What Are the Consequences of Phosphorylation and Hyperphosphorylation of Ryanodine Receptors in Normal and Failing Heart?

John H.B. Bridge, Eleonora Savio-Galimberti

Marks and colleagues published some influential results in 2000, suggesting that protein kinase A phosphorylation regulates ryanodine receptor (RyR2) open probability (P0\text{sq}). The authors attributed this to the dissociation of the RyR-associated regulatory protein FKBP12.6. They went on to propose that, in failing hearts, RyR2 is hyperphosphorylated, which leads to defective channel function by increasing sensitivity to Ca-induced activation of the molecule. Moreover, these effects were attributable to phosphorylation of a single amino acid, serine 2809 (S2809). There are 4 of these serine molecules at location 2809 in the human RyR2 molecule, 1 on each subunit of the tetramer. Much subsequent work has referred to the 2809 residue, which is found in rabbits. Marks and colleagues further suggested that phosphorylation of S2809 was also responsible for the normal effects of adrenergic stimulation on excitation–contraction coupling (ECC) and that hyperphosphorylation was responsible for abnormal ECC during failure.

The RyR is a tetramer that forms the largest membrane ion channel. In muscle, it is found in the sarcoplasmic reticulum (SR), where it functions as the Ca release channel. The heart isoform is designated RyR2. The pore sequences are largely found in membrane spanning domains. There is a large cytosolic domain. Although hyperphosphorylation refers, by definition, to the occupation of all the putative phosphorylation sites in a molecule in the context of the work by Marks and colleagues, hyperphosphorylation refers to 3 or perhaps 4 of the serine residues. In RyRs, there are a large number of consensus phosphorylation sites. RyRs are associated with a number of proteins, and these include anchoring protein phosphatases and kinases, as well as the regulatory protein FKBP12.6. From the standpoint of this editorial, this last protein is perhaps the most interesting, and it is considered to be responsible for stabilizing the state of conductance of the molecule. It is plausible that phosphorylation of RyRs is essential for their function. However, the proposal that phosphorylation of Serine2808/9 is responsible for much of the regulation of RyR2, as well as providing a basis for the effects of the adrenergic stimulation in health and disease, has come under some criticism.

Benkusky et al. were the first to challenge the proposals of Marks and colleagues. Benkusky et al. generated a knock-in mouse model in which serine 2808 was replaced by an alanine (S2808A). They evaluated the \( \beta \)-adrenergic responses of these mice in both isolated cells and Langendorff preparations. These included Ca transients and sparks, which the authors measured in isolated cells and Ca currents in bilayers. In Langendorff preparation, they measured parameters such as developed pressure. Although these authors do not dispute the response to RyR2 phosphorylation as a mechanism to respond to chronic stress, their results failed to support the idea that the phosphorylation that S2808 significantly impacts maladaptive remodeling following chronic stress. Lehnart and Marks criticized these results on a number of grounds, including the fact that many of physiological measurements did not adequately take into consideration such important physiological determinants as temperature and pacing rate. They also pointed out that Ca transients alone are not a particularly good indication of whether or not ECC has been affected in the knock in. They suggested that a more direct measure of RyR2 function after adrenergic stimulation and its effect on ECC is the gain function, which is essentially the Ca transient (eg, rate of upstroke) divided by the Ca current that triggers it.

In a recent issue of Circulation Research, MacDonnell et al challenge the idea that the regulation of cardiac contractility by adrenergic stimulation involves phosphorylation of the RyRs at S2808/9. They used a knock-in mouse in which S2808 was replaced with an alanine (S2808A), which, in theory, cannot be phosphorylated. In this study, MacDonnell et al have carefully compared the responses of both wild-type and S2808A mice to a number of carefully measured physiological parameters during both normal and adrenergic stimulation. In vivo studies using echocardiography suggested that the effect of isoproterenol (ISO) on heart rate and ejection fraction were similar in wild-type and S2808A knock-in mice. Again, in isolated heart studies, there were no differences between wild type and mutants with respect to heart rate, magnitude of generated pressure and its rate of decay all of which were increased in a similar manner by ISO. These authors recognized that by eliminating an essential residue from RyR2 that can be phosphorylated an adaptive response may result. This could increase the abundance of other proteins essential to ECC. These include L-type Ca channels, Na/Ca exchanger (NCX), and SERCA. However, no differences in the abundance of these proteins could be.

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detected by Western blot analysis. Most importantly, these authors measured key parameters associated with ECC. These include the characteristics of L-type Ca currents: SR Ca release and ECC gain. It appears that the density and voltage-dependent inactivation of L-type Ca current did not differ between wild-type and knock-in mice. Moreover, these quantities were increased to the same extent when exposed to ISO. Among other things, the amplitude of triggered cytosolic Ca transients in wild-type and mutant mice when treated with ISO increased to the same extent. Finally, ECC gain was calculated at each voltage step in wild-type and knock-in mice under basal conditions and in the presence of ISO. Changes in ECC gain were identical. It may be objected that the similarity in gains between wild-type and knock-in mice were spurious. Thus, one could imagine the Ca transient and Ca current were proportionally increased by ISO in, for example, the wild type in such a way that the gain remained unchanged. However, the results from MacDonnell clearly indicate that this is not the case. In the light of these results, it is rather difficult to accept that phosphorylation of serine 2808 can explain the inotropic responses of heart to β-adrenergic stimulation. A caveat to this conclusion is that phosphorylation of some other undisclosed phosphorylation epitopes could explain these results. However, it is difficult to see how this could occur without manifesting its effects on Ca release and gain. It remains interesting that Marks and colleagues observed hyperphosphorylation of RyR2 in heart failure. It would be of interest to see whether or not mouse failure models that are either wild type or mutated show significant differences.

There is little doubt that β-adrenergic stimulation of heart cells and tissue profoundly affects ECC. It does so by a number of well-documented mechanisms. These include an increase in the magnitude of $I_{c, o}$, which is a significant component of the trigger, and increase in SERCA activity, which will tend to increase SR Ca content. Depending on the extent of adrenergic stimulation, there may be a transient or permanently increased change in SR Ca content. MacDonnell et al and a number of studies from various independent laboratories indicate that protein kinase A–mediated phosphorylation of RyRs has no significant effect on RyR gating.

At this point, it is worth considering some mechanisms that may govern Ca homeostasis in heart cells, particularly during adrenergic stimulation. If, by any mechanism, $P_{oRyR}$ is increased, it will lead to an increased release flux of Ca, which will of course lead to increased contraction. With increased trigger flux resulting from adrenergic stimulation, this effect could be quite significant. However, if this increase in SR content ultimately stabilizes, it must do so when net transsarcolemmal influx and efflux are matched. The increased SR release flux will stimulate increased extrusion by NCX, and this process will continue until transmembrane fluxes again come into balance. It, therefore, seems that an increase in $P_{oRyR}$ is essential for an inotropic response to ISO. However, 2 facts should be considered. The first is that SR luminal proteins including calsequestrin can regulate $P_{o}$. Secondly, the relationship between SR content and Ca release is extremely steep. Thus, small changes in SR Ca content can produce quite large changes in release fluxes and this constitutes part of the homeostatic mechanisms that control SR Ca content proposed by Eisner and colleagues. However, if release flux is increased and that released Ca is extruded by NCX it will reduce SR Ca content. This will have a secondary effect of reducing release flux and therefore the extent of Ca extrusion by NCX. Therefore, there will be a flux imbalance. With Ca influx unchanged or augmented there will be a net gain of Ca which will restore SR Ca content. Therefore it appears that increases in $P_{o}$ alone are likely to produce transitory increases in SR content, as MacDonnell et al acknowledged. Extreme effects of adrenergic stimulation will overcome these homeostatic mechanisms in which SR Ca content will increase uncontrollably and lead to the spontaneous and possibly arrhythmogenic release of SR Ca.

MacDonnell et al voice additional concerns. It appears that a Ca leak seems to be characteristic of certain forms of heart failure, and this too may be a consequence of hyperphosphorylation. The first issue concerns the mechanism by which hyperphosphorylation produces increases in $P_{oRyR}$. Wehrens and Marks have suggested this arises because the stabilizing protein FKBP12.6 dissociates from RyRs, which, in turn, produces an increase in $P_{oRyR}$. A number of groups, however, have suggested that this dissociation of the FKBP12.6 does not influence $P_{oRyR}$ (Xiao et al.). However, this issue remains controversial. A second problem is that if indeed $P_{o}$ does increase and induces a leak, Wehrens and Marks have suggested that this would lead to a decline in SR Ca content. The biophysical consequences of this leak are rather difficult to predict. However, in view of the extensive theory on Ca homeostasis worked out by Eisner’s group, this seems unlikely. An increase in leak will initially reduce the SR content, which, in turn, will reduce release flux and subsequently Ca extrusion. Again, SR content will be restored by the mismatched transsarcolemmal Ca fluxes. On the other hand, if SR content declines in heart failure as a result of some defect in the ability of the SR to maintain its Ca load, this can only be stabilized when influx matches efflux. It is here that the diastolic Ca leak may compensate the reduced release flux and subsequent Ca extrusion, so that fluxes remain in balance. The latter point has been argued by Bridge and Savio.

In summary, it seems clear from the results of MacDonnell et al that the effects of phosphorylation on RyR2 do not require the existence of a serine residue and that it is unlikely that the physiological responses of ECC to adrenergic stimulation can be explained by only phosphorylation of 1 or more serine residues. It will be of considerable interest to learn the consequences of prolonged adrenergic stimulation in the mouse knock-in S2808/9A because this may resolve some of the disagreements that surround the existence and consequences of hyperphosphorylating RyR2.

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References


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