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Corresponding Author: Dr. W. J. Lederer, M.D., Ph.D.

Corresponding Author's Institution: UMBI - University of Maryland Biotechnology Institute

First Author: Stephan E Lehnart, M.D.

Order of Authors: Stephan E Lehnart, M.D.; W. J. Lederer, M.D., Ph.D.

An antidote for calcium leak: targeting molecular arrhythmia mechanisms

Stephan E. Lehnart^{1,2*} and W. J. Lederer¹

¹ University Medical Center Goettingen, Center of Molecular Cardiology, Depts. of Cardiology & Pulmonology and Pharmacology, Germany; ² University of Maryland Biotechnology Institute, Medical Biotechnology Center, Baltimore, MD, U.S.A.

* Corresponding author

University Medical Center (UMG)

Center of Molecular Cardiology

Dept. of Cardiology & Pulmonology

Robert-Koch-Str. 40

37075 Goettingen

Germany

Email: slehnart@med.uni-goettingen.de

Phone: +49 551 39-10574

Fax: +49 551 39-10650

Ca²⁺ dependent arrhythmias

Ca²⁺ dependent arrhythmias were first appreciated as a distinct cellular signaling process when triggered extrasystoles were linked to abnormally elevated Ca²⁺ concentrations in the sarcoplasmic reticulum (SR) store. Thus large elevations of [Ca²⁺]_{SR} (or "SR Ca²⁺ overload") were shown to be linked to both early and delayed afterdepolarizations [1-6]. However, only recently have changes in Ca²⁺ sparks, the primary elemental SR Ca²⁺ release events, become associated with a molecular mechanism of SR Ca²⁺ leak and arrhythmogenesis (Fig. 1A). Specifically, missense mutations of the cardiac ryanodine receptor (RyR2) have been associated with an increased Ca²⁺ spark frequency and cell-wide arrhythmogenic Ca²⁺ waves that could underlie extrasystoles and lead to arrhythmias [7-9]. However, the molecular and physiological explanation of cellular Ca²⁺ instability and arrhythmogenesis does remain contentious [10, 11]. The debate has focused on issues of SR Ca²⁺ load, the open probability (P_o) and posttranslational modification of RyR2 Ca²⁺ release channels, and the concept of abnormal SR "Ca²⁺ leak".

In this issue of *The Journal of Molecular and Cellular Cardiology*, Knollmann and associates [12] investigate the surprising new antiarrhythmic properties of a well-known "type 1" antiarrhythmic drug, flecainide, better known for its ability to block cardiac Na⁺ channels (Na_v1.5) [13]. They reported previously that flecainide may be an effective antiarrhythmic agent in the treatment of a prototype Ca²⁺ dependent arrhythmia -- catecholaminergic polymorphic ventricular tachycardia (CPVT) [14]. CPVT is caused by mutations of either RyR2 or the SR Ca²⁺ binding protein calsequestrin, which may regulate RyR2 from the SR luminal side. According to the new study flecainide has dual antiarrhythmic actions: 1) it affects the intracellular SR Ca²⁺ release channel (RyR2) as well as 2) the sarcolemmal channel Na_v1.5 which mediates membrane depolarization (See Fig. 1B). The proposed mode of action of flecainide on RyR2 is novel: flecainide decreases Ca²⁺ leak by blocking the RyR2 / SR Ca²⁺ release channel in the open state. How would open channel block of RyR2 reduce the propensity for single cells to produce arrhythmogenic Ca²⁺ waves and for the heart to exhibit Ca²⁺ dependent arrhythmias in individuals carrying a CPVT mutation?

Calsequestrin mediated arrhythmia syndromes

In order to investigate the efficacy of flecainide in the treatment of Ca²⁺ dependent arrhythmias, calsequestrin deficient cells from the CASQ2^{-/-} mouse heart were employed. Indeed, earlier work by Knollmann and colleagues has established that the CASQ2^{-/-} mouse displays SR Ca²⁺ leak-induced ventricular tachycardias [15]. Calsequestrin2 is a low-affinity, high-capacity Ca²⁺ binding protein inside the SR store and may inhibit RyR2 Ca²⁺ leak at increased SR Ca²⁺ loads [15, 16]. Calsequestrin may also contribute to the development of the SR ultrastructure and the physical volume of the store [15]. The link to ventricular arrhythmias has been discovered in families with CPVT and CASQ2 mutations [17], and patients homozygous for mutant CASQ2 alleles were predicted to lack protein function [18] analogous to the CASQ2^{-/-} mouse heart used by Knollmann and associates [12]. Indeed, in vitro characterization of CASQ2 mutants showed reduced SR Ca²⁺ content and afterdepolarizations [19] and mouse models carrying human missense mutations showed stress-induced ventricular arrhythmias [20, 21]. Strikingly, different CASQ2 mutant hearts showed decreased mutant calsequestrin2 protein levels and Ca²⁺ triggered spontaneous Ca²⁺ waves [20]. Interestingly, in the CASQ2^{R33Q/R33Q} mouse model the width of the junctional SR has been found dilated by 2-fold [22]. In contrast, the CASQ2^{-/-} mice showed nonspecific increases in SR volume (+50%) [15]. If indeed mutant calsequestrin2 and/or associated protein changes cause destabilization of the RyR2 channel closed state as suggested earlier [16], treatment with flecainide may be confirmed by future studies in

arrhythmia models with mutant RyR2 or RyR2 dysregulated by other disease mechanisms to document drug efficacy beyond the extremely rare CASQ2 mutant CPVT2 syndrome.

The dynamic nature of calcium mediated arrhythmia mechanisms

SR Ca²⁺ load and SR Ca²⁺ leak. Increased SR Ca²⁺ leak leads to CPVT through a transient and cAMP dependent excess increase of the intrinsic RyR2 open probability (P_o) [7]. While under physiological conditions, catecholaminergic stress surges lead to increased $[Ca^{2+}]_{SR}$ load and increased intracellular Ca²⁺ transients (inotropy), in CPVT2 due to calsequestrin mutations [8] intracellular Ca²⁺ instability produces arrhythmogenic Ca²⁺ waves from increased SR leak leading to overall lower $[Ca^{2+}]_{SR}$ levels [20]. Importantly, increased SR Ca²⁺ leak and decreased SR load have been documented in the CASQ2^{-/-} deficient background as well [15].

Tetracaine and Caffeine. How will pharmacological agents affect the above relationships between leak and load? Tetracaine decreases the sensitivity of RyR2 to be activated by $[Ca^{2+}]_i$ at the cost of increasing $[Ca^{2+}]_{SR}$ at all steady-state conditions, an action thought to be due to tetracaine's ability to "block" the RyR2 channel pore. Therefore partial inhibition of SR Ca²⁺ release by tetracaine shifts the relationship towards increased $[Ca^{2+}]_{SR}$ and a reduced RyR2 $[Ca^{2+}]_i$ sensitivity [23]. On the other hand, low doses of caffeine, a classic activator of RyR2, drastically increase the sensitivity for any given $[Ca^{2+}]_i$, lower the threshold for Ca²⁺ waves and decrease $[Ca^{2+}]_{SR}$ [24]. Tetracaine has been found to exert anti-arrhythmic actions in catecholamine stimulated cardiomyocytes [25]. Tetracaine by inhibiting occurrence of intracellular Ca²⁺ waves shifts the cardiomyocyte Ca²⁺ leak-load balance from diastolic leak to systolic SR Ca²⁺ release at increased $[Ca^{2+}]_{SR}$ loads [12, 25].

Flecainide. In contrast to the above agents, flecainide appears to produce early termination of increased Ca²⁺ spark occurrence in leaky CASQ2^{-/-} cells. As an acute leak termination mechanism open state block of RyR2 is proposed by Knollmann and colleagues [12]. Interestingly, RyR2 open state block manifests through an increase in Ca²⁺ spark frequency which does not change spark-mediated SR leak (see Fig. 1, Hilliard et al. 2009 and below). These flecainide effects occur through an immediate increase in short-lived channel closures which result in decreased RyR2 mean burst duration and decreased P_o during burst activity. Also, and different to tetracaine, there is no apparent change in average closed times.

Previous investigations have established that the probability of RyR2 channel activity modification by ryanoids is significantly influenced by the transmembrane holding potential and drug charge [26]. At pH 7.4, solubilized flecainide may exist mainly in the ionized form, an important characteristic for use-dependent block of Na_v1.5 [27]. Since the RyR2 single channels have been recorded at positive potentials (+40 mV), the effects of flecainide could have been overestimated. Nonetheless, in permeabilized and low Ca²⁺ clamped wild-type rat cells (EGTA 0.36 mM) flecainide decreased the spark amplitude and width (the product of which is referred to as 'spark mass'), indicating inhibitory action against potentially pro-arrhythmogenic Ca²⁺ spark activity. Still through increased spark frequency, flecainide may lead to an increase in steady-state $[Ca^{2+}]_i$ which has been excluded by ratiometric Ca²⁺ imaging. In summary, shorter and smaller Ca²⁺ sparks occur from RyR2 block by flecainide and this may inhibit propagation of arrhythmogenic Ca²⁺ waves.

A analogy to global warming? CPVT may be viewed as analogous to "global warming:" CPVT conditions predispose the myocytes to undergo wild swings of intracellular Ca²⁺ dysregulation. Under certain stimulated conditions CASQ2^{-/-} cells produce excess changes in $[Ca^{2+}]_{SR}$ leading to dangerous storms of intracellular Ca²⁺ waves, a key measure of Ca²⁺-

dependent arrhythmogenesis. Surprisingly, flecainide inhibits the development of these storms possibly due to its unique effects on RyR2 gating. By simultaneously blunting excess swings of intracellular Ca^{2+} instability while decreasing the propensity of $\text{Na}_v1.5$ (I_{Na}) to contribute to aberrant diastolic membrane depolarizations, flecainide may inhibit two principal mechanisms of cellular arrhythmogenesis (Figure 1B).

Flecainide versus non-blocking RyR2 inhibitors. Are there similarities between the mode(s) of action between the RyR2 ion channel reagent JTV519 (and more recent RyR2 specific derivatives) that resemble the actions of flecainide? (for review see Lehnart 2007 [28]) JTV519 has inhibitory effects on I_{Na} and I_{Ca} ($\text{Ca}_v1.2$) which may decrease $[\text{Ca}^{2+}]_{\text{SR}}$ relative to untreated cells and thereby decrease Ca^{2+} wave propensity [29]. Additionally, JTV519 and in particular recent RyR2-selective derivatives like S107 have been found to exhibit specific effects on RyR2 gating behavior: stabilizing the protein kinase A (PKA) phosphorylated channel closed state through increased binding of the calstabin2 subunit [7]. The selective RyR2 stabilizing drugs like S107 do not show channel block activity and in that respect have a mode of action that is significantly different from flecainide [7, 30]. In this context the new flecainide results, while important, need further investigation to broaden our understanding of other flecainide actions on the RyR2 macromolecular channel complex in control hearts, CPVT hearts and $\text{CASQ2}^{-/-}$ hearts. In brief, flecainide appears to be an ion channel blocking agent (significantly different from RyR2 stabilizing analogs showing closed state stabilization) that produces many short-lasting, partial closed states which appear to preserve overall SR Ca^{2+} fluxes and thus do not affect $[\text{Ca}^{2+}]_{\text{SR}}$ load under the tested conditions.

Summary. Knollmann and colleagues provide intriguing evidence that flecainide may inhibit RyR2 calcium leak at the subcellular level by altering Ca^{2+} spark behavior and thereby reducing the linkage between the local Ca^{2+} flux during a Ca^{2+} spark and the activation of a nearby Ca^{2+} spark site that is needed to produce arrhythmogenic Ca^{2+} waves. This new antiarrhythmic mechanism provides special insight into a drug that was largely ignored after the "pro-arrhythmic" label it received following the CAST-I trial [31]. However, we also learn importantly from this study that thoughtful and mechanistic re-examination of cellular and subcellular behavior has enabled us to learn important new features of cardiac arrhythmic disease and treatment.

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Disclosure Statement

No conflicts to disclose.

Figure Legends

Figure 1. Mechanisms of Ca^{2+} dependent arrhythmias. **A.** In CPVT, RyR2 or CASQ2 mutations lead to an increased propensity for RyR2 Ca^{2+} leak which under stress conditions result in Ca^{2+} signaling instability, particularly as $[\text{Ca}^{2+}]_{\text{SR}}$ increases occur. Such increases in $[\text{Ca}^{2+}]_{\text{SR}}$ and molecular destabilization of the channel complex sensitize the RyR2 to $[\text{Ca}^{2+}]$; and

enable a given Ca^{2+} spark to abnormally trigger neighboring junctional SR release sites to produce regenerative Ca^{2+} sparks which would not occur under healthy control conditions. Abnormally increased Ca^{2+} spark activity may lead to intracellular Ca^{2+} waves and promote electrical membrane instability (afterdepolarizations) as well as multi-cellular electrical instability underlying arrhythmia 'triggers'. **B.** Flecainide blocks the burst activity of RyR2 and maybe the activation of neighboring junctional SR Ca^{2+} release sites by reducing the Ca^{2+} spark signaling mass [12]. The mechanism by which the Ca^{2+} spark mass is decreased is through open channel block which decreases RyR2 mean burst time even while the Ca^{2+} spark rate is increased [12]. Also, flecainide inhibits membrane depolarization by blocking $\text{Na}_v1.5$. The molecular actions of flecainide thus constitute a novel antiarrhythmic mechanism.

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Figure
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