Can Novel Therapies for Arrhythmias Caused by Spontaneous Sarcoplasmic Reticulum Ca\textsuperscript{2+} Release be Developed Using Mouse Models?

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Ventricular arrhythmias are a significant cause of premature death in Western society and occur both in the absence and presence of heart disease. For example, in heart failure close to 50% of patients die of lethal forms of ventricular arrhythmias. In spite of a large amount of basic and clinical investigations, current pharmacotherapy for ventricular arrhythmias is inadequate, demonstrating the need for novel therapeutic approaches.

Abnormal automaticity is responsible for induction and maintenance of different types of ventricular tachycardias. The form of abnormal automaticity that results from abnormal release of Ca\textsuperscript{2+} from a Ca\textsuperscript{2+} overloaded sarcoplasmic reticulum (SR), referred to as a “triggered arrhythmia”, is the topic of this editorial. In vitro studies have shown that increasing the SR Ca\textsuperscript{2+} load beyond a critical level causes spontaneous opening of the SR Ca\textsuperscript{2+} release channel (ryanodine receptor; RYR) and results in spontaneous SR Ca\textsuperscript{2+} release.\textsuperscript{1} The subsequent increase in cytosolic [Ca] activates an inward current through the sarcolemmal Na\textsuperscript{+} / Ca\textsuperscript{2+} exchanger which causes membrane depolarization.\textsuperscript{1,2} Spontaneous SR Ca\textsuperscript{2+} release during the diastolic interval causes depolarization (delayed after depolarizations; DADs) which has been shown to result in action potentials in single cells and in the intact heart when the aberrant release is adequately synchronized within and among cardiac myocytes.\textsuperscript{3,4} Factors that lead to DADs include increased heart rate and catecholamine stress, which both induce triggered arrhythmias by increasing SR Ca\textsuperscript{2+} loading.\textsuperscript{5}

Whereas many molecules are involved in SR Ca\textsuperscript{2+} overload (DAD) related arrhythmias, abnormal opening of the RYR is an essential component. Therefore, factors that increase the likelihood that RYR will open abnormally during diastole (when it should be closed to allow for replenishment of SR Ca\textsuperscript{2+} stores) should predispose the heart to arrhythmias. Similarly, factors that prevent these types of RYR openings should prevent these types of arrhythmias. In the present issue of Circulation Research, Cerrone et al\textsuperscript{5} show that a mutation (R4496C; causing a change in a single amino acid) in RYR, that is known to be associated with catecholaminergic polymorphic ventricular tachycardia (CPVT) in humans, causes what appears to be identical arrhythmias in a RYR-R4496C “knock in” mouse model. This study provides novel support for the idea that alterations in the properties of RYR are sufficient to induce the types of triggered ventricular arrhythmias that are known to be responsible for sudden death in CPVT patients. These authors also show that mice harboring the RYR-R4496C mutation have structurally normal hearts and apparently normal basal physiology, but ventricular arrhythmias can be more easily induced by exercise, catecholamine, and caffeine than in normal hearts.\textsuperscript{5} Cerrone et al present data that convincingly demonstrates that the types of arrhythmias induced (bidirectional ventricular tachycardia) are identical to those seen in patients harboring like mutations. Therefore, this RYR-R4496C mouse model\textsuperscript{5} should be extremely valuable in studies to define the molecular alterations caused by these mutations that predispose myocytes within the ventricle to spontaneous SR Ca\textsuperscript{2+} release and associated life-threatening arrhythmias. This model could also be valuable for the development of novel therapies to prevent Ca\textsuperscript{2+} overload related arrhythmias in both CPVT and other arrhythmia prone (such as congestive heart failure) patients.

The mechanisms responsible for normal (action potential-induced) SR Ca\textsuperscript{2+} release as well as those factors that lead to abnormal (spontaneous, Ca\textsuperscript{2+} overload-related) release are well established. Normal SR Ca\textsuperscript{2+} release in ventricular myocytes occurs when Ca\textsuperscript{2+} influx through the L-type Ca\textsuperscript{2+} channel (LTCC), during the early portions of the action potential, causes elevated [Ca\textsuperscript{2+}] in the diffusion limiting space between the T-tubule and the junctional SR.\textsuperscript{6,7} This causes Ca\textsuperscript{2+} binding to cytoplasmic portions of RYR which induces channel opening (Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release).\textsuperscript{8,9} Spontaneous SR Ca\textsuperscript{2+} release can be induced in normal myocytes (with normal RYR) by inducing SR Ca\textsuperscript{2+} overload with rapid pacing, inhibition of the Na pump with cardiac glycosides, or catecholamine exposure.\textsuperscript{1} These studies show that the normal RYR is sensitive to the [Ca\textsuperscript{2+}] in the SR lumen and when this [Ca\textsuperscript{2+}] is sufficiently elevated, RYR opening is induced, resulting in increased [Ca\textsuperscript{2+}] in the T-tubule-junctional SR space which activates neighboring RYR to induce localized spontaneous SR Ca\textsuperscript{2+} release (spontaneous Ca\textsuperscript{2+} spark).\textsuperscript{10} Mutations of the RYR that are known to exist in humans, like those reported by Cerrone et al,\textsuperscript{5} appear to alter the properties of RYR so that they are “hypersensitive” to those factors that induce spontaneous SR Ca\textsuperscript{2+} release.\textsuperscript{11,12} Increasing RYR sensitivity to luminal SR...
[Ca\(^{2+}\)] appears to be a likely mechanism for the enhanced likelihood for spontaneous SR Ca\(^{2+}\) release in patients with known RYR mutations.\(^{13,14}\)

Patients, and now mice, that harbor RYR mutations exhibit an increased propensity to develop triggered arrhythmias during or after exercise,\(^{13,14}\) with a clearly documented role for catecholamine induction. The molecular basis for catecholamine induction of these arrhythmias has been explored\(^{11,12}\) but has not been clearly established. One explanation is that catecholamines induce arrhythmias (in normal hearts and those with RYR mutants) by increasing SR Ca\(^{2+}\) loading\(^1\) through protein kinase A (PKA)-mediated increases in Ca\(^{2+}\) influx (LTCC phosphorylation) and increases in SR Ca\(^{2+}\) uptake (phospholamban phosphorylation). Spontaneous SR Ca\(^{2+}\) release would occur at lower SR Ca\(^{2+}\) loads in myocytes with mutated RYR having increased luminal Ca\(^{2+}\) sensitivity. Another possibility is that PKA-mediated phosphorylation has different effects on mutant and wild-type RYR, producing mutant channels with increased Ca\(^{2+}\) sensitivities, making them more likely to open inappropriately and produce spontaneous Ca\(^{2+}\) release. In this regard, the Marks laboratory\(^{15,16}\) has championed the hypothesis that catecholamine related arrhythmias result, at least in part, from PKA-mediated “hyperphosphorylation” of RYR which by disassociation of FKBP12.6 (also termed Calstabin) from RYR, induces changes in RYR properties that lead to enhanced channel openings and spontaneous SR Ca\(^{2+}\) release. Importantly, in recent studies, this group has shown that drugs which stabilize RYR-FKBP12.6 binding can reduce catecholamine induced arrhythmias in mice.\(^{16}\) Whether or not this approach will also be antiarrhythmic in mice with the RYR-R4496C and catecholamine induced displacement is yet to be performed. FKBP12.6 appears to bind normally to RYR-R4496C mutation is clearly an important experiment yet to be performed. FKBP12.6 appears to bind normally to RYR-R4496C and catecholamine induced displacement is not different than in wild-type RYR,\(^{17}\) yet Cerrone et al\(^8\) show that arrhythmias are more readily induced by exercise and catecholamines. In addition, the new study by Cerrone et al\(^8\) shows that caffeine, which does not appear to induce displacement of FKBP12.6 from normal or mutated RYR,\(^{17}\) has a greater effect on arrhythmia induction in RYR-R4496C mice. Therefore, the molecular mechanisms responsible for enhanced RYR-mediated spontaneous SR Ca\(^{2+}\) release in RYR-R4496C mice versus those with PKA- “hyperphosphorylated” (normal) RYR may ultimately turn out to be somewhat different. The fact that the arrhythmias seen in the FKBP12.6 KO mice are different (no bidirectional Vr) than those with the RYR-R4496C mutation suggests differences in the underlying mechanisms. Given the scientific activity related to this topic and the model systems now in hand these mechanistic questions should soon be resolved. More importantly, at least to this investigator, is the fact that the available mouse models can reliably reproduce the catecholamine induced, RYR-mediated arrhythmias that are known to be a significant cause of sudden death in patients with and without RYR mutations. Therefore, these mouse models appear to be appropriate systems to explore novel therapies for catecholamine-related cardiac arrhythmias that ultimately begin with the abnormal opening of the cardiac RYR.

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