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Extinguishing intracellular calcium leak – a promising anti-arrhythmic approach

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Ventricular arrhythmias remain a leading cause of sudden cardiac death (SCD) in the United States. Triggered activity (TA) is one of the major mechanisms causing tachyarrhythmias, the other two common causes being automaticity and reentry.[1] TA occurs when one or multiple spontaneously generated heart beats originate from an action potential that produces an afterdepolarization large enough to reach threshold membrane potential.[2] Historically, afterdepolarizations have been characterized as early (EAD) or delayed (DAD) depending on whether they originate before or after completion of repolarization, respectively.[3] The cellular origin of DADs has long been attributed to abnormal Ca^{2+} release from the sarcoplasmic reticulum (SR), leading to activation of inward Na^{+}/Ca^{2+}-exchanger current (I_{NCX}). Several recent studies also have implicated abnormal SR Ca^{2+} release as a mechanisms underlying EADs, in addition to the 'traditional' mechanism involving reactivation of the L-type Ca^{2+} current by changes in membrane potential.[4, 5]

The principal Ca^{2+} release channel on the SR is the type 2 ryanodine receptors (RyR2), which is activated by the relatively small amount of Ca^{2+} that enters the cytosol via voltage-dependent L-type Ca^{2+} channels during phase 2 of the action potential. This process of Ca^{2+}-induced Ca^{2+} release (CICR) then results in a quantitatively much greater release of Ca^{2+} from the SR, which triggers myocyte contraction.[6] Relaxation occurs during diastole when cytosolic Ca^{2+} is returned into the SR by the Ca^{2+}-ATPase (SERCA2a) or extruded from the cell by NCX. An alternative theory for activation of RyR2 has been proposed by Jiang *et al.*[7], who suggested that RyR2 can open independent of Ca^{2+} influx as a result of 'store overload-induced Ca^{2+} release' (SOICR). At this time, there is little or no solid experimental evidence for this SOICR phenomenon and its relevance for cardiac arrhythmias.[8] However, it is clear that the level of SR Ca^{2+} loading sensitizes RyR2 channels to release, although the molecular mechanisms responsible for this feature remain unclear.

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In patients with 'catecholaminergic polymorphic ventricular tachycardia' (CPVT), inherited mutations in RyR2 cause TA and lethal tachyarrhythmias.[9, 10] Single channel recordings have revealed that these pathogenic RyR2 mutations increase the probabilistic opening of RyR2.[10] Abnormal mutant RyR2 openings have also been shown to initiate spontaneous SR Ca^{2+} sparks and arrhythmogenic SR Ca^{2+} waves in ventricular myocytes from mice with RyR2 mutations.[5, 11] Importantly, these abnormal SR Ca^{2+} release events occur in the absence of elevated SR Ca^{2+} contents.[5, 12] It is believed that local elevations of [Ca^{2+}] at one Ca^{2+} release unit can trigger Ca^{2+} release of neighboring RyR2 clusters, resulting in subcellular propagation and the formation of Ca^{2+} waves. Thus, abnormal Ca^{2+} waves underlying afterdepolarizations can occur as a result of 'leaky' RyR2 even in the absence of Ca^{2+} store overload. Under certain conditions such as elevated adrenergic tone, these Ca^{2+} waves occur in a sufficient number of myocytes leading to TA at the tissue level, which can lead to arrhythmia induction.[11, 13]

In view of the important role of 'leaky' RyR2 in the development of afterdepolarizations and TA, a new class of antiarrhythmic drugs was developed that normalizes RyR2 channel functions without completely blocking the channel during CICR.[6] The first example of a compound in this class is the benzothiazepine derivative JTV519 (also known as K201), which was shown to reduce RyR2 open probability and prevent ventricular tachycardia in a mouse model of FKBP12.6 deficiency.[14] Lehnart *et al*.[15] also demonstrated that JTV519 normalizes channel dysfunction caused by CPVT-linked missense mutations in RyR2. Subsequently, several JTV519 derivatives (referred to as 'Rycals') have been developed with improved RyR2-specificity, reduce off-target effects, and enhance oral availability.[16] Compound S107 was shown to suppress premature ventricular contractions (PVCs) and spontaneous sustained VT in *mdx* mice, a mouse model of Duchenne's muscular dystrophy.[17]

The class 1c antiarrhythmic drug flecainide was also shown to reduce the open probability of RyR2 in a calsquestrin-deficient (*Casq2*−/−) mouse model of CPVT and to reduce the occurrence of ventricular arrhythmias in CPVT patients.[18] Hillard *et al.*[19] subsequently demonstrated that flecainide reduces salutatory Ca^{2+} wave propagation between adjacent Ca^{2+} release units by inhibition of open state RyR2, without affecting SR Ca^{2+} content. Moreover, flecainide was shown to suppress spontaneous Ca^{2+} release events in RyR2 mutant mice heterozygous for CPVT-linked mutation R4496C (R4496/+).[20]

Most recently, Dr. Chen's group reported that the nonselective beta-blocker carvedilol and three related analogues (VK-II-86, CS-I-34 and CS-I-59) suppressed ventricular tachyarrhythmias in the R4496/+ mouse model of CPVT by reducing RyR2 open probability.[21] Unlike other beta blockers, carvedilol suppressed the occurrence of spontaneous Ca^{2+} waves in ventricular myocytes isolated from R4496/+ mice. However, the mechanisms by which carvedilol analogous suppressed SR Ca^{2+} release and afterdepolarizations remained unclear.

In this issue of Heart*Rhythm*, Maruyama *et al.*[22] tested the hypothesis that a new carvedilol analogue VK-II-36 prevents TA through the suppression of EADs. Optical mapping studies of [Ca^{2+}] and voltage in the epicardial surface of ventricles of R4496/+ mice revealed EADs

and DADs coinciding with SCaEs at artificially slowed heart rates. Interestingly, the carvedilol analogue VK-II-36 prevented both types of afterdepolarizations, indicating that 'leaky' RyR2 were responsible for both EADs and DADs in this model. The findings are consistent with an important role for SR Ca^{2+} 'leak' in the pathogenesis of EADs, and they confirm that reactivation of L-type Ca^{2+} channels is not necessarily required for EAD genesis.

The paper also represents the first demonstration of antiarrhythmic effects of the carvedilol analogue VK-II-36 in a large animal model. In a rabbit model of acquired long QT syndrome induced by I_{Kr} blocker R-4031, VK-II-36 reduced action potential duration (APD) and dispersion. Those effects appeared to be caused by inhibition of abnormal SR Ca^{2+} release events, since the effects of VK-II-36 were primarily on the regions with long APDs. On the other hand, it remains to be established whether VK-II-36 has 'off target' effects on ion channels other than RyR2, which could modulate APD independent of VK-II-36's effect on SR Ca^{2+} release. In addition, it would be interesting to determine in future studies how VK-II-86 compares to other carvedilol analogues in terms of its dose response, RyR2 selectivity, and oral availability.[21]

The findings of the paper by Maruyama *et al*.[22] also are significant because they suggest that RyR2-modulating antiarrhythmic drugs could prevent triggered activity associated with EADs, not just DADs. It remains to be established whether this is true for carvedilol derivatives only, or for the entire class of RyR2 modulators that also includes JTV519, S107, and flecainide. The clinical indications for carvedilol derivatives and RyR2 modulators in general go beyond the rare inherited arrhythmia syndrome, as RyR2 gain-of-function activity has been causally linked to atrial fibrillation and lethal ventricular arrhythmias in failing hearts in various animal models. The lack of beta-blocking effects of the carvedilol analogue represents a favorable feature and suggests that these drugs could prevent arrhythmias without negative inotropic or chronotropic effects. Thus, inhibition of abnormal SR Ca^{2+} release via RyR2 represents a promising target for antiarrhythmic drug development.

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References

1. Vaquero M, Calvo D, Jalife J. Cardiac fibrillation: from ion channels to rotors in the human heart. Heart Rhythm. 2008; 5:872–879. [PubMed: 18468960]
2. Priori SG, Chen SR. Inherited dysfunction of sarcoplasmic reticulum Ca2+ handling and arrhythmogenesis. Circ Res. 2011; 108:871–883. [PubMed: 21454795]
3. Hoffman BF, Cranefield PF. The Physiological Basis of Cardiac Arrhythmias. Am J Med. 1964; 37:670–684. [PubMed: 14242077]
4. Choi BR, Burton F, Salama G. Cytosolic Ca2+ triggers early afterdepolarizations and Torsade de Pointes in rabbit hearts with type 2 long QT syndrome. J Physiol. 2002; 543:615–631. [PubMed: 12205194]

5. Chelu MG, Sarma S, Sood S, et al. Calmodulin kinase II-mediated sarcoplasmic reticulum Ca2+ leak promotes atrial fibrillation in mice. J Clin Invest. 2009; 119:1940–1951. [PubMed: 19603549]
6. Wehrens XH, Lehnart SE, Marks AR. Ryanodine receptor-targeted anti-arrhythmic therapy. Annals of the New York Academy of Sciences. 2005; 1047:366–375. [PubMed: 16093511]
7. Jiang D, Xiao B, Yang D, et al. RyR2 mutations linked to ventricular tachycardia and sudden death reduce the threshold for store-overload-induced Ca2+ release (SOICR). Proc Natl Acad Sci U S A. 2004; 101:13062–13067. [PubMed: 15322274]
8. Prosser BL, Ward CW, Lederer WJ. Subcellular Ca2+ signaling in the heart: the role of ryanodine receptor sensitivity. The Journal of general physiology. 2010; 136:135–142. [PubMed: 20660656]
9. Laitinen PJ, Brown KM, Piippo K, et al. Mutations of the cardiac ryanodine receptor (RyR2) gene in familial polymorphic ventricular tachycardia. Circulation. 2001; 103:485–490. [PubMed: 11157710]
10. Wehrens XH, Lehnart SE, Huang F, et al. FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. Cell. 2003; 113:829–840. [PubMed: 12837242]
11. Kannankeril PJ, Mitchell BM, Goonasekera SA, et al. Mice with the R176Q cardiac ryanodine receptor mutation exhibit catecholamine-induced ventricular tachycardia and cardiomyopathy. Proc Natl Acad Sci U S A. 2006; 103:12179–12184. [PubMed: 16873551]
12. Uchinoumi H, Yano M, Suetomi T, et al. Catecholaminergic polymorphic ventricular tachycardia is caused by mutation-linked defective conformational regulation of the ryanodine receptor. Circ Res. 2010; 106:1413–1424. [PubMed: 20224043]
13. Xie LH, Weiss JN. Arrhythmogenic consequences of intracellular calcium waves. Am J Physiol Heart Circ Physiol. 2009; 297:H997–H1002. [PubMed: 19561309]
14. Wehrens XH, Lehnart SE, Reiken SR, et al. Protection from cardiac arrhythmia through ryanodine receptor-stabilizing protein calstabin2. Science. 2004; 304:292–296. [PubMed: 15073377]
15. Lehnart SE, Wehrens XH, Laitinen PJ, et al. Sudden death in familial polymorphic ventricular tachycardia associated with calcium release channel (ryanodine receptor) leak. Circulation. 2004; 109:3208–3214. [PubMed: 15197150]
16. Bellinger AM, Reiken S, Carlson C, et al. Hypernitrosylated ryanodine receptor calcium release channels are leaky in dystrophic muscle. Nat Med. 2009; 15:325–330. [PubMed: 19198614]
17. Fauconnier J, Thireau J, Reiken S, et al. Leaky RyR2 trigger ventricular arrhythmias in Duchenne muscular dystrophy. Proc Natl Acad Sci U S A. 2010; 107:1559–1564. [PubMed: 20080623]
18. Watanabe H, Chopra N, Laver D, et al. Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. Nat Med. 2009; 15:380–383. [PubMed: 19330009]
19. Hilliard FA, Steele DS, Laver D, et al. Flecainide inhibits arrhythmogenic Ca2+ waves by open state block of ryanodine receptor Ca2+ release channels and reduction of Ca2+ spark mass. J Mol Cell Cardiol. 2010; 48:293–301. [PubMed: 19835880]
20. Kang G, Giovannone SF, Liu N, et al. Purkinje cells from RyR2 mutant mice are highly arrhythmogenic but responsive to targeted therapy. Circ Res. 2010; 107:512–519. [PubMed: 20595652]
21. Zhou Q, Xiao J, Jiang D, et al. Carvedilol and its new analogs suppress arrhythmogenic store overload-induced Ca2+ release. Nat Med. 2011; 17:1003–1009. [PubMed: 21743453]
22. Maruyama M, Xiao J, Zhou Q, et al. Carvedilol analogue inhibits triggered activities evoked by both early and delayed afterdepolarizations. Heart Rhythm. 2012