECC properties were phosphorylation of the LTCCs and the SR Ca^{2+}-ATPase by cAMP-dependent protein kinase A (PKA) which results in both elevated release of Ca^{2+} from the SR (by increasing the number of couplons activated and by enhancing the amount of Ca^{2+} released per couplon) and dramatic reductions in the duration of the Ca^{2+} transient (Bers, 2014).

Subsequent studies revealed that RyR2 properties also change in response to sympathetic stimulation as well as with other physiological and pathophysiological stimuli, although theoretical and experimental work suggested that these changes alone have minor direct effects on ECC and Ca^{2+} cycling (Eisner *et al.* 2009). The earliest, and most controversial, RyR2 regulatory mechanism proposed was a PKA-dependent 'hyper'-phosphorylation of RyR2 at position S2808 (2809 in humans; Marx *et al.* 2000). This mechanism contends that S2808 phosphorylation induces disassociation of the stabilizer FK-binding protein (FKBP)12.6 from RyR2 channels leading to destabilization of the RyR2 closed state and thereby enhanced SR Ca^{2+} leak. The cornerstone of this mechanism was a knockin mouse lacking a phosphorylation site at position S2808 (S2808A) in the *RYR2* gene. These mice are protected from myocardial-induced heart failure and have reduced ECC/contractility. All the critical predictions of the S2808 hypothesis have been challenged (Houser, 2010; Bers, 2012) and an article in this issue of *The Journal of Physiology* by Liu *et al.* (2014) not only adds to the litany of naysayers, but supports an antithesis. Specifically, when mice with a genetic form of dilated cardiomyopathy (resulting from calsequestrin haploid deficiency plus SERCA1 over-expression) are also heterozygous for the S2808A knockin mutation (i.e. have one S2808 and one A2808 allele), heart disease is accelerated in conjunction with increased SR Ca^{2+} leak, indicative of destabilized RyR2 channels. Importantly, mice lacking both S2808 phosphorylation sites (A2808–A2808) were less negatively affected than the S2808–A2808 mice, while treatment of S2808–S2808 cardiomyocytes with a phosphatase caused transitory increases in SR Ca^{2+} leak (Liu *et al.* 2014).

Though the relevance of Liu *et al.*'s mouse model to human heart disease can be questioned, the findings are compatible with a number of previous results. For example, baseline phosphorylation of S2808 appears to be upwards of ~80%, suggesting that S2808 phosphorylation might be necessary for normal structure and function of RyR2 channels. If correct, then the loss of S2808 phosphorylation might disrupt normal channel structure/function/stability as occurs in many inherited disease- and arrhythmia-causing RyR2 mutations. However, this might be overly simplistic since, as the authors demonstrate, intermediate levels of S2808 phosphorylation appear to produce the greatest degree of Ca^{2+} disregulation. The authors speculate, with supporting Ca^{2+} spark distributions, that a potentially relevant complicating factor in understanding the pattern of results is the regional heterogeneity of SR Ca^{2+} release expected from 'combinatorial variations in the monomer composition of individual RyR2 tetramers' when phosphorylation levels are intermediate. However, even if single channel RyR2 studies can identify variable properties of heterogeneously phosphorylated RyR2 channels, this concept still must be reconciled with the fact that couplons typically contain many (15 to upwards of several hundred) RyR2 channels, making stochastic regional heterogeneity improbable.

The results of Liu *et al.* (2014) provide some provocative new concepts to consider regarding the role of S2808 phosphorylation, as well as other phosphorylation sites, in RyR2 function and regulation. Some immediate questions might be whether S2808–A2808 mice also fare poorly in other forms of heart disease or how the effects of phosphorylation heterogeneity are influenced by RyR2 phosphorylation sites, and vice versa. The new data further suggest that any process generating heterogeneity of RyR2 monomer phosphorylation, regardless of the site, may also destabilize RyR2 channels; such a process could conceivably be a factor in the post-exercise period when there is routinely increased triggered activity

 DOI: 10.1113/jphysiol.2014.272575

and arrhythmia vulnerability. Finally, the results of Liu *et al.* illustrate the need to pay careful attention to the level and dynamics of channel phosphorylation when performing RyR2 studies and suggest that variable phosphorylation underlies the previous discrepancies between RyR2 studies.

References

Bers DM (2012). Ryanodine receptor S2808 phosphorylation in heart failure: smoking gun or red herring. *Circ Res* **110**, 796–799.

Bers DM (2014). Cardiac sarcoplasmic reticulum calcium leak: basis and roles in cardiac dysfunction. *Annu Rev Physiol* **76**, 107–127.

Eisner DA, Kashimura T, O'Neill SC, Venetucci LA & Trafford AW (2009). What role does modulation of the ryanodine receptor play in cardiac inotropy and arrhythmogenesis? *J Mol Cell Cardiol* **46**, 474–481.

Houser SR (2010). Does protein kinase A-mediated phosphorylation of the cardiac ryanodine receptor play any role in adrenergic regulation of calcium handling in health and disease? *Circ Res* **106**, 1672–1674.

Liu B, Ho H-T, Velez-Cortes F, Lou Q, Valdivia C, Knollmann B, Valdivia H & Gyorke S (2014). Genetic ablation of ryanodine receptor 2 phosphorylation at Ser-2808 aggravates Ca^{2+}-dependent cardiomyopathy by exacerbating diastolic Ca^{2+} release. *J Physiol* **592**, 1957–1973.

Marx SO, Reiken S, Hisamatsu Y, Jayaraman T, Burkhoff D, Rosemblit N & Marks AR (2000). PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* **101**, 365–376.