Mechanisms of ryanodine receptor 2 dysfunction in heart failure

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In the Review by Dridi and colleagues (Intracellular calcium leak in heart failure and atrial fibrillation: a unifying mechanism and therapeutic target. *Nat. Rev. Cardiol.* https://doi.org/10.1038/s41569-020-0394-8 (2020))[1], the authors discuss the role of Ca^{2+} leak via cardiac ryanodine receptor 2 (RYR2) as a shared pathological mechanism in heart failure and atrial fibrillation. We agree with the authors' overall conclusion that Ca^{2+} leak from the sarcoplasmic reticulum is a key trigger of cardiac arrhythmia but disagree with their interpretation of the underlying mechanisms.

The authors propose a simplified mechanism, in which cAMP-dependent protein kinase (PKA)-mediated phosphorylation of RYR2 at Ser2808 is increased in heart failure and atrial fibrillation, 'hyperphosphorylation' of Ser2808 dissociates the FK506-binding protein 12.6 (FKBP12.6) subunit (called 'calstabin 2' almost exclusively by the authors), FKBP12.6-dissociated RYR2 becomes dysfunctional, and RYR2-mediated Ca^{2+} leak promotes arrhythmia and impairs contractility[1]. This mechanism has been advanced by the authors not only in heart failure but also in inherited arrhythmias, muscular dystrophy, seizures, cognitive dysfunction and diabetes mellitus (reviewed previously[2]).

Although the authors address some controversies, they do not acknowledge that every component of their hypothesis is challenged by an increasing number of equally compelling studies from multiple laboratories. For example, the claim that Ser2808 is the only PKA phosphorylation site in RYR2 is based on their study in which recombinant RYR2-Ser2808Ala channels treated with PKA are not phosphorylated, whereas RYR2-Ser2030Ala channels, which lack another proposed PKA phosphorylation site, are unaffected[3]. These results are not independently reproducible: other groups have shown that PKA also phosphorylates RYR2 at Ser2030 in vitro and during the normal β-adrenergic response[4], and ablation of cardiac PKA prevents phosphorylation of RYR2 at both Ser2808 and Ser2030 in isoprenaline-treated hearts[5]. Moreover, transgenic mice expressing RYR2-Ser2030Ala have an incomplete β-adrenergic response[6]. Although the role of Ser2030 in regulating RYR2 function in normal and diseased hearts is incompletely understood, the evidence does not allow a potential role to be disregarded, which the authors do.

The authors further claim that RYR2-Ser2808Ala mice generated in their laboratory have a blunted β-adrenergic response and slowed progression to heart failure and that a RYR2-Ser2808Ala mouse generated in our laboratory supports their hypothesis. This statement is incorrect. We acknowledge that our RYR2-Ser2808Ala mouse has a slightly preserved fractional shortening after transverse aortic constriction[7], but all other indicators of cardiac remodelling are not significantly different from control mice. Therefore, this single observation isolated from all other data does not lend validation to the authors' hypothesis. Overall, our RYR2-Ser2808Ala mouse has a normal β-adrenergic response[8] and similar progression to heart failure to that of control mice following transverse aortic constriction[7] or myocardial infarction[9]. The results are the same even in RYR2-Ser2808Ala mice of different genetic backgrounds[10].

The authors explain that the multiple exposed cysteine residues in RYR2 mean that oxidation of the channel could be partially responsible for the discrepancies between experimental outcomes. However, the authors disregard the multiple predicted phosphorylation sites in this ~5,000-residue protein and minimize the problem to a single amino acid in a phosphorylation 'hotspot', next to a Ca^{2+}/calmodulin-dependent protein kinase II phosphorylation site (Ser2814) and two other possible phosphorylation sites (Thr2810 and Ser2811).

The overall mechanism proposed by Dridi and colleagues, although compelling, is not widely accepted in the field, and multiple disagreements remain to be addressed. Therefore, we invite readers to explore the underlying literature critically and to make their own objective inferences. We also encourage researchers in the field to continue addressing these issues in the laboratory, because experimental validation by independent research groups is paramount to furthering our knowledge about RYR2 regulation. The reagents developed in our laboratory to address these issues are freely available to all interested researchers.

There is a reply to this letter by Dridi, H. et al. *Nat. Rev. Cardiol.* https://doi.org/10.1038/s41569-020-00444-w (2020).

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Competing interests
The authors declare no competing interests.