Leaky ryanodine receptors cause delayed afterdepolarizations and ventricular arrhythmias

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This editorial refers to 'Mutant ryanodine receptors in catecholaminergic polymorphic ventricular tachycardia generate delayed afterdepolarizations due to increased propensity to Ca$^{2+}$ waves'† by J. Paavola et al., on page 1135

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic disease characterized by the absence of structural heart disease, syncope, and sudden cardiac death. Typically, acceleration of the heart rate during physical exercise or emotional distress provokes an increasing number of ventricular premature complexes followed by runs of bidirectional or polymorphic ventricular tachycardia (VT). During clinical testing, about 30–50% of the patients will reproducibly develop VT following exercise testing or catecholamine injection.1,2

The ECG morphology of ventricular tachyarrhythmias observed in patients with CPVT resembles that of VTs commonly described in digitalis toxicity (which is associated with cellular calcium overload), and in metabolic disturbances as seen in severe HF (which is associated with high adrenergic tone).3 In conditions of cytoplasmic Ca$^{2+}$ overload or enhanced β-adrenergic signalling, cardiac myocytes exhibit greater ectopic activity. It has therefore been suggested that arrhythmias in CPVT are mediated by triggered activity and delayed afterdepolarizations (DADs), which are defined as oscillations in the membrane potential themselves may not be sufficient to initiate triggered arrhythmias. Indeed, the amplitude of a DAD needs to be of sufficient amplitude before it can trigger an action potential. Moreover, premature ventricular complexes can only lead to arrhythmias in the presence of a susceptible substrate (e.g., a ventricle that is partially refractory, or has conduction abnormalities).7

Clinical evidence for DADs in patients with CPVT

In contrast to conventional electrode catheter recordings, MAP recordings provide precise information not only about the local activation time, but also about the entire repolarization time course. Electrophysiological data for the occurrence of DADs in patients with CPVT have been scarce in the literature, with the exception of a case report by Nakajima et al.2 In this study of a 14-year-old male with CPVT, endocardial MAP recordings at the inflow of the right ventricle revealed 'humps' following the previous action potential, which is consistent with DADs. The amplitude of these signals increased following the infusion of isoproterenol, and the DADs were frequently followed by ventricular premature beats and bidirectional VT.2

The studies by Paavola et al.6 significantly extend these findings by demonstrating the occurrence of DADs in genotyped patients with CPVT. Endocardial MAP recordings were performed in 15 CPVT patients with known RyR2 mutations and 12 age-matched controls. Under baseline conditions, DADs were observed in 20% (three out of 15) of the CPVT patients, whereas no DADs were found in control subjects. Since none of these patients exhibited arrhythmias or symptoms at rest, it is conceivable that DADs by themselves may not be sufficient to initiate triggered arrhythmias. Moreover, premature ventricular complexes can only lead to arrhythmias in the presence of a susceptible substrate (e.g., a ventricle that is partially refractory, or has conduction abnormalities).7

Following low dose epinephrine infusion, 27% (four out of 15) of the CPVT patients showed DADs. Epinephrine increased the amplitude and the slope of DADs, and occasionally led to premature ventricular beats.6 These findings indicate that larger DADs evoked by epinephrine stimulation are more capable of triggering action potentials and premature ventricular beats. On the other hand, it is surprising that not more patients exhibited DADs in this study, considering that most patients (80%; 12 out of 15) of this cohort had experienced exercise-related syncope or cardiac arrest. One possible explanation is that DADs were not detected as MAP recordings were only obtained from one site (i.e., the right ventricular septum). Another possibility is that the epinephrine dose administered during electrophysiological
Ionic mechanisms underlying DADs in CPVT

DADs occur after the repolarization of the preceding action potential has been completed, and are caused by aberrant release of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR) intracellular Ca\(^{2+}\) stores.\(^8\) Spontaneous SR Ca\(^{2+}\) release events that cause DADs in single myocytes often begin in a small region of the cell. The locally elevated Ca\(^{2+}\) then diffuses to adjacent calcium-release units where it activates other Ca\(^{2+}\) release channels, which induces Ca\(^{2+}\) release that propagates throughout the cell (Figure 1). The resulting elevation of cytosolic Ca\(^{2+}\) concentrations may activate the Na\(^+\)/Ca\(^{2+}\) exchanger, which leads to extrusion of Ca\(^{2+}\) from the cell in exchange for an influx of Na\(^+\) into the cell.\(^9\) Triggered activity arises when the depolarization threshold is reached, resulting in a ventricular premature complex.

Strong evidence for a causal link between defective RyR2 gating and DADs was obtained in cardiomyocytes isolated from knockin mice, heterozygous for the CPVT-associated RyR2 mutations R4496C or R176Q.\(^5,10\) Action potential recordings from R4496C/+/- mice revealed DADs that appeared more frequently following isoproterenol stimulation.\(^5\) Measurements of intracellular Ca\(^{2+}\) concentrations demonstrated spontaneous SR Ca\(^{2+}\) release events in myocytes isolated from R176Q/+/- mice.\(^10\) Although these Ca\(^{2+}\) release events were observed under baseline conditions, the frequency and amplitude of these DAD triggers were enhanced following isoproterenol stimulation. Thus, studies in both patients and mice with inherited RyR2 mutations have clearly demonstrated that abnormal intracellular Ca\(^{2+}\) release through mutant RyR2 channels can initiate DADs, which could trigger lethal ventricular arrhythmias.

**Figure 1** In patients with CPVT, intracellular Ca\(^{2+}\) leak via mutant RyR2 channels (RyR2) on the sarcoplasmic reticulum (SR) membrane activate the Na\(^+\)/Ca\(^{2+}\)-exchanger (NCX) on the transverse tubular (TT) membrane. The extrusion of Ca\(^{2+}\) via NCX provides a transient inward current (I\(_{Na}\)). If the membrane potential reached the threshold depolarization level, an extra action potential may be triggered. This premature ventricular complex could result in bidirectional (bVT) or polymorphic ventricular tachycardia commonly observed in CPVT. Modified from Wehrens et al.\(^7\) and Aizawa et al. \(^\text{Int Heart J} \text{2006;47:381–89.}\)

**RyR2 defects associated with CPVT-linked mutations**

Ryanodine receptors are ion channels located on the SR membrane, responsible for Ca\(^{2+}\) release from the SR. Channel complexes are comprised of four pore-forming RyR2 subunits, each of which is associated with various proteins that modulate channel function.\(^11\) For example, activation of β-adrenergic receptors by catecholamines results in the activation of cAMP-dependent protein kinase A (PKA) bound to the RyR2 macromolecular complex. Activated PKA phosphorylates serine 2808 on RyR2, which decreases the binding affinity of the channel-stabilizing subunit calstabin2 (also known as FKBP12.6). This sequence of signalling events renders RyR2 more sensitive to Ca\(^{2+}\)-dependent activation, enabling enhanced intracellular Ca\(^{2+}\) release during stress or exercise.\(^11\)

Paavola et al.\(^6\) expressed mutant RyR2 channels in HEK293 cells and recorded calcium release events using confocal microscopy to study the mechanisms by which genetic mutations cause enhanced SR Ca\(^{2+}\) release. HEK293 cells expressing CPVT-mutant RyR2 channels showed spontaneous Ca\(^{2+}\) release events at lower concentrations of cAMP than cells transfected with wild-type RyR2 channels. These findings are consistent with the occurrence of DADs in patients carrying the same RyR2 mutations. It is very likely that the DADs are initiated by diastolic SR Ca\(^{2+}\) 'leakage' through CPVT-mutant RyR2 channels. The single channel properties of P2328S, Q4201R, and V4653F mutant RyR2 channels have been previously characterized using the planar lipid bilayer system.\(^12\) PKA-phosphorylated CPVT-mutant channels exhibited abnormally high open probabilities at low cytosolic Ca\(^{2+}\) concentrations, which are in agreement with the increased likelihood of DADs observed in HEK293 cells expressing these mutant RyR2 channels.

Several molecular mechanisms have been proposed for the abnormal diastolic activity of mutant RyR2 channels. Jiang et al.\(^13\) have suggested that mutant RyR2 channels may be more sensitive to luminal (SR) Ca\(^{2+}\) activation by a mechanism referred to as 'store-overload induced Ca\(^{2+}\) release'. Wehrens et al.\(^14\) have shown that CPVT-associated RyR2 mutations reduce the affinity for the channel-stabilizing subunit calstabin2 (FKBP12.6), a phenomenon that was also observed for the P2328S, Q4201R, and V4653F mutant channels.\(^12\) In addition, it has been proposed that CPVT mutations alter interactions between domains within the channel complex, leading to 'domain unzipping' and a reduced threshold to diastolic SR Ca\(^{2+}\) leakage.\(^15\) The finding that DADs also occur under baseline conditions in patients with CPVT argues in favour of an intrinsic RyR2 channel defect (i.e. an increased sensitivity to activation by Ca\(^{2+}\) or stimulation by cAMP), and appears to be inconsistent with the store-overload hypothesis (as SR overload is not likely to occur under resting conditions). Additional studies in patients with CPVT and in cardiomyocytes expressing CPVT-mutant RyR2 channels are warranted to further
explore the triggers of DADs and mechanisms underlying SR Ca\(^{2+}\) leak through mutant RyR2 channels.

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**References**


