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 Ca²⁺ from the sarcoplasmic reticulum (SR), which in turn could cause delayed afterdepolarizations¹ 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 Ca²⁺ 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 Ca²⁺ influx through the cardiac DHPR via Ca²⁺-induced Ca²⁺ 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 showed that the CRU name only a few, that make up the calcium release unit (CRU).² 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 Ca²⁺ 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 Ca²⁺, increased sensitivity to caffeine and halothane, reduced internal Ca²⁺ stores, and a reduced sensitivity to Ca²⁺ and Mg²⁺ inhibition. This has led to the hypothesis that the cardiac RyR channelopathies are likely to result in an increased diastolic Ca²⁺, slowed relaxation after an action potential, and arrhythmogenic Ca²⁺ waves.

It has been previously shown by Jiang et al¹³ 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) Ca²⁺ concentrations. However, at normal and elevated Ca²⁺ concentrations, there was no difference in the open probability between wt or mutant channel. Their findings, at Ca²⁺ 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 al¹⁴ report for the first time the effects that the expression of CPVT RyR2s have on cardiac cells. Using HL-1 cardiomyocytes transfected with wt or three CPVT (S2246L, N4104K, and R4497C) RyR2 cDNAs, they overexpressed RyR2 by ~2-fold. This did not suppress native wRyR2 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 Ca²⁺ 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 Ca²⁺ 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 al¹⁵ 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-

© 2003 American Heart Association, Inc.

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

See related article, pages 531-540

Not All Sudden Death Is the Same

P.D. Allen
precise nature of these defects remains to be defined. The work of George et al. is a critical step in understanding the mechanism by which these mutations in the 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, 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.

References

2. Fabiato A. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. Am J Physiol. 1983;245:C1–C14.

**Key Words:** sudden death ■ sarcoplasmic reticulum ■ ryanodine receptors ■ ventricular tachycardia ■ FKBP12.6
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
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/93/6/484

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org//subscriptions/