Does Protein Kinase A–Mediated Phosphorylation of the Cardiac Ryanodine Receptor Play Any Role in Adrenergic Regulation of Calcium Handling in Health and Disease?

Steven R. Houser

Cardiac myocyte ryanodine receptors (sarcoplasmic reticulum [SR] Ca release channel; cardiac ryanodine receptor [RyR2]) are localized in the junctional SR, in close proximity to L-type Ca channels (LTCCs) embedded in the membranes of the transverse (T)-tubules. This signaling microdomain has been termed the couplon, because it is here that excitation–contraction coupling takes place. As the heart fills with blood during diastole, RyR2 is stabilized in a closed state, allowing Ca uptake by the SR Ca ATPase to pump Ca from the cytoplasm into the SR, providing the primary source of Ca to activate the contractile apparatus during the next heart beat (systole). During systole, the cardiac action potential depolarizes the T-tubules and causes the opening of LTCCs. Ca influx through LTCCs elevates the [Ca] within the cytoplasmic space between the T-tubular and SR membrane, which promotes Ca binding to neighboring RyR2s, inducing some to open. Ca then moves out of the SR lumen into the subsarcolemmal space and the additional elevation of [Ca] induces a regenerative opening of other RyRs in the couplon. The resulting localized Ca signal is called a Ca spark. The action potential synchronizes these local Ca release events throughout the cell (termed Ca-induced Ca release) to produce the global cardiac [Ca] transient. The stabilized closed state and Ca-dependent opening of RyR2 during diastole and systole are essential for normal cardiac diastolic and systolic function. Destabilizing the behavior of RyR2 would be expected to have a negative impact on the function of the heart.

Approximately 10 years ago, the Marks laboratory reported that protein kinase (PK)A-mediated (hyper)phosphorylation of RyR2 at S2809 in the failing heart caused increased RyR Ca sensitivity and abnormal channel activity. Based on a series of provocative experiments, this group suggested that PKA-mediated RyR2-S2809 phosphorylation is involved in the normal increase in cardiac contractility produced by sympathetic stimulation (one example would be to increase cardiac contractility during exercise). They also proposed that in severe pathological stress, persistent adrenergic activity caused RyR2-S2809 hyperphosphorylation and destabilization of RyR2 function, resulting in diastolic leakage of Ca from the SR and a propensity for arrhythmias. The mechanism for these effects was shown to involve hyperphosphorylation of RyR2 at S2809, which leads to FK-binding protein (FKBP)12.6 (normally bound to RyR) and proposed as a stabilizer of its function) dissociation from RyR, resulting in abnormal RyR activity. The RyR-S2809 hyperphosphorylation and loss of the putative FKBP12.6-stabilizing effect appeared to be critical to the development and progression of heart failure, because prevention of these processes significantly improved cardiac function after myocardial infarction and reduced stress-related arrhythmias. As would be expected, these reports sparked a host of studies to confirm the original observations and further define the role of RyR2 in heart failure and life threatening arrhythmias. Some of these studies have not been able to confirm critical features of the original RyR-S2809 hyperphosphorylation hypothesis.

In this issue of Circulation Research, the Bers laboratory refutes an essential element of the PKA RyR-S2809 hyperphosphorylation hypothesis. Guo et al specifically explored the portion of the hypothesis that predicts that PKA hyperphosphorylation of RyR2 at S2809 causes FKBP12.6 to dissociate from RyR2 and thereby produce a leaky SR. In a superb series of experiments in cardiac myocytes, these authors show that FKBP12.6 (the putative RyR2 stabilizer) is found in much lower abundance than RyR. Therefore, FKBP12.6 was only bound to a small percentage (∼15%) of RyRs. Importantly, Guo et al convincingly demonstrate that FKBP12.6 does not dissociate from RyR2 with PKA-mediated phosphorylation of RyR-S2809. They also show that FKBP12 is more abundant than FKBP12.6, is tightly bound to RyR2, does not dissociate with PKA phosphorylation of RyR2, and does not affect RyR2 function. To me, these are definitive experiments. The authors should be commended because they developed the novel, quantitative techniques to assess FKBP12.6- and FKBP12-RyR binding. All of their results support the conclusion that FKBP12.6 binding to RyR is not influenced by PKA phosphorylation of RyR-S2809. A particular strength of this new study is that the investigators developed techniques that allowed RyR binding and unbinding of FKBP12.6 and FKBP12 to be directly visualized over time, in real cardiac myocytes, in the absence and presence of PKA activators. The quantitative approaches developed for these experiments were much more rigorous than have been used previously.

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leaves little doubt that PKA-mediated RYR phosphorylation at S2809 does not cause FKBP12.6 (or FKBP12) to dissociate from RyR2. These results are consistent with those published by others.25 It is also noteworthy that the Bers group11,12 and others18 have previously shown that PKA-mediated hyperphosphorylation of RyR-S2809 plays no role in SR Ca leak; therefore, in some respects, the results of Guo et al23 are not surprising. Our group24 has also shown that PKA phosphorylation of RyR at S2809 makes no contribution to normal sympathetic regulation of cardiac function. These and many other reports11–24 question the validity of the RyR2-S2809 hyperphosphorylation-FKBP12.6 hypothesis.

One of the results of the study by Guo et al23 leaves open the possibility that FKBP12.6 binding to RyR2 might play a small role in regulation RyR2 activity in normal and diseased cardiac myocytes. In this regard, Guo et al were able to show that FKBP12.6 binding to RyR2 reduces channel activity (FKBP12 had no effect) by a small amount, explaining results of some previous experiments in which the expression of FKBP12.6 was increased.25,26 Collectively, these results suggest that changes in FKBP12.6 abundance would alter the number of RyR-FKBP12.6 complexes, and this might have some small physiological or pathological relevance. However, if Guo et al are correct, that FKBP12.6 is in relatively low abundance in normal myocytes (bound to less than 15% of RyR2), it is difficult to see how FKBP12.6 could be a critical RyR2 stabilizer, because most RyRs in the normal myocyte would not have bound FKBP12.6, and excitation–contraction coupling and diastolic Ca regulation in these myocytes are very stable.

To this reader, much of the data in the literature that have tested the RyR2-S2809 hyperphosphorylation hypothesis are mutually exclusive. The experiments by Guo et al23 are a key example. These new studies show that PKA-mediated hyperphosphorylation of RyR2-S2809 does not dissociate FKBP12.6 from RyR2, although the data from the Marks laboratory4–10 show robust dissociation. I see no technical explanation for the differences, and both results cannot be correct. To this investigator, there are now much more data refuting than supporting the hypothesis that PKA-mediated RyR-S2809 phosphorylation is a critical regulator of RyR2 function in health and disease. This opinion is not meant to downplay the potential role of abnormal RyR function in the disturbed Ca handling of the failing heart or in lethal cardiac arrhythmias.27 There is compelling evidence from the Bers laboratories that calcium/calmodulin kinase (CaMKII)-mediated RyR2 phosphorylation is responsible for SR Ca leak.11–13 There are also data suggesting that both PKA- and CaMKII-mediated RyR phosphorylation are both necessary for abnormal RyR2 behavior.28 Given the clinical significance of this topic, there is a need to continue to advance this field.

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