HHS Public Access

Author manuscript

Circ Arrhythm Electrophysiol. Author manuscript; available in PMC 2016 June 01.

Published in final edited form as:

Circ Arrhythm Electrophysiol. 2015 June ; 8(3): 529–530. doi:10.1161/CIRCEP.115.002916.

Dual V_m/Ca Imaging of Premature Ventricular Contractions: Bridging the Gap of Anatomical Scales

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Keywords

Editorial; arrhythmia (mechanisms)

The role of pathological Ca^{2+} handling in mediating arrhythmia has been and continues to be a subject of intense experimental and theoretical investigation. Advances in dual optical mapping of membrane potential V_m and intracellular $[Ca^{2+}]_i$ coupled with improved mathematical models of myocyte Ca^{2+} handling have begun to reveal the role of $[Ca^{2+}]_i$ in mediating certain types of arrhythmia.

In particular, pathological diastolic Ca^{2+} release from the sarcoplasmic reticulum (SR) leads to Ca^{2+} extrusion via the Na-Ca exchanger (NCX). Because NCX is electrogenic, exchanging 3 Na^+ ions for 1 Ca^{2+} ion, Ca^{2+} extrusion leads to pathological diastolic membrane depolarization (termed delayed after-depolarization - DAD). If the magnitude of the DAD is sufficiently large, voltage-gated Na channels are activated and a triggered action potential ensues.[1] Indeed, the role of diastolic Ca^{2+} release and DADs in mediating both atrial and ventricular arrhythmias has now been well established in several cardiac pathologies, including catecholaminergic polymorphic ventricular tachycardia (CPVT) [2-4] and heart failure of different etiologies. [5,6]

Systolic Ca^{2+} elevation, on the other hand, can give rise to early afterdepolarizations (EADs) that occur in phase II/III of the action potential. EADs are also dangerously arrhythmogenic, leading to both triggered action potentials as well as prolongation of action potential duration (APD), enhancing dispersion of repolarization, a prerequisite for reentrant arrhythmias including Torsades de Pointe (TdP). EADs are particularly important in diseases of delayed repolarization, such as inherited and drug-induced LQT syndromes.[7] The mechanisms governing EADs, however, are disputed.

One theory posits that during delayed repolarization, L-type Ca^{2+} channels enter a state where both the activation and inactivation gates are open, allowing for I_{CaL} window current, which can give rise to EADs.[8] Thus, in contrast to DADs, this mechanism of EAD

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Conflict of Interest Disclosures: None.

generation does not directly involve SR Ca^{2+} handling. However, there is also significant evidence that EADs can occur through the same mechanism as DADs: via spontaneous SR Ca release and subsequent activation of depolarizing I_{NCX}.[9]

In this issue of *Circulation: Arrhythmia and Electrophysiology*, Kim *et al*. provide convincing evidence for the latter mechanism of EAD generation in a rabbit model of drug-induced LQT syndrome.[10] In particular, both high- and low- spatial resolution optical mapping of V_m and Ca^{2+} were performed in isolated rabbit hearts treated with dofetilide to delay repolarization. Kim and colleagues observed small 'islands' (~ 1 mm^2) of systolic Ca^{2+} elevation that preceded membrane depolarization by approximately 12 ms. This observation supports Ca^{2+} elevation mediated by SR Ca^{2+} release rather than opening of L-type Ca^{2+} channels, as I_{CaL} might be expected to have a faster impact on V_m. To further support this hypothesis, the authors administered the ryanodine receptor (RyR) stabilizing drug K201, which completely abolished ectopy in all treated hearts, while it was shown to have no effect on I_{CaL} at the concentrations used. The authors conclude that intracellular Ca^{2+} becomes elevated during delayed repolarization, which increases SR Ca^{2+} load as well as RyR sensitivity (which depends on both cytosolic and luminal Ca^{2+}). Thus, spontaneous systolic SR Ca^{2+} release occurs and may therefore represent a novel therapeutic target for arrhythmia suppression in LQT.

Despite these interesting and convincing findings, the results of this study raise several intriguing questions. The first, perhaps, is what dictates where the 'islands' of early Ca^{2+} elevation arise and are these islands sufficiently large to overcome the source-sink mismatch to produce a triggered action potential? With high-resolution optical mapping, Kim and colleagues observed small Ca^{2+} islands of approximately 1 mm^2 (upon earliest appearance) that then grew and fused with other islands to create large areas of Ca^{2+} elevation. The authors report that the islands did not appear to correspond to any particular anatomical feature and perhaps represent areas of altered Ca^{2+} handling protein expression. Indeed, increased expression of SERCA and/or decreased expression or phosphorylation of phospholamban may lead to locally increased SR Ca^{2+} load and, therefore, increased probability of spontaneous SR Ca^{2+} release.

Ectopic activity and triggered action potentials occurred following the emergence of Ca^{2+} islands, so at some point, the source-sink mismatch was indeed overcome. But at what point did this occur? Making a crude assumption that Ca^{2+} islands that are 1 mm in diameter (as observed on the epicardial surface) may represent approximately 1 mm^3 volume of tissue, this works out to approximately 20,000-30,000 myocytes (assuming myocyte volume = 30pL[11] and 30% extracellular space). Recent theoretical predictions have indicated that the critical number of cells required to generate an EAD of sufficient magnitude to produce a triggered action potential in healthy tissue is ≈700,000.[12] This number is reduced considerably to ≈230,000 required cells under conditions of reduced repolarization reserve,[13] yet this number is still an order of magnitude higher than roughly calculated here (≈20k-30k).

There are several potential explanations for this apparent discrepancy. The first is that the Ca^{2+} island dimensions reported by Kim and colleagues represent the size of the islands

when they were first detectable at the surface of the heart during high-resolution optical mapping, which depends on sensitivity, resolution and depth penetration of the imaging methodology. However, these early islands may not yet have generated sufficient depolarizing current to trigger a propagating action potential. Indeed, there was a delay (~12 ms) between emergence of Ca^{2+} islands and the V_m upstroke. Thus, it is possible, that sufficient depolarizing current was not achieved until the Ca^{2+} islands were much larger and fused with neighboring islands. Second, it is difficult to ascertain whether the reduction in repolarization reserve is similar between the experiment and simulation. [10,12] Furthermore, EAD generation in the model was not produced via spontaneous SR Ca^{2+} release, but rather through modification of membrane currents, thus the mechanism of EAD generation between model and experiment may be different. Finally, it may be that physiological factors not accounted for in the model are contributing to EAD generation and propagation and that the simulations have over-estimated the number of cells required. Regardless of the possible differences between the experiment and theoretical predictions, the issue of source-sink mismatch is a critical one and an area that may benefit from tight integration and iteration between model and experiment.

Another intriguing result reported by Kim *et al*. is the effectiveness of the RyR stabilizer K201 in suppressing ectopic activity in the rabbit model of drug-induced LQT. Treatment options for patients with inherited LQT are limited, depend on genotype/phenotype, and may include β-blockers, sodium channel blockers, potassium supplementation, or ICD implantation.[14] β-blockers are particularly effective in patients with LQT1, presumably because they prevent adrenergic-induced QT prolongation, as well as providing rate control.[14] In light of the findings here, β-blockers are potentially capable of preventing SR Ca^{2+} overload and release, as an additional mechanism of EAD prevention. Thus, RyR stabilization may potentially provide an exciting new therapeutic target for LQT syndromes.

In conclusion, dual imaging of V_m and $[Ca^{2+}]_i$ presents an important pathway for dissecting spatio-temporal mechanisms of Ca^{2+}-mediated ectopic activity leading to lethal arrhythmias. To fully comprehend the mechanism of DADs and EADs, we need tools to infer cellular events in intact tissue preparations, because isolated cell lacks many fundamental features of tissue, such as cell-cell coupling, conduction, extracellular matrix, etc. Kim *et al*. presented particularly powerful dual imaging methodology, which is capable of spanning several anatomical scales of excitation-contraction coupling in intact heart preparation. Future development of this approach will hopefully allow full three-dimensional reconstruction of sites of origin of Ca^{2+}-mediate DADs and EADs.

Acknowledgments

Funding Sources: CMR is supported in part by the US National Institutes of Health (R01 HL111600) and the American Heart Association (12SDG9010015). IRE is supported by National Institutes of Health (R01 HL114395 and R01 HL115415).

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