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Leaky "Feet" and Sudden Death

P.D. Allen

Increases in intracellular Ca^{2+} are crucial signaling events in many cell types. The cardiac isoform of the (sarco)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 sarcolemmal 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

1. Giannini G, Sorrentino V. Molecular structure and tissue distribution of ryanodine receptors calcium channels. *Med Res Rev*. 1995;15:313–323.
2. Sorrentino V. The ryanodine receptor family of intracellular calcium release channels. *Adv Pharmacol*. 1995;33:67–90.
3. Yang H-T, Tweedie D, Wang S, Guia A, Vinogradova T, Bogdanov K, Allen PD, Stern MD, Lakatta EG, Boheler KR. The ryanodine receptor modulates the spontaneous beating rate of cardiomyocytes during development. *Proc Natl Acad Sci U S A*. June 27, 2002; 10.1073/

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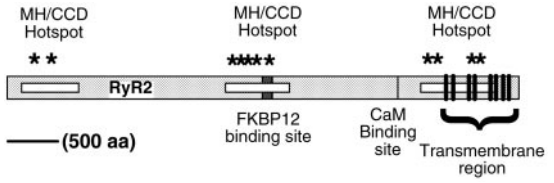

Linear RyR2 protein sequence. Asterisks indicate site of reported RyR2 mutation. White boxes show MH/CCD hot spots in RyR1.

pnas.142651999. Available at: http://www.pnas.org. Accessed July 9, 2002.

4. Marx SO, Reiken S, Hisamatsu Y, Gaburjakova M, Gaburjakova J, Yang YM, Rosemblit N, Marks AR. Phosphorylation-dependent regulation of ryanodine receptors: a novel role for leucine/isoleucine zippers. *J Cell Biol*. 2001;153:699–708.
5. Marks AR, Reiken S, Marx SO. Progression of heart failure: is protein kinase A hyperphosphorylation of the ryanodine receptor a contributing factor? *Circulation*. 2002;105:272–275.
6. Brandt A, Schleithoff L, Jurkat-Rott K, Klingler W, Baur C, Lehmann-Horn F. Screening of the ryanodine receptor gene in 105 malignant hyperthermia families: novel mutations and concordance with the in vitro contracture test. *Hum Mol Genet*. 1999;8:2055–2062.
7. Deufel T, Sudbrak R, Feist Y, Rubsam B, Du Chesne I, Schafer KL, Roewer N, Grimm T, Lehmann-Horn F, Hartung EJ, et al. Discordance, in a malignant hyperthermia pedigree, between in vitro contracture-test phenotypes and haplotypes for the MHS1 region on chromosome 19q12-13.2, comprising the C1840T transition in the RYR1 gene. *Am J Hum Genet*. 1995;56:1334–1342.
8. Keating KE, Quane KA, Manning BM, Lehane M, Hartung E, Censier K, Urwyler A, Klausnitzer M, Muller CR, Heffron JJ, et al. Detection of a novel RYR1 mutation in four malignant hyperthermia pedigrees. *Hum Mol Genet*. 1994;3:1855–1858.
9. Tiso N, Stephan DA, Nava A, Bagattin A, Devaney JM, Stanchi F, Larderet G, Brahmbhatt B, Brown K, Bauce B, Muriago M, Basso C, Thiene G, Danieli GA, Rampazzo A. Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). *Hum Mol Genet*. 2001;10:189–194.
10. Tong J, Oyamada H, Demaurex N, Grinstein S, McCarthy TV, MacLennan DH. Caffeine and halothane sensitivity of intracellular Ca^{2+} release is altered by 15 calcium release channel (ryanodine receptor) mutations associated with malignant hyperthermia and/or central core disease. *J Biol Chem*. 1997;272:26332–26339.
11. Priori SG, Napolitano C, Tiso N, Memmi M, Vignati G, Bloise R, Sorrentino VV, Danieli GA. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2001;103:196–200.
12. Laitinen PJ, Brown KM, Piippo K, Swan H, Devaney JM, Brahmbhatt B, Donarum EA, Marino M, Tiso N, Viitasalo M, Toivonen L, Stephan DA, Kontula K. Mutations of the cardiac ryanodine receptor (RyR2) gene in familial polymorphic ventricular tachycardia. *Circulation*. 2001;103:485–490.
13. Lopez JR, Gerardi A, Lopez MJ, Allen PD. Effects of dantrolene on myoplasmic free $[Ca^{2+}]$ measured in vivo in patients susceptible to malignant hyperthermia. *Anesthesiology*. 1992;76:711–719.
14. Mickelson JR, Louis CF. Malignant hyperthermia: excitation-contraction coupling, Ca^{2+} release channel, and cell Ca^{2+} regulation defects. *Physiol Rev*. 1996;76:537–592.
15. Jiang D, Xiao B, Zhang L, Chen SRW. Enhanced basal activity of a cardiac Ca^{2+} release channel (ryanodine receptor) mutant associated with ventricular tachycardia and sudden death. *Circ Res*. 2002;91:218–225.
16. Yang T, Fessenden JD, Ta TA, Mukherjee S, Pessah IN, Allen PD. Caffeine, 4-CMC and K^+ sensitivity of intracellular Ca^{2+} release is altered by six RyR-1 mutations associated with malignant hyperthermia. *Biophys J*. 2002;82:641a. Abstract.

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