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Calcium Leaks: Initiator of Atrial Fibrillation?

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The concerted intracellular movement of calcium (Ca) from sarcoplasmic reticular (SR) stores to the cytosol and resequestration back into the SR is crucial not only to the excitation-coupling process but also to a number of signaling cascades that utilize Ca either as a second/tertiary messenger or as an end-effector.^{1–3} Consequently, the release and trafficking of intracellular Ca is regulated at multiple levels, including the release of SR Ca through the SR Ca release channel – or, the ryanodine receptor (RyR).^{1;4} In terms of the excitation-contraction coupling process in cardiac myocytes, “trigger” Ca that enters the cells through the L-type Ca channels activates RyR channels, causing the release of Ca from SR stores, providing Ca to the myofilaments that is required for contraction.¹ Abnormalities in the amount of released Ca – either too much or too little – not only affect contraction of the myocytes, but also can be arrhythmogenic.⁵

Failure to terminate SR Ca release during each cardiac cycle results in a Ca “leak”, making additional Ca available to the myofilaments. There are many factors that can cause prolonged release of Ca from the SR, including inappropriate availability of trigger Ca from the L-type Ca channels, prolonged opening of the RyR channels, or failure of the RyR channels to close completely. In this regard, the closing of the RyR channels is regulated in part by the binding of the RyR channels to a small, 12.6 kDa peptide, named FKBP12.6, or calstabin-2.^{4;6–8} In normal physiology, the opening probability of the RyR channels during the resting membrane phase of the action potential is low and associated with the close coupling of calstabin-2 with the RyR complex.^{6;9;10} Phosphorylation of the RyR channels by protein kinase A (cyclic AMP dependent), which occurs at a site that is in close proximity to the calstabin-2 binding site, has been reported to dissociate the RyR – calstabin-2 complex and increases SR Ca release.^{6;7} Point mutations in RyR that adversely influence efficient binding to calstabin-2 have been identified in patients susceptible to exercise-induced (likely to be catecholaminergic) ventricular tachycardia.⁹ Dissociation of calstabin-2 from the RyR complex, either due to increased protein kinase A-mediated phosphorylation of RyR or due to genetic defects in calstabin-2 availability and/or binding to RyR is associated with arrhythmogenicity.^{9;10} For example, in ventricular myocytes isolated from calstabin-2 knockout mice, inward ionic currents and Ca leak from the SR were recorded during diastole, when neither should have been observed.¹⁰ These electrogenic abnormalities with calstabin-2 deficiency at the level of the myocyte were associated with an abnormal alternating pattern of action potentials *in vivo*. Conversely, transgenic overexpression of calstabin-2 was associated with a reduction in the incidence of stimulation-induced tachycardia in mice that were pretreated with isoproterenol.¹¹ Concomitantly, myocytes isolated from calstabin-2 overexpressing mice

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exhibited fewer Ca release events.¹¹ Taken together, abnormalities in either binding of calstabin-2 to RyR and/or abundance of calstabin-2 can lead to Ca leak from the SR and form the basis of triggered arrhythmogenic foci.

In this issue, Sood *et al.* extend the findings of past studies on ventricular arrhythmias to stimulation-induced atrial fibrillation (AF) and SR Ca leak in atrial myocytes of mice transgenically deficient in calstabin-2.¹² While there were no differences in atrial geometry and baseline electrophysiological parameters (heart rate, refractory periods, and sinus node recovery times) between the calstabin-2 deficient mice and wild type littermates, provocative stimulation within the right atrium induced short bursts of AF in a greater percentage of the calstabin-2 deficient mice. Concomitantly, an increase in Ca leak from the SR and spontaneous Ca release events during recovery from rapid stimulation were noted in atrial myocytes from calstabin-2 deficient mice when compared to myocytes obtained from the wild type mice. Using tetracaine at a dose that is known to affect RyR channel closing, AF inducibility and SR Ca leak were reduced in the *in vivo* and *in vitro* studies, respectively. Unfortunately, the authors do not show an *ex vivo* correlate to inducible arrhythmias in the form of afterdepolarizations in recorded myocyte action potentials. It is of interest, however, to note that the inducible electrophysiological abnormalities were observed in the absence of differences in right atrial geometry, atrial ultrastructure, or in the abundance of several key ionic processes responsible for Ca homeostasis.

AF, which is the most common reason for hospitalizations for arrhythmias, is often progressive, with episodes that go from being paroxysmal to persistent and then permanent. In addition to atrial dilation and remodeling of the atrial myocytes and the extracellular matrix that occurs with persistent and/or permanent AF, alterations in processes responsible for Ca homeostasis, including L-type Ca channels, RyR channel functionality, and Ca uptake into the SR, have been documented.¹³ Moreover, the severity of changes in atrial structure and ionic processes worsen as a function of AF duration, suggesting a time-dependent component to AF perpetuation and the putative mechanisms responsible for AF maintenance. Nevertheless, as with most other pathological conditions, it is often difficult to discern events that precipitate an increased vulnerability to AF initiation. In this light, it is interesting to note that Sood *et al.* report that enhanced AF inducibility in the calstabin-2 deficient mice occurred despite the mRNA and protein levels of RyR channels, SR Ca reuptake channels, and sodium calcium exchanger being similar to that of the wild type mice.¹² Moreover, there was no evidence of inappropriate atrial fibrosis, a finding that is commonly reported in AF patients and in animal models of AF. Taken together, it is possible that a reduction in calstabin-2 and/or inefficient binding of calstabin-2 to RyR channels may provide a mechanistic underpinning for AF initiation through the formation of arrhythmogenic foci and localized, self-terminating pathways. With increasing AF durations, defects in the other key Ca homeostatic processes and remodeling of the atrial myocardium are likely to become manifest leading to the progressive severity of AF episodes.

AF is a disease process that predominantly affects the elderly. AF prevalence increases from 0.1% in adults younger than 50 years to 9% in persons aged 80 years or older. Given that the life of mice is approximately 2 years, the mice used in the study by Sood *et al.* (3 to 5 months) likely corresponded to “young adult” or “middle-aged”; an age at which spontaneous AF would not be anticipated in humans.¹² It would be of interest to determine whether calstabin-2 deficiency would be associated with the development of spontaneous AF in aged mice (15 – 18 month old). Nevertheless, Sood *et al.* have provided evidence that Ca leak and/or release through the RyR channels occurred in the calstabin-2 deficient mice. Prolonged leakage of SR Ca could culminate in a slow, but steady, increase in cytosolic Ca concentrations, which in turn may alter Ca-dependent functioning of a number of ion channels and the efficiency of transduction of intracellular signaling cascades that depend on Ca as the second messenger.

^{1,3} As an example, Ca is required for activation of some of the protein kinase C isozymes, which in turn can modulate synthesis of proteins, including of those that constitute and/or regulate increased deposition of the extracellular matrix. Therefore, studies examining whether the age-dependence of ion channel function and alterations in extracellular matrix content and composition are differentially modulated in the setting of calstabin-2 deficiency are warranted. Nevertheless, studies such as the one by Sood *et al.* are provocative in that the results provide an electrogenic basis for AF initiation.¹² Further understanding of the long-term effects of calstabin-2 deficiency on other ionic processes and/or development of spontaneous AF will likely provide further insight into pathogenesis and progression of AF.

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