Increases in intracellular Ca$^{2+}$ are crucial signaling events in many cell types. The cardiac isoform of the (sarc)endoplasmic reticulum Ca$^{2+}$ release channel or ryanodine receptor (RyR2) is an important component of this signaling pathway in a wide variety of both excitable (nerve, smooth muscle, and heart) and nonexcitable (parotid, pancreas, and adrenal medulla) cells and is a critical component of excitation-contraction coupling in the heart.1,2 The absence of RyR2 in knockout mice leads to an early embryonic lethal phenotype because its function is essential for regulation of the intrinsic beating rate, and this early lethality has prevented studying its absence in other cell types.3

Unlike skeletal muscle, where excitation-contraction coupling is mediated through a mechanical coupling between its RyR isoform, RyR1, and the skeletal isoform of the sarcoplasmic slow voltage-gated Ca$^{2+}$ channel (dihydropyridine receptor, DHPR), in cardiac muscle, Ca$^{2+}$ release through RyR2 is caused by the inward Ca$^{2+}$ flux through the cardiac DHPR via Ca$^{2+}$-induced Ca$^{2+}$ release (CICR). It also appears that, at least in heart, RyR2 is part of a larger macromolecular complex containing phosphorylases, phosphatases, and the immunophilin FKBP12.6, which regulate the level of CICR.4,5

Because of its large size (>200-kB gene, ~15-kB mRNA), it is not surprising that the ryanodine receptor is a likely target for mutation. There are >30 reported missense mutations in the RyR1 gene that have been associated with alterations of Ca$^{2+}$ homeostasis and are the cause of central core disease (CCD) and malignant hyperthermia (MH).6–10 More recently, 11 missense mutations have been associated with a group of closely associated cardiomyopathies that are characterized by early sudden death: arrhythmogenic right ventricular cardiomyopathy (ARVD2), familial polymorphic ventricular tachycardia, and catecholaminergic polymorphic ventricular tachycardia.9,11,12 Interestingly, the RyR2 mutations associated with cardiomyopathies are clustered in the same hot spots as the RyR1 mutations associated with MH and CCD (see Figure). These skeletal RyR channelopathies are associated with high resting myoplasmic Ca$^{2+}$, increased sensitivity to caffeine and halothane, reduced internal Ca$^{2+}$ stores, and a reduced sensitivity to Ca$^{2+}$ and Mg$^{2+}$ inhibition.13,14 This has led to the hypothesis that the cardiac RyR channelopathies are likely to result in an increased diastolic Ca$^{2+}$ and potential arrhythmogenic Ca$^{2+}$ waves.

In this issue of Circulation Research, Jiang et al.15 report on the possible mechanism for catecholaminergic polymorphic ventricular tachycardia, examining the biophysics of heterologously expressed RyR2 channels carrying one of the reported clinical mutations. The authors demonstrate that substitution of an arginine at position 4496 with either a neutral (A) polar (C) or negatively charged (E) amino acid progressively increased the open probability of RyR2 at low Ca$^{2+}$ concentrations. The clinical mutation R4496C was also shown to induce a higher frequency of spontaneous Ca$^{2+}$ waves in transfected cells than wild type. This suggests the possibility that the mutated channels increased activity of the channel during diastole in the heart and increased the Ca$^{2+}$ load thereby increasing the frequency of propagated Ca$^{2+}$ waves leading to arrhythmias. The hypothesis that this syndrome has a similar phenotype to MH/CCD is supported by the fact that, similar to the findings in the present study, Yang et al.16 have seen increased ryanodine binding at very low Ca$^{2+}$ concentrations in 6 human MH/CCD mutations expressed in dyspedic myotubes. One difference that separates R4496C from the RyR1 MH/CCD mutations is in its lack of difference from wild type in terms of Ca$^{2+}$ inhibition. This, however, may be due to the fact that RyR2 has an intrinsically lower sensitivity to Ca$^{2+}$ inhibition than RyR1 and this masked the potential difference.

The work of Jiang et al.15 is a great beginning toward our understanding the mechanisms that cause this syndrome. Hopefully, it is only a beginning. Unfortunately, HEK cells are not heart cells, and they lack both critical components that regulate RyR2 in vivo and regular depolarization with Ca$^{2+}$ entry. What now cries out to be done is to repeat the present experiments on least representative mutations from the other two hot spots, including evaluation of the effect of the mutation on total internal Ca$^{2+}$ stores to complete the analogy with MH/CCD. Then, it will be crucial (1) to express these and other hot spot mutants in neonatal cardiac cells and examine the possible alterations in spontaneous Ca$^{2+}$ release activity and sparks in vitro and (2) to create either transgenic or knock-in mice expressing the mutated proteins and examine their phenotype in vivo.

References

Linear RyR2 protein sequence. Asterisks indicate site of reported RyR2 mutation. White boxes show MH/CCD hot spots in RyR1.
Leaky "Feet" and Sudden Death

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