See related article, pages 531–540

Not All Sudden Death Is the Same

P.D. Allen

Sudden death as a result of cardiac arrhythmia is probably the most common symptom associated with cardiac disease. It occurs not only in people with known cardiac disease, most notably congestive heart failure, but also in young, apparently healthy individuals who have no apparent structural heart disease. Frequently, in this latter group, these fatal arrhythmias are associated with exercise and increased β-adrenergic stimulation. One possible mechanism for how these arrhythmias could occur in otherwise "normal" individuals is an aberrant release of Ca^{2+} from the sarcoplasmic reticulum (SR), which in turn could cause delayed afterdepolarizations[1] that can trigger potentially fatal ventricular arrhythmias.

Unlike skeletal muscle, where excitation-contraction coupling (EC coupling) is intermittent and mediated through a mechanical coupling between the slow voltage-gated Ca^{2+} channel (dihydropyridine receptor, DHPR) in the sarcolemma and the skeletal isoform of the large-conductance calcium release channel in the SR (ryanodine receptor, RyR), RyR1 in cardiac muscle EC coupling is rhythmic and the cardiac isoform of RyR (RyR2) is activated by the inward Ca^{2+} influx through the cardiac DHPR via Ca^{2+}-induced Ca^{2+} release (CICR).[2] In the heart, RyR2 does not act in a vacuum but rather is part of a macromolecular complex containing the immunophilin FKBP12.6, phosphorylases, and phosphatases,[3] in addition to the DHPR and several other proteins including calsequestrin, triadin, junctin, and junctophilin, to name only a few, that make up the calcium release unit (CRU).[4] Heart failure has been associated with disruption of this macromolecular complex secondary to hyperphosphorylation of RyR2 and the associated dissociation of FKBP12.6.[5] Interestingly, mice that carry two null alleles for FKBP12.6 have been shown to have exercise- and catecholamine-induced fatal ventricular arrhythmias suggesting that this is a crucial subunit for controlling ventricular Ca^{2+} homeostasis.[6]

Recently, 11 missense mutations of RyR2 and one missense mutation of calsequestrin have been associated with a group of closely associated cardiomyopathies that are characterized by early sudden death: catecholaminergic polymorphic ventricular tachycardia (CPVT), arrhythmogenic right ventricular cardiomyopathy (ARVD2), and familial polymorphic ventricular tachycardia.[7–10] Interestingly, the RyR2 mutations associated with these cardiomyopathies are clustered in the same hot spots as the more than 50 missense mutations in RyR1 that are associated with malignant hyperthermia (MH) and central core disease (CCD).[11,12] Like CPVT and ARVD2, MH individuals have normal muscle histology and have a "normal" phenotype until triggered by exposure to a triggering agent or stress. MH mutations are associated with a high resting free 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. This has led to the hypothesis that the cardiac RyR channelopathies are likely to result in an increased diastolic Ca^{2+}, slowed relaxation after an action potential, and arrhythmogenic Ca^{2+} waves.

It has been previously shown by Jiang et al[13] that, when studied in lipid bilayers, one CPVT RyR2 mutation (R4496C) expressed in HEK293 cells has an increased open probability at low (5 nmol/L) Ca^{2+} concentrations. However, at normal and elevated Ca^{2+} concentrations, there was no difference in the open probability between *wt* or mutant channel. Their findings, at Ca^{2+} concentrations above 150 nmol/L, were confirmed by Wehrens et al,[6] who studied three CPVT mutations (S2246L, R2474S, and R4497C) expressed in the same heterologous cell line. In the latter study, it was demonstrated that the CPVD RyR2s were more sensitive to protein kinase A (PKA) phosphorylation, and it was suggested, based on an in vitro binding study, that CPVT RyR2s had a lower affinity for FKBP12.6. In this issue of *Circulation Research*, George et al[14] report for the first time the effects that the expression of CPVT RyR2s have on cardiac cells. Using HL-I cardiomyocytes transfected with *wt* or three CPVT (S2246L, N4104K, and R4497C) RyR2 cDNAs, they overexpressed RyR2 by ≈2-fold. This did not suppress native *wt*RyR2 expression, and thus the mix of RyRs expressed mimics the clinical heterozygous situation. Interestingly, they demonstrated that in unstimulated cells, beating frequency was not increased in CPVT RyR2-expressing cultures; furthermore, the endoplasmic reticulum Ca^{2+} load was increased in all transfected cells, suggesting that, if CPVT RyR2s were leaky, there was a mechanism to completely compensate for such leakiness. As would be expected, they demonstrated that caffeine and 4-chloro-*m*-cresol (4CmC) sensitivity was shifted to the left in CPVT RyR2-expressing cells. They also showed that the Ca^{2+} release amplitude was higher and relaxation time was longer in these cells after exposure to these direct RyR agonists. They did not show a decrease in the amount of FKBP12.6 associated with the membrane fractions of these cells. This finding would have been predicted by Tiso et al[15] from yeast two-hybrid studies but is the opposite of what has been suggested from studies of some of the same heterologously expressed CPVT RyR2s.[6] They did find that CPVT RyR2-expressing cells were more sensi-

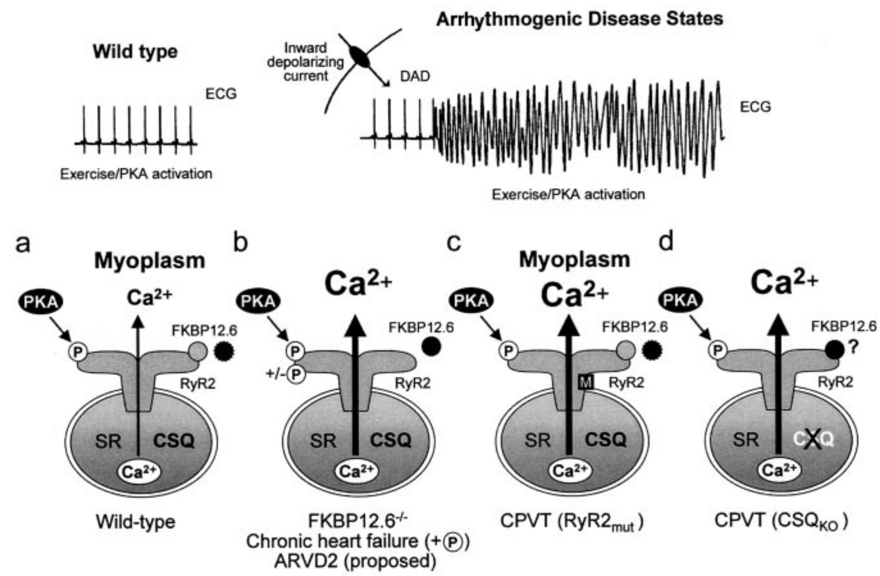
The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

From the Department of Anesthesia, Brigham and Women's Hospital, Boston, Mass.

Correspondence to P.D. Allen, MD, PhD, Professor of Anesthesia, Department of Anesthesia, Brigham and Women's Hospital, 75 Francis St, Boston, MA 02115. E-mail allen@zeus.bwh.harvard.edu

(*Circ Res.* 2003;93:484-486.)

***Circulation Research* is available at http://www.circresaha.org**
DOI: 10.1161/01.RES.0000093184.27194.42



a, During exercise- or catecholamine-induced stress, *wt*RyR2 releases more Ca^{2+}, increasing myocardial contractility and an increased heart rate with a normal ECG. The proposed mechanism for this increase in Ca^{2+} release is partial dissociation of the FKBP12.6 from RyR2 due to phosphorylation of RyR2 by PKA. In arrhythmogenic disease states (b through d), the amount of Ca^{2+} release is greater, and this can lead to an elevated diastolic myoplasmic Ca^{2+}. This elevation in myoplasmic Ca^{2+} can in turn lead to diastolic afterdepolarizations (DADs) that can initiate fatal ventricular tachyarrhythmias and cause sudden death. This increase in SR Ca^{2+} release can be mediated through a number of mechanisms. b, In FKBP12.6 knockout animals, chronic heart failure, where RyR2 is hyperphosphorylated, and possibly in the syndrome ARVD2, FKBP12.6 is either absent or completely dissociated from RyR2. c, In CPVT hearts, the amount of dissociation of FKBP12.6 is similar to control, but some other factor associated with the mutation in RyR2 increases SR Ca^{2+} release. d, In CPVT caused by the absence of cardiac calsequestrin, the phosphorylation state of RyR2 and the degree of dissociation of FKBP12.6 are unknown, but the phenotype is the same as b and c.

tive to β-adrenergic receptor (β-AR) stimulation by either isoproterenol or forskolin and had prolonged Ca^{2+} transients under this circumstance, but this sensitivity was not due to differences either in the amount of RyR2 phosphorylation or the magnitude of the loss of FKBP12.6 from CPVT-expressing microsomes from what was seen for control cells or for cells overexpressing *wt*RyR2. It is certainly possible that the mechanism for their abnormal Ca^{2+} release after catecholamine stimulation is an increased sensitivity of CPVT RyR2s to PKA, as shown by Wehrens et al,[6] but they clearly show that the mechanism for this increased sensitivity, if real, cannot simply be attributed to a lowered affinity for these CPVT RyR2s for FKBP12.6. At present, their findings strongly indicate that there must be an FKBP12.6-independent defect(s) in the regulation of CPVT RyR2s in HL-1 cardiomyocytes that leads to increased Ca^{2+} release on cell stimulation and the arrhythmias that could ensue. The precise nature of these defects remains to be defined.

The work of George et al[14] is a critical step in understanding the mechanism by which these mutations in the RyR2 cause sudden death and shows the necessity of doing structure-function studies in appropriate cell lines or animal models. Currently, chronic administration of β-blockers is used in the management of CPVT, but due to the many intracellular targets of the β-AR signaling cascade, the results of their study indicate that precise in situ regulation of mutant RyR2 channel function represents an attractive and feasible therapeutic strategy. To develop novel therapeutic strategies in the management of CPVT/ARVD, it will be necessary to know the precise molecular mechanisms by which CPVT mutations in RyR2 cause the augmented Ca^{2+} release. The next step must be to develop an animal model(s) to facilitate studies of CPVT RyR structure function under normal physiological conditions and its regulation by Ca^{2+} and Mg^{2+}. Attractive as a unifying hypothesis involving loss of FKBP12.6 might be for heart failure, CPVT, and ARVD,[16] it is unlikely that all RyR2 channelopathies that cause sudden death have a common molecular mechanism leading to Ca^{2+} overload (Figure). Furthermore, similar cardiac phenotypes do not even have to be based on the same pathogenesis, as has been shown for patients with CPVT associated with the absence of cardiac calsequestrin rather than a mutation of RyR2.[17]

References

1. Fozzard HA. Afterdepolarizations and triggered activity. *Basic Res Cardiol*. 1992;87(suppl 2):105–113.
2. Fabiato A. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *Am J Physiol*. 1983;245:C1–C14.
3. 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.
4. Franzini-Armstrong C, Protasi F, Ramesh V. Shape, size, and distribution of Ca^{2+} release units and couplons in skeletal and cardiac muscles. *Biophys J*. 1999;77:1528–1539.
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. Wehrens XH, Lehnart SE, Huang F, Vest JA, Reiken SR, Mohler PJ, Sun J, Guatimosim S, Song LS, Rosemblit N, D'Armiento JM, Napolitano C, Memmi M, Priori SG, Lederer WJ, Marks AR. FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. *Cell*. 2003;113:829–840.

7. 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.
8. Priori SG, Napolitano C, Memmi M, Colombi B, Drago F, Gasparini M, DeSimone L, Coltorti F, Bloise R, Keegan R, Cruz Filho FE, Vignati G, Benatar A, DeLogu A. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2002;106:69–74.
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. 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.
11. 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.
12. Loke J, MacLennan DH. Malignant hyperthermia and central core disease: disorders of Ca^{2+} release channels. *Am J Med*. 1998;104:470–86.
13. Jiang D, Xiao B, Zhang L, Chen SR. 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.
14. George CH, Higgs GV, Lai FA. Ryanodine receptor mutations associated with stress-induced ventricular tachycardia mediate increased calcium release in stimulated cardiomyocytes. *Circ Res*. 2003;93:531–540.
15. Tiso N, Salamon M, Bagattin A, Danieli GA, Argenton F, Bortolussi M. The binding of the RyR2 calcium channel to its gating protein FKBP12.6 is oppositely affected by ARVD2 and VTSIP mutations. *Biochem Biophys Res Commun*. 2002;299:594–598.
16. Most P, Koch WJ. Sealing the leak, healing the heart. *Nat Med*. 2003;9: 993–994.
17. Postma AV, Denjoy I, Hoorntje TM, Lupoglazoff JM, Da Costa A, Sebillon P, Mannens MM, Wilde AA, Guicheney P. Absence of calsequestrin 2 causes severe forms of catecholaminergic polymorphic ventricular tachycardia. *Circ Res*. 2002;91:21–26.

KEY WORDS: sudden death ■ sarcoplasmic reticulum ■ ryanodine receptors ■ ventricular tachycardia ■ FKBP12.6

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

Not All Sudden Death Is the Same

P.D. Allen

Circ Res. 2003;93:484-486
doi: 10.1161/01.RES.0000093184.27194.42
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571