Calmodulin: a gatekeeper for ryanodine receptor function in the myocardium

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This editorial refers to ‘Dissociation of calmodulin from cardiac ryanodine receptor causes aberrant Ca$^{2+}$ release in heart failure’ by M. Ono et al., doi:10.1093/cvr/cvq108

The ryanodine receptor (RyR) releases intracellular Ca$^{2+}$ from the sarcoplasmic reticulum (SR) of beating cardiomyocytes following Ca$^{2+}$ influx via the voltage-dependent sarcolemmal Ca$^{2+}$ channel. This crucial cellular event, named ‘Ca$^{2+}$-induced Ca$^{2+}$ release’ and discovered three decades ago by Fabiato and Fabiato, is mandatory for the rhythmic contraction as well as for maintaining the force–frequency relationship of the myocardium. RyR type 1 (RyR1), cardiac RyR2, and RyR3 have been the subject of intensive investigation for many years. More recently, Ikemoto and Yamamoto provided evidence of structure–function relationships in the RyRs. They reported that the N-terminal and central domains of RyRs form an interacting domain pair. Unzipping and zipping actions of such a domain pair determine the opening and closing probabilities of the RyR Ca$^{2+}$ channel, respectively. A deregulation of this domain pair leads to a defect in heart function. Unzipping the N-terminal from the central domain leads to RyR2 hyperactivation and Ca$^{2+}$ leak from the SR, ultimately causing heart failure. Mutations in the three domains of RyR2 were reported to cause defective inter-domain interactions and, in turn, abnormal Ca$^{2+}$ cycling, leading to severe heart failure and lethal arrhythmia.

Although a functional defect in RyR2 is associated with Ca$^{2+}$ leak from the SR, a hyperactive RyR2 increasing its opening probability following phosphorylation by PKA or Ca$^{2+}$/calmodulin (CaM)-dependent kinase leads to arrhythmogenesis. A regular PKA-dependent phosphorylation leads to a positive inotropic effect. In contrast, the mechanism of the adverse effect of PKA-induced hyperphosphorylation of the channel was elucidated a few years ago. Such a phosphorylation dissociates the channel-stabilizing protein calstabin 2 or FKBP12.6, triggering a Ca$^{2+}$ leak.

Although CaM, a Ca$^{2+}$-binding protein, was the first protein discovered to interact with RyR2, its mode of action in both normal and pathological situations remains unclear. CaM, acting directly by binding to the RyR2 at residues 3583–3603, inhibits RyR2 both at physiological and higher, pathological Ca$^{2+}$ concentrations.

A recent report from Meissner’s laboratory revealed that a mouse with a mutation in the CaM-binding domain of RyR2, located between the switch domain and Ca$^{2+}$ channel, develops severe hypertrophic cardiomyopathy. As RyR2 defects in Ca$^{2+}$ transport function are at the crossroad of cardiac genetic and acquired diseases, this raises a still unanswered question as to the status of CaM binding to RyR2 in these diseases.

Ono et al. investigated the potential role of CaM on abnormal Ca$^{2+}$ leak in heart failure. The authors used rapid ventricular cardiac pacing as a means of inducing heart failure in the dog, a reliable and well-established animal model. They isolated SR and cardiomyocytes from both diseased and control myocardium. Using both the CaM-SANPH cross-linking technique on RyR2 immunoblots and the CaM-alexa Fuor488-binding test in saponin-permeabilized isolated cardiomyocytes, they found that the affinity of CaM binding to RyR2 was dramatically decreased in failing hearts when compared with healthy hearts. This observation points indeed to a crucial role of CaM bound to SR RyR2 as a gatekeeper of the Ca$^{2+}$ channel.

To investigate the role of CaM in Ca$^{2+}$ release from the SR, they monitored the frequency of Ca$^{2+}$ sparks in saponin-permeabilized isolated cardiomyocytes. They found a higher spark frequency in cells isolated from failing heart than in those from healthy hearts. The difference between the two experimental models was reversed by adding saturating CaM or dantrolene.

Next, they carried out mechanistic experiments (i.e. labelling the N-terminal domain of RyR2 by DPc10) to test the effect of CaM on the interaction mode between the central and N-terminal domains. Dantrolene, targeting the domain pair, but not CaM was able to switch the unzipped domain pair to a zipped configuration in both FK506-treated normal SR and SR from failing heart. The authors thus concluded that CaM restored the function of RyR without affecting the configuration of the domain pair.

There is still an important question that has not been addressed in the study of Ono et al. CaM also activates RyR function through activation of CaM kinase and subsequent phosphorylation of the channel. Although the authors used KN93 to inhibit the kinase, they did not look at the phosphorylated status of RyR2, which might modulate CaM affinity of the receptor or/and the configuration of the domain pair. Interestingly, a recent report from Andrew Marks’ laboratory shows that CaM kinase δ-induced phosphorylation of RyR is crucial for the maintenance of the force–frequency relationship in the heart.

Matsuzaki and colleagues previously demonstrated that a tight interaction between the N-terminal and central domain (zipped...
configuration) was required to stabilize the RyR Ca$^{2+}$ channel and that this interaction, lost in heart failure (unzipped configuration), could be reversed by both K201 and dantrolene. Ono et al. (the same team) have now added one more step in the understanding of abnormal Ca$^{2+}$ release (i.e. leak visualized by spontaneous sparks) from the SR in the failing heart. They uncovered that CaM, bound or unbound to RyR, is at the crossroad between normality and disease and that the switch between the two states depends not only on the zipped or unzipped configuration of the domain pair of RyR. Thus, restoration of CaM binding to RyR2 or a reversal of the unzipped into the zipped configuration of the domain pair of RyR2 or both might be potent therapeutic approaches in heart failure.

Conflict of interest: none declared.

References
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