Primary electrical diseases of the heart such as the Long QT Syndrome (LQTS), Short QT Syndrome (SQTS), Brugada Syndrome (BrS), and Catecholaminergic polymorphic ventricular tachycardia (CPVT) are inherited monogenic disorders caused by mutations in ion channel genes (i.e., channelopathies), calcium handling proteins, or related molecules that occur in the absence of overt structural abnormalities. Because these disorders are typically associated with a high incidence of ventricular tachyarrhythmias and sudden cardiac death (SCD), they are the subject of intense investigation. Although collectively, monogenic diseases underlie a minority of SCD cases in the general population, elucidation of the underlying mechanisms by which they promote electrical instability has provided a wealth of knowledge regarding the role of ion channel dysfunction in electrical remodeling and arrhythmogenesis at multiple levels of integration, linking single amino acid mutations in ion channel genes to electrical dysfunction at the intact cell, organ, and system levels.

In recent years, numerous investigations have focused on mechanisms by which altered ion channel function and action potential properties can promote arrhythmias at the multicellular network level in various animal models of LQTS, SQTS, and BrS. In this issue of Circulation Research, Cerrone et al provide a strong mechanistic link between a known CPVT causing mutation and electrical instability arising from the His-Purkinje network of the intact heart in a tour de force study using high-resolution optical mapping, cellular electrophysiological measurements, a variety of pharmacological tools, and numerical simulations.

Catecholaminergic Polymorphic Ventricular Tachycardia

CPVT is a heritable disorder that presents clinically as exercise- or stress-induced ventricular arrhythmias, syncope, or SCD. Electrocardiographically, patients with CPVT exhibit polymorphic VT (PVT), bidirectional VT with an alternating QRS axis (Bi-VT), and ventricular fibrillation (VF). Several mutations in the cardiac Ryanodine Receptor (RyR2) and Calsequestrin (CSQ) have been identified in affected patients with CPVT. Because both RyR2 and CSQ are key calcium handling proteins involved in excitation-contraction (EC) coupling, it was postulated that abnormal intracellular calcium cycling is a determinant of CPVT-related arrhythmias.

Calcium-Handling Proteins in Health and Disease

EC coupling is a well-described fundamental principal by which the ionic (excitation) properties of a myocyte tightly coordinate its mechanical (contractile) function. The process is initiated by the transient opening of voltage-gated L-type calcium channels after depolarization of the cellular membrane. Calcium entry through these channels, which are strategically localized at the invaginations of the T-tubular network, in close spatial proximity to the sarcoplasmic reticulum (SR), activates a regenerative positive feedback process of calcium-induced calcium release from the SR through RyR2, resulting in tropomyosin translocation and myofilament contraction. Elevated free cytosolic calcium levels are then restored by the rapid reuptake of calcium into the SR by SERCA2a and extrusion to the extracellular space by the electrogenic sodium-calcium exchanger which generates a net depolarizing transient inward current. Disease-induced malfunction of several EC coupling proteins results in mechanical and electrical dysfunction at the cellular level that might be transduced to the entire organ to form lethal arrhythmias. Although arrhythmias that are dependent on changes in EC coupling proteins are commonly associated with structural heart disease such as heart failure or myocardial ischemia, they also occur in young, apparently healthy individuals, presumably by predisposing to delayed afterdepolarizations (DADs). In fact, DAD-induced triggered activity might constitute an important class of arrhythmias that underlie SCD in various forms of familial cardiomyopathies, including CPVT and arrhythmogenic right ventricular dysplasia (ARVD), both of which are characterized by mutations in calcium handling genes, most notably RyR2. At the molecular level, Wehrens et al elegantly demonstrated that mutant RyR2 channels found in patients with CPVT have a decreased binding affinity for FKBP12.6, a calcium regulatory protein, which normally acts to reduce the open channel probability of RyR2, thereby preserving SR calcium load during diastole. Although, at rest, the function of mutant RyR2 channels was identical to that of their wild-type counterparts, mutant channels exhibited enhanced dissociation from FKBP12.6 in response to β-adrenergic stimulation.
These authors argued that protein kinase A (PKA)-mediated phosphorylation of a single amino acid residue (Serine-2808) within RyR2 was sufficient for rendering RyR2 channels defective and enhancing the diastolic SR calcium leak, which could be effectively reversed by pharmacologically increasing the affinity of FKBP12.6 for RyR2.5

CAMKII Versus PKA-Mediated Phosphorylation of RyR2
The specific molecular mechanism and signaling pathways that lead to the pathological hyperphosphorylation of RyR2 have been recently challenged.6–13 For example, Chen and colleagues showed that phosphorylation of RyR2 by PKA at Ser-2030 did not dissociate FKBP12.6 from it.8 These authors subsequently identified an alternative phosphorylation site (Ser-2030), which they argued was the principal mediator of PKA phosphorylation of RyR2.7 Furthermore, Valdivia and colleagues have recently demonstrated that genetic ablation of Ser-2030 on RyR2 failed to alter the β-adrenergic responsiveness of mice and did not modify their progression toward heart failure.6 Priori and coworkers,14 using the same mouse model studied by Cerrone et al,14 found that K201, an agent that enhances the binding of FKBP12.6 to RyR2, failed to abrogate arrhythmias induced by caffeine or epinephrine in vivo and did not prevent the generation of DADs and triggered activity in isolated cardiomyocytes, arguing against an important role for the interaction between FKBP12.6 and RyR2 in the mechanism of arrhythmias in this specific animal model of CPVT.14 More recently, Curran et al12 demonstrated that β-adrenergic stimulation enhances diastolic SR calcium leak in a manner that is dependent on Ca2+-Calmodulin–dependent protein kinase II (CaMKII) but not PKA.

Elucidating the molecular mechanisms and signaling pathways that lead to the hyperphosphorylation of RyR2 has clear implications for the development of novel therapeutic agents.5 However, regardless of the specific molecular targets, it has become clear that enhanced diastolic calcium leak through RyR2 (either as a consequence of its dissociation from FKBP12.6 or as a result of a direct effect on RyR2 or related proteins within a larger macromolecular complex) is a major contributor to DADs and calcium handling defects in promoting delayed afterdepolarizations and CPVT-related arrhythmias.

Purkinje Fibers as a Source of DADs in CPVT
The present study by Cerrone et al3 goes a long way in demonstrating how a single amino acid substitution in RyR2 that causes DADs in isolated cardiomyocytes can also result in typical CPVT-related arrhythmias in the intact mouse heart. Remarkably, these arrhythmias were not present at baseline, but were only evoked on challenge with caffeine, isoproterenol, or epinephrine which presumably uncovered the otherwise “silent” intracellular calcium instability, in a manner analogous to the “multi-hit” hypothesis.

A major contribution of the present study3 is the detailed description of the focal nature and source of CPVT-related arrhythmias, including MVT, Bi-VT, and PVT, which consistently arose from deep layers. The observation that all arrhythmias exhibited a typical epicardial breakthrough pattern that emanated from 1 (MVT) or 2 (Bi-VT and PVT) epicardial breakthrough patterns that could be readily converted to MVT with a wide QRS complex on administration of Lugol’s solution to the RV cavity, they most certainly originated in the His-Purkinje network and not the myocardial wall.

The Perfect Storm
The findings of Cerrone et al3 highlight the importance of evaluating arrhythmias at the intact organ level, because the occurrence of DADs in isolated cardiomyocytes does not translate directly into sustained arrhythmias or even triggered beats in the intact heart. Moreover, this study illustrates the importance of understanding the dynamic interplay between passive and active membrane properties in the ultimate control of membrane potential, the genesis of afterdepolarizations, and the successful propagation of DADs across the myocardium (Figure). Clearly, the RyR2 mutation described here did not give rise to random ectopic foci across the ventricle that would have quickly degenerated into VF. Instead, these arrhythmias were dependent on an intact His-Purkinje network. Hence, it is clear from the present study that the threshold for DAD generation is lower in Purkinje fibers compared with intact myocardial tissue, which
could be attributable to unique differences in the intrinsic ionic properties (ie, a reduced repolarizing reserve) of Purkinje compared with myocardial cells, especially in the absence of β-adrenergic stimulation as elegantly demonstrated by Nattel and coworkers. Therefore, whereas β-adrenergic stimulation enhances the propensity of calcium leak, it also increases the density of the slowly activating delayed rectifier potassium current, which might act to suppress the development of DADs. Moreover, whereas myocardial cells are tightly coupled electrically to one another, Purkinje fibers are relatively insulated from the myocardiun thereby reducing the electrotonic sink that they encounter. This results in greater modulation of membrane potential by a given amount of current allowing DADs to encounter. This results in greater modulation of membrane potential by a given amount of current allowing DADs to propagate throughout the myocardium.

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None.

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