Published in final edited form as:

J Mol Cell Cardiol. 2012 February ; 52(2): 304–311. doi:10.1016/j.yjmcc.2011.06.024.

Dynamic local changes in sarcoplasmic reticulum calcium: physiological and pathophysiological roles

Eric A. Sobie¹ and W.J. Lederer²

¹Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine, New York, NY, USA

²BioMET, Center for Biomedical Engineering & Technology, University of Maryland, Baltimore, MD USA

Abstract

Evidence obtained in recent years indicates that, in cardiac myocytes, release of Ca^{2+} from the sarcoplasmic reticulum (SR) is regulated by changes in the concentration of Ca^{2+} within the SR. In this review, we summarize recent advances in our understanding of this regulatory role, with a particular emphasis on dynamic and local changes in SR $[\text{Ca}^{2+}]$. We focus on five important questions that are to some extent unresolved and controversial. These questions concern: (1) the importance of SR $[\text{Ca}^{2+}]$ depletion in the termination of Ca^{2+} release; (2) the quantitative extent of depletion during local release events such as Ca^{2+} sparks; (3) the influence of SR $[\text{Ca}^{2+}]$ refilling on release refractoriness and the propensity for pathological Ca^{2+} release; (4) dynamic changes in SR $[\text{Ca}^{2+}]$ during propagating Ca^{2+} waves; and (5) the speed of Ca^{2+} diffusion within the SR. With each issue, we discuss data supporting alternative viewpoints, and we identify fundamental questions that are being actively investigated. We conclude with a discussion of experimental and computational advances that will help to resolve controversies.

Keywords

Ca^{2+} spark; Ca^{2+} wave; Ca^{2+} transient; arrhythmia; triggered activity; ventricular myocyte; mathematical modeling

Introduction

In the heart, release of Ca^{2+} from the sarcoplasmic reticulum (SR), through a mechanism known as Ca^{2+} -induced Ca^{2+} release (CICR), is centrally involved in both physiological and pathophysiological processes. Each time the heart beats, Ca^{2+} entering cardiac myocytes through L-type Ca^{2+} channels triggers release of Ca^{2+} through ryanodine receptors (RyR) in the SR membrane, and the resulting increase in cytosolic $[\text{Ca}^{2+}]$ enables robust contraction. However, since the trigger for SR Ca^{2+} release is Ca^{2+} itself, a small amount of released Ca^{2+} can, under pathological conditions, trigger CICR in a regenerative manner. This Ca^{2+}

© 2010 Elsevier Ltd. All rights reserved.

Editorial Correspondence: Eric A. Sobie, Mount Sinai School of Medicine, One Gustave Levy Place, Box 1215, New York, NY 10029, USA; Tel (212) 659-1706; FAX: (212) 831-0114; eric.sobie@mssm.edu.

Disclosures: None to declare

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

release, which occurs in the form of a propagating Ca^{2+} wave, can potentially depolarize the cell membrane and initiate dangerous cardiac arrhythmias.

Work performed over the past two decades has demonstrated that cytoplasmic control of CICR depends primarily on local rather than global (cell-wide) changes in $[\text{Ca}^{2+}]$, as described in detail by Cannell and Kong in this issue [1]. An important structural feature that enables this local control is the fact that RyRs are not distributed uniformly in the SR membrane but instead are arranged in clusters [2-4]. Ca^{2+} release from an individual cluster of RyRs can be visualized experimentally as a localized increase in fluorescence known as a Ca^{2+} spark [5], and Ca^{2+} transients in healthy cells consist of the nearly synchronous triggering of thousands of such units. Ca^{2+} sparks are primarily triggered by local openings of L-type Ca^{2+} channels [6, 7], although recent work suggests that L-type openings may work in concert with local entry of Ca^{2+} through $\text{Na}^{+}\text{-Ca}^{2+}$ exchangers [8, 9]. Pathological spontaneous Ca^{2+} waves also consist of Ca^{2+} sparks [10]. In this case, waves occur when spontaneous Ca^{2+} sparks trigger additional events; therefore, local coupling between RyR clusters determines whether or not these potentially deadly events occur [11]. Finally, in pathological conditions such as heart failure, a frequent observation is a loss of the close coupling between L-type Ca^{2+} channels and RyR clusters, due in part to remodeling of the T-tubule system and subsequent “orphaning” of RyR clusters [12-15]. As noted in a recent review [16], these structural derangements reduce the synchrony of triggered Ca^{2+} transients and may contribute to an increased propensity for arrhythmias. Interestingly, the process of T-tubule remodeling during disease causes CICR in ventricular cells to more closely resemble CICR in atrial [17, 18] or Purkinje myocytes [19, 20], cells for which asynchronous Ca^{2+} release represents normal physiology. All of these examples highlight the importance of local cytosolic signaling in the regulation of SR Ca^{2+} release in heart cells.

More recently, it has become clear that dynamic changes in the amount and distribution of Ca^{2+} in the SR also play important regulatory roles. By analogy with regulation by cytoplasmic $[\text{Ca}^{2+}]$, it seems logical that local rather than global changes will be determinative. Largely for technical reasons, however, our understanding of the role played by local changes in SR $[\text{Ca}^{2+}]$ has lagged behind our understanding of local changes in cytosolic $[\text{Ca}^{2+}]$. As a result several key questions are still open. The goals of this review are to highlight recent advances in our understanding of the importance of local changes in SR $[\text{Ca}^{2+}]$, and to identify issues that remain unresolved and perhaps somewhat controversial.

While we focus here primarily on regulation of release by dynamic and local changes in SR $[\text{Ca}^{2+}]$, other reviews in this issue (e.g. Cannell and Kong [1]) and elsewhere address related matters of importance. For instance, average SR $[\text{Ca}^{2+}]$ content is well known to affect the “gain” of CICR [21, 22], to determine a threshold for spontaneous Ca^{2+} waves [23, 24], and to influence the rate of Ca^{2+} “leak” from the SR in quiescent cells.[25, 26] In addition, modulation of RyR sensitivity is known to produce, over the course of several beats, offsetting changes in SR $[\text{Ca}^{2+}]$ through a process termed “autoregulation” [22, 24]. The important studies that provided these insights are not covered in detail. We also, in the interest of brevity, do not discuss beat-to-beat changes in Ca^{2+} transient amplitude, or alternans, even though evidence suggests that SR $[\text{Ca}^{2+}]$ is critically important for the development of some [27, 28] but perhaps not all forms of alternans [29, 30]. See recent reviews for more discussion of this interesting and clinically relevant topic [31, 32].

We will focus on 5 inter-related questions related to the regulatory role played by dynamic local changes in SR $[\text{Ca}^{2+}]$:

1. What is the role of changes in SR $[\text{Ca}^{2+}]$ in regulating SR Ca^{2+} release?

2. What is the extent of local SR Ca^{2+} depletion during release events such as Ca^{2+} sparks?
3. How do dynamic changes in SR $[\text{Ca}^{2+}]$ influence the probability of pathological Ca^{2+} release?
4. How do transient and local changes in SR $[\text{Ca}^{2+}]$ influence Ca^{2+} waves?
5. How fast is Ca^{2+} diffusion within the SR?

1) What is the role of changes in SR $[\text{Ca}^{2+}]$ in regulating SR Ca^{2+} release?

For many years after the discovery of Ca^{2+} sparks, mechanisms underlying spark termination remained one of the primary unsolved questions, as review articles published around the turn the century highlighted [33-35]. Some studies speculated that because SR $[\text{Ca}^{2+}]$ should decline during release, depletion could contribute to the termination process [36]. However, since a considerable reserve of $[\text{Ca}^{2+}]$ remained in the SR after release [37], and pharmacological agents could produce extremely long-lasting local SR Ca^{2+} release events [5], it was clear that Ca^{2+} exhaustion, or the SR simply running out of Ca^{2+} , was not the only answer.

Even if SR $[\text{Ca}^{2+}]$ did not deplete to zero during Ca^{2+} release, a hint that dynamic decreases in SR $[\text{Ca}^{2+}]$ might be important came from the observation that SR luminal $[\text{Ca}^{2+}]$ affects RyR gating. In planar lipid bilayer experiments, it was shown that RyR open probability is considerably greater at high than at low SR $[\text{Ca}^{2+}]$ [38, 39]. The idea that dynamic changes in SR $[\text{Ca}^{2+}]$ contribute to termination gained considerable momentum in 2002 with two important publications. Along with coworkers, we published a mathematical modeling study demonstrating that a termination mechanism based on local depletion of SR $[\text{Ca}^{2+}]$ could in principle account for diverse experimental results [40]. Roughly simultaneously, Terentyev et al published data consistent with this idea [41]. Specifically, these authors increased the buffering power of the SR, an intervention designed to slow depletion of SR $[\text{Ca}^{2+}]$ without affecting cytosolic regulatory processes such as Ca^{2+} -dependent inactivation. Because this prolonged the duration of Ca^{2+} sparks, it indicated the importance of depletion for release termination.

Importantly, several subsequent studies have provided additional support for this hypothesis. The development of fluo-5N as an indicator for SR $[\text{Ca}^{2+}]$ [42, 43] made it possible to observe local depletion of JSR $[\text{Ca}^{2+}]$, or so-called Ca^{2+} “blinks” [44]. Short-term changes in the expression of calsequestrin (CSQ), the primary SR Ca^{2+} buffer, altered the duration of Ca^{2+} release [45], as required by the hypothesis, and a series of studies on mutant forms of CSQ provided additional confirmatory evidence [46-48]. Partial inhibition of RyRs, which increased baseline SR $[\text{Ca}^{2+}]$ and slowed the rate of depletion, lengthened the duration of local release, dramatically in some cases [49, 50]. Moreover, studies on regenerative Ca^{2+} waves suggested that dynamic changes in SR $[\text{Ca}^{2+}]$ needed to be considered to explain the properties of these events. [51, 52]. Finally, studies performed at both the cellular [53] and Ca^{2+} spark [54, 55] levels have indicated that SR $[\text{Ca}^{2+}]$ refilling plays an important role in the recovery of Ca^{2+} release. Together these observations have provided compelling support for the idea that changes in SR $[\text{Ca}^{2+}]$ influence release, and specifically that local depletion of SR $[\text{Ca}^{2+}]$ is critical for release termination.

At the molecular level, however, mechanisms of sensing changes in local SR $[\text{Ca}^{2+}]$ remain somewhat unclear. Planar lipid bilayer studies have shown that isolated RyRs have luminal binding sites for Ca^{2+} and that channel gating displays an intrinsic dependence on SR $[\text{Ca}^{2+}]$ [56]. The effects of luminal $[\text{Ca}^{2+}]$ on RyR open probability, however, are much greater in the presence of CSQ than in its absence, implying that CSQ may serve as the main link

between SR $[Ca^{2+}]$ and RyR gating [57, 58]. There is disagreement, however, about whether the primary effect of CSQ on RyRs is activating [57] or inhibitory [58]. In addition, although changes in the expression of accessory proteins such as triadin and junctin clearly influence SR Ca^{2+} release [59, 60], the role of these proteins in sensing SR $[Ca^{2+}]$ remains incompletely understood. Determining these regulatory interactions, and how they may be modified physiologically and in disease states, is likely to be a significant focus of research over the next few years.

Regardless of the molecular mechanisms, it is clear that local depletion of SR $[Ca^{2+}]$ regulates release. One of the key questions therefore becomes: by how much does SR $[Ca^{2+}]$ deplete during local release events such as Ca^{2+} sparks?

2) What is the extent of local SR Ca^{2+} depletion during release events such as Ca^{2+} sparks?

The extent of JSR depletion during release events can be estimated using either mathematical models or experimental measurements of SR $[Ca^{2+}]$. Simulations of depletion [40, 54, 61] require assumptions of important variables such as JSR volume, JSR buffer concentration, RyR cluster size, the Ca^{2+} flux carried through each open RyR, and the rate of JSR refilling from neighboring network SR. With the development of fluorescent indicators such as fluo-5N for SR $[Ca^{2+}]$ measurements [42, 43], it became possible to directly measure changes in SR $[Ca^{2+}]$. The JSR depletion signal during a Ca^{2+} spark has been detected and named a Ca^{2+} “blink” [44]. Despite the obvious appeal of assessing JSR depletion using fluo-5N recordings, it is important to emphasize that the interpretation of such experimental records requires its own set of assumptions such that converting from fluorescence to local SR $[Ca^{2+}]$ is not straightforward. Possible issues with both modeling and experimental estimates of the extent of JSR depletion are discussed below.

Some back-of-the-envelope calculations are useful and provide an important context for the interpretation of interpret modeling and experimental results. The volume of each JSR unit can be calculated by assuming that the JSR is shaped like a disk or “pancake.” If the JSR lumen has a thickness of 32 nm, then a JSR unit with radius 100 nm will have a volume of 1×10^{-18} L (aL), and a JSR unit with radius 200 nm will have a volume of 4 aL. (Based on estimates of RyR size and packing in arrays [62], these JSR units would be able to hold 25 and 100 RyRs, respectively). At a free SR $[Ca^{2+}]$ concentration of 1 mM, the larger volume of 4 aL will contain only 2400 free Ca^{2+} ions. Of course, most Ca^{2+} ions in the JSR are likely to be bound to CSQ rather than free. This buffering may increase the total $[Ca^{2+}]$ content of the JSR by a factor of 20, but probably not by more than this. Carefully calibrated measurements of total SR content using caffeine application place strong constraints on the total amount of Ca^{2+} stored in the SR. If total SR $[Ca^{2+}]$ is 100 $\mu\text{mol/L}$ cytosol [63], cytosolic volume is 20 times total SR volume [64], and JSR is 10% of the total SR volume [64], then total JSR $[Ca^{2+}]$ is roughly 20 mM. Thus, somewhere in the neighborhood of 50,000 Ca^{2+} ions are present in each JSR compartment before local Ca^{2+} release. Planar lipid bilayer measurements of RyR permeation under physiological conditions estimate that each open channel carries ~ 0.5 pA of current [65], a flux that corresponds to approximately 1500 Ca^{2+} ions per millisecond. Thus, 8 open RyRs carrying 0.5 pA each would completely deplete a JSR compartment in fewer than 4 ms. This simple calculation makes two things apparent. The first is that an RyR current of 0.5 pA can only represent the initial or maximal flux through each open RyR. As the JSR depletes, the Ca^{2+} flux through each open RyR must also decrease. The second key point is that the JSR is unlikely to contain all of the Ca^{2+} ions that are released during each Ca^{2+} spark, and JSR refilling from neighboring NSR regions will occur during Ca^{2+} release. Slow refilling will allow for nearly complete depletion whereas fast refilling will limit the extent of SR depletion, and quantifying the refilling rate is therefore quite important (see below). In our present mathematical modeling,

we constrain refilling based on measurements of repetitive Ca^{2+} sparks from individual RyR clusters. These data indicate that Ca^{2+} spark amplitude, which models suggest is proportional to total JSR [Ca^{2+}], recovers with a time constant of 90 ms [54, 55]. It should be noted, however, that current mathematical models do not capture the considerable heterogeneity in refilling speed that has been observed experimentally [50].

As a direct result of the parameter constraints just discussed, estimates of JSR depletion based on mathematical models have consistently predicted strong depletion of roughly 90% [40, 54, 61]. In contrast, measurements of Ca^{2+} blinks imply that the JSR only depletes by ~50% during a typical Ca^{2+} spark [44, 47, 50], similar to the extent of SR depletion measured at the cellular level during Ca^{2+} transients [37, 43]. It is possible that the mathematical models overestimate the extent of depletion due to an error in one of the assumptions discussed above. However, it is also possible that blinks underestimate the extent of JSR depletion because these experimental signals are, like all measurements, imperfect representations of reality. Some issues that should be considered are the following. First, the point spread function of a typical confocal microscope is a prolate spheroid with typical dimensions (full-width at half maximum) of 400 nm in the lateral dimensions and 800-1000 nm along the optical axis. Thus, a single pixel records from a volume of roughly 0.2 fL, and the local JSR may not always coincide with the microscope plane of focus. Because the microscope recording volume is 50-100 times greater than the volume of each JSR unit therefore, it seems likely that most of the fluorescence recorded during a Ca^{2+} blink originates outside the JSR unit immediately adjacent to the RyRs, even after accounting for the fact that SR occupies 3% of the total cell volume [64]. If Ca^{2+} depletion in this neighboring network SR is less than the depletion in the local JSR, then the blink will underestimate the local depletion. A second issue concerns spatial averaging. In an ideal recording environment, the amplitude of a local signal would be determined by plotting fluorescence from a single pixel. However, confocal fluorescence recordings are quite noisy, and investigators routinely average over several pixels to generate the time course plots that are displayed in publications. This has the effect of further degrading the spatial resolution of the measurements.

Third, dye may be distributed non-uniformly within the SR, perhaps as a result of fluo-5N binding to proteins such as CSQ, since fluo-5N images routinely appear brighter at Z-lines compared with the regions between Z-lines [43, 44]. A fourth potential issue relates to dye kinetics. Calibration experiments have suggested that the K_d for Ca^{2+} of fluo-5N, which is 70 μM in free solution, is likely to be larger (~400 μM) in the cellular environment due to dye-protein interactions [43]. These steady-state measurements, however, could not assess how proteins influence the kinetics of Ca^{2+} binding to fluo-5N. If Ca^{2+} unbinding from fluo-5N is slow in the cellular environment, this would also cause fluo-5N signal to underestimate the true extent of JSR depletion. A tool that would help to address these issues would be a mathematical model that simulates not only Ca^{2+} release and refilling, but also processes such as binding to the indicator, blurring by the confocal microscope, and noise, in order to estimate quantitatively how particular changes in [Ca^{2+}] are translated into measurable signals.

Given these caveats, a disagreement between 90% depletion as predicted by mathematical models [40] versus 50% depletion as inferred from measurements [50] strikes us as a point of contention that is not insurmountable. It seems relatively easy to envision how small changes in a few important assumptions may bring these divergent estimates into agreement.

3) How do dynamic changes in SR $[Ca^{2+}]$ influence the probability of pathological Ca^{2+} release?

If local depletion and refilling of SR $[Ca^{2+}]$ contribute, respectively, to release termination and recovery, then changes in depletion or refilling should have consequences for both normal and pathological SR Ca^{2+} release. Indeed, several experimental results suggest that acceleration of SR $[Ca^{2+}]$ refilling may abbreviate release refractoriness and increase the risk of spontaneous release in the form of propagating Ca^{2+} waves. This pathological Ca^{2+} release has been termed SOICR (store overload induced Ca^{2+} release) in some publications [66, 67]. Although these studies have provided novel insight into the consequences of RyR mutations, it is important to emphasize that SOICR does not constitute an alternative or independent phenomenon [68]. Instead, it is a manifestation of two mechanisms described above: (1) Ca^{2+} waves are produced when sparks trigger additional sparks through CICR in the cytosol; and (2) SR $[Ca^{2+}]$, through its modulation of RyR gating, affects the probability that this occurs.

Several studies provide evidence that dynamic changes in SR $[Ca^{2+}]$ influence pathological Ca^{2+} release. For instance, a recent study in intact rat hearts showed that an increase in either extracellular $[Ca^{2+}]$ or pacing rate, which presumably caused faster uptake of Ca^{2+} into the SR via SERCA (Sarco-endoplasmic reticulum Ca^{2+} ATPase), reduced the average latency to the appearance of regenerative Ca^{2+} waves [69]. Alterations in SERCA activity can also influence local Ca^{2+} signaling, as increased SERCA activity (through ablation of phospholamban) can accelerate Ca^{2+} spark decay [70], SERCA inhibition can slow Ca^{2+} spark decay [71], and increased SERCA activity can cause faster restitution of Ca^{2+} spark amplitude [55].

Additional evidence comes from experimental models of catecholaminergic polymorphic ventricular tachycardia (CPVT), an arrhythmia disorder caused by mutations in either RyR or CSQ. Several *in vitro* studies have shown that certain CPVT-causing mutations in CSQ cause faster recovery of SR $[Ca^{2+}]$ and abbreviated refractoriness after release. These changes are likely to contribute to the increased propensity for pathological Ca^{2+} waves and arrhythmias caused by these mutations [46, 47]. Similarly, cardiac-specific CSQ deletion in mice produces a robust experimental model of CPVT [72, 73]. In particular, the CSQ-null mouse generated by Knollmann's group [72], which has been intensively studied over the past few years, exhibits several interesting features that raise questions for future work. For instance, deletion of the primary SR Ca^{2+} buffer would be expected to cause faster recovery of SR $[Ca^{2+}]$ after release. Indeed, a preliminary study has documented accelerated Ca^{2+} release restitution in CSQ-null mice [74]. This effect, when combined with increased diastolic leak of Ca^{2+} observed in cells from these hearts [72], can contribute to the increased propensity ventricular arrhythmias in these mice. However, an interesting observation in the initial study [72] was an increase in the volume of network SR. The functional consequences of this change remain unknown. Similarly, in a recently-generated knock-in model, mice homozygous for the R33Q mutation in CSQ exhibited not only the CPVT phenotype of enhanced spontaneous Ca^{2+} release and ventricular arrhythmias, but also unexpected structural changes, including increased JSR volumes and decreased levels of junctin and triadin [75]. How these changes either contribute to, or protect against, arrhythmias is not yet clear.

Thus, although many experimental models exhibit ventricular arrhythmias and an increased propensity for pathological Ca^{2+} waves, much remains to be determined about mechanisms controlling release refractoriness in these models. For example, can accelerated release restitution in CSQ-null mice be explained entirely from faster recovery of free SR $[Ca^{2+}]$, or does CSQ regulation of RyR gating also need to be considered? To address such questions, methods that examine Ca^{2+} release refractoriness at the spark level are likely to be quite

useful. We developed a method to generate repetitive Ca^{2+} sparks from individual RyR clusters and, through careful analysis of the data, infer separate time courses describing recoveries of Ca^{2+} spark amplitude and triggering probability [54]. In a follow-up study using this protocol, we showed that RyR sensitivity influences the recovery of spark triggering but does not affect the recovery of spark amplitude [55]. It is quite likely that these types of experiments can help to illuminate mechanisms that elevate diastolic Ca^{2+} spark rate and arrhythmia risk in disease models.

4) How do transient and local changes in SR $[\text{Ca}^{2+}]$ influence Ca^{2+} waves?

The previous section focused on recovery of SR $[\text{Ca}^{2+}]$ after release, and how this influences the probability of pathological spontaneous release in the form of a propagating Ca^{2+} wave. A closely related question is the following: how does SR $[\text{Ca}^{2+}]$ change during a Ca^{2+} wave as a function of space and time, and how do these changes either encourage or discourage wave propagation? Provocative experimental results were presented by Keller et al [76]. In guinea pig myocytes, these authors found that partial inhibition of SERCA immediately decreased the velocity of Ca^{2+} wave propagation without affecting wave amplitude or cytosolic $[\text{Ca}^{2+}]$ prior to the wave. Since waves propagate via CICR when Ca^{2+} sparks trigger additional sparks, acute SERCA inhibition would be expected to reduce uptake in the region between adjacent RyR clusters, increase cytosolic $[\text{Ca}^{2+}]$ at the unactivated cluster, and thereby accelerate rather than retard Ca^{2+} waves. To explain this surprising result (which has not been observed by all groups [77]), Keller et al proposed that during propagation of waves, $[\text{Ca}^{2+}]_{\text{SR}}$ may increase locally at unactivated sites, thereby sensitizing these RyR clusters and speeding propagation [76].

This intriguing hypothesis is difficult to test directly, however, due to technical issues such as those discussed above: small SR volumes, limited spatial resolution, and inadequate signal-to-noise. These types of ideas can, however, be addressed through mathematical modeling [61, 78, 79]. For instance, Ramay et al recently implemented a model that calculates changes in both cytosolic and SR $[\text{Ca}^{2+}]$ during Ca^{2+} waves (Fig. 1), and examined systematically how dynamic local concentration changes are influenced by parameters controlling diffusion and SERCA activity [61]. Interestingly, the speed of Ca^{2+} diffusion within the SR was the most important determinant of changes in SR $[\text{Ca}^{2+}]$ occurring ahead of Ca^{2+} waves. If SR Ca^{2+} diffusion was extremely fast ($300 \mu\text{m}^2/\text{s}$), SR $[\text{Ca}^{2+}]$ always decreased at unactivated sites. However, sensitization waves as proposed by Keller et al could occur with moderate ($60 \mu\text{m}^2/\text{s}$) or slow ($12 \mu\text{m}^2/\text{s}$) SR Ca^{2+} diffusion, with slower diffusion causing more pronounced sensitization. This study [61] illustrates the usefulness of modeling for delineating how physical or geometric variables can influence concentration changes during SR Ca^{2+} release. It also points to the potential importance of SR Ca^{2+} diffusion in determining physiological responses.

5) How fast is Ca^{2+} diffusion within the SR?

As noted above, mathematical modeling suggests that the effective Ca^{2+} diffusion coefficient within the SR ($D_{\text{Ca,SR}}$) significantly influences dynamic local changes in SR $[\text{Ca}^{2+}]$ during Ca^{2+} waves. In recent years two groups have attempted to quantify the speed of diffusion [80, 81]; unfortunately, the estimates obtained in these two studies differed by nearly an order of magnitude, making the value of $D_{\text{Ca,SR}}$ a prominent unresolved and controversial issue. Wu & Bers combined SR fluo-5N measurements with local caffeine application in rabbit ventricular myocytes and calculated that $D_{\text{Ca,SR}}$ is approximately $60 \mu\text{m}^2/\text{s}$ [81]. In contrast, Swietach et al, in rat and guinea pig myocytes, combined cytosolic fluo-3 measurements with repeated local caffeine applications and concluded that $D_{\text{Ca,SR}}$ is much lower, roughly $8 \mu\text{m}^2/\text{s}$ [80]. The reasons for this discrepancy are not fully

understood; however, as noted above, such a quantitative difference can have important implications for the changes in SR $[Ca^{2+}]$ that occur during Ca^{2+} release.

More recently, Bers and coworkers have obtained additional data using fluo-5N to support their earlier suggestion of moderately fast SR Ca^{2+} diffusion [82]. Specifically, these authors demonstrated that during Ca^{2+} sparks, gradients of $[Ca^{2+}]$ exist between JSR and NSR regions. However, during global release in which many Ca^{2+} sparks are triggered, gradients between JSR and NSR regions could not be detected. The experiments in this study were complemented with mathematical modeling. The simulations could approximately reproduce the experimental results if $D_{Ca,SR} = 60 \mu m^2/s$ was assumed, but could not reproduce the results if much slower diffusion (i.e. $8 \mu m^2/s$, as estimated by Swietach et al [80]) was assumed, thereby supporting the conclusion of moderately fast diffusion.

These results, however, raise other important questions. If Ca^{2+} diffusion within the SR is relatively fast, then how can recovery of SR $[Ca^{2+}]$, assessed either directly as Ca^{2+} blinks [50] or indirectly through the recovery of Ca^{2+} spark amplitude [54, 55], be relatively slow? $D_{Ca,SR}$ of $60 \mu m^2/s$ would imply that after release termination, SR $[Ca^{2+}]$ recovers quickly [81]. In fact, however, this same group observed Ca^{2+} blinks that recovered with time constants averaging 161 ms [50], much slower than one might expect assuming fast SR Ca^{2+} diffusion.

One possible answer is that the duration of local SR Ca^{2+} release in heart cells may, under some conditions, be much longer than previously appreciated. Several studies have assumed that the peak of the Ca^{2+} spark corresponds to the termination of SR Ca^{2+} release, which would imply a release duration of 10-20 ms [83, 84]. Although data in amphibian skeletal muscle support such an assumption [85, 86], in heart cells the release duration and the Ca^{2+} spark time-to-peak probably do not coincide. Simulations demonstrated that when SR $[Ca^{2+}]$ depletes during sparks, release termination can occur after the Ca^{2+} spark peak [40]. Blink measurements subsequently showed, consistent with the modeling results, a longer time to blink nadir than time to spark peak [44, 50]. More recently, direct evidence has been obtained for the persistence of small amounts of SR Ca^{2+} release during the decaying phase of Ca^{2+} sparks. By simultaneously imaging SR and cytosolic $[Ca^{2+}]$, Brochet et al were able to visualize small release events that would have otherwise been impossible to distinguish from noise [87]. Through this method they unequivocally demonstrated that SR release events, even very small ones, are accompanied by significant and measurable SR $[Ca^{2+}]$ depletion (Fig. 2A). They also showed a strong correlation between spark decay and blink recovery (Fig. 2B), although, it should be noted, such a correlation has not been observed in all studies [50]. Brochet et al [44] further obtained the novel result that increasing cytosolic buffering abbreviates both Ca^{2+} sparks and the corresponding Ca^{2+} blinks (Fig. 2C). Such a result would only be possible if local CICR maintains release from a few RyRs well past the peak of the spark. These authors hypothesized that such events are facilitated by irregularities in the structure of the RyR cluster, perhaps involving “rogue” RyRs [88]. Similarly, Picht et al, in their simulations, assumed a Ca^{2+} spark release flux that decayed with a time constant of 74 ms [82]. Thus, both recent experimental studies support the idea of extended SR release during sparks [82, 87].

We suspect, however, that biological reality will ultimately prove to be somewhat more complicated. Essentially, every model put forward to date has strengths, but each may also suffer from one or more weaknesses. Fig. 3 schematically illustrates important tradeoffs. For instance, our simulations from nearly a decade ago (Fig. 3A) produced a release flux that terminated relatively quickly (< 30 ms), and we assumed relatively slow Ca^{2+} transfer between network and junctional SR [40]. This model successfully matched spark time courses, and, with minor parameter changes, can recapitulate the relatively slow recoveries

of Ca^{2+} spark amplitude [54, 55] and free JSR [Ca^{2+}] [50, 82]. This model has been criticized, however, for its prediction of severe (90%) JSR depletion (but see question 2 above). Alternatively, a model that assumes fast termination and fast NSR to JSR transfer would predict less depletion (Fig. 3B). This model, however, would predict unrealistically fast recovery of JSR [Ca^{2+}] after termination. Finally, the solution arrived at by Picht et al (Fig. 3C) was to assume fast transfer between NSR and JSR and slow termination (> 100 ms). This model was able to simultaneously reproduce limited SR depletion during Ca^{2+} sparks, fast diffusion within the SR, and slow recovery of JSR [Ca^{2+}]. Such extended release fluxes, however, should manifest themselves as relatively long Ca^{2+} sparks, and the correspondence of this model with experimental Ca^{2+} spark data therefore remains to be determined.

Fig. 3D shows numerical simulations of Ca^{2+} spark time courses to illustrate two extreme cases. a release flux that terminates quickly (90% decay in 33 ms; left) and a more slowly-decaying release flux (90% decay in 180 ms; right). The Ca^{2+} spark produced by the latter is clearly extremely long compared with the former, as evidenced by the full durations at half maximum indicated in Fig. 3D. Although much more extensive simulations are required to address these issues in greater quantitative detail, this exercise at least illustrates the difficulty of reproducing a variety of results with any single model.

Summary and future directions

The past several years have provided important new insight into the regulatory roles of dynamic local changes in SR [Ca^{2+}]. Evidence suggests that such signals are important for terminating SR Ca^{2+} release, establishing the release refractory period, determining the propensity of myocytes for regenerative Ca^{2+} waves, and influencing Ca^{2+} wave velocity. Important variables that affect these processes, and are therefore important to quantify, include the extent of local SR depletion during release and the speed of Ca^{2+} diffusion within the SR. In this review we have attempted to both summarize important recent results and to emphasize issues that require further study. We conclude with a brief discussion of experimental methods and advances in modeling techniques that together will be instrumental in addressing important unresolved questions.

Experimentally, the development of fluorescent dyes located within the SR was clearly a major advance [42, 43], and, more recently, protocols for simultaneous measurements of SR and cytosolic [Ca^{2+}] have also moved the field forward. Two important technical details, however, should be mentioned. One is that, at present, most measurements of SR [Ca^{2+}] are made in permeabilized cells to avoid contamination from cytosolic fluo-5N. The permeabilization process, however, is known to dramatically increase the rate of spontaneous Ca^{2+} sparks, and the use of permeabilized cells may have contributed to some of the quantitative disagreements mentioned above. A second technical detail concerns species. For reasons that remain largely a mystery, it has proven extremely difficult to measure SR [Ca^{2+}] using fluo-5N in cells from rats or mice. Thus, the studies on Ca^{2+} blinks referenced above were performed on myocytes from rabbits and/or cats whereas rats and mice are frequently used for Ca^{2+} spark studies. Although fundamental mechanisms of Ca^{2+} release regulation appear to be similar across mammalian species, this may be a source of quantitative differences.

Thus, an SR targeted dye that could be used in all mammalian species under identical conditions would likely help to resolve certain controversies. An even more useful tool would be a dye that localized exclusively to either junctional or network SR. Finally, advanced microscopy technologies that overcome the diffraction barrier and provide

nanometer scale resolution are likely to allow for improved signals and more precise quantification of important variables.

In terms of computation, phenomena for which local concentration changes are important, such as cardiac SR Ca^{2+} release, are inherently more difficult and expensive to simulate than processes that only need to consider average concentrations. As a result, modeling studies performed to date have made simplifying assumptions to make problems tractable, and most can be grouped into one of the following categories: (1) simulations of individual events (i.e. sparks) that may consider stochastic gating of channels but do not integrate these events with larger-scale phenomena such as action potentials [40]; (2) simulations that consider spatial details but ignore aspects such as stochastic channel gating [61, 89, 90]; (3) simulations that link SR Ca^{2+} release to cellular phenomena such as action potentials but ignore local regulatory aspects [91]. Only a handful of studies have attempted to consider a wide range of phenomena within a single model [11, 30, 92]. An important active area of research is the development of methods that speed computations by simulating an entire population of RyR clusters but do not sacrifice critical aspects such as local JSR depletion and stochastic Ca^{2+} spark triggering [93-95]. Further developments in this area will soon allow for the seamless integration of realistic Ca^{2+} spark models with models of larger scale phenomena such as membrane currents and intracellular signaling.

We note also that the accuracy of mathematical models depends on the assumptions that underlie them. Two models intended to simulate the same process may exhibit fundamentally different behavior due to different underlying assumptions, although predicting emergent behavior from the details of the model equations is generally not straightforward. Methods such as parameter sensitivity analysis, which allow for systematic model comparisons [96, 97], and methods to constrain model parameters based on systematic comparisons with data [98, 99] will therefore become increasingly important as models simultaneously proliferate and gain complexity. In cardiac physiology, these types of methods are currently applied primarily to models of ionic currents and action potentials; applications of such ideas to models of CICR, Ca^{2+} sparks, and Ca^{2+} waves are likely to yield both novel insights and improved mathematical representations. The purpose of models, after all, is to simplify and illuminate rather than to complicate and confuse.

References

1. Cannell MB, Kong CH. Local control in cardiac E-C coupling. *J Mol Cell Cardiol.* 2011
2. Chen-Izu Y, McCulle SL, Ward CW, Soeller C, Allen BM, Rabang C, et al. Three-dimensional distribution of ryanodine receptor clusters in cardiac myocytes. *Biophys J.* 2006; 91:1–13. [PubMed: 16603500]
3. Baddeley D, Jayasinghe ID, Lam L, Rossberger S, Cannell MB, Soeller C. Optical single-channel resolution imaging of the ryanodine receptor distribution in rat cardiac myocytes. *Proc Natl Acad Sci U S A.* 2009; 106:22275–80. [PubMed: 20018773]
4. Franzini-Armstrong C, Protasi F, Ramesh V. Shape, size, and distribution of Ca^{2+} release units and couplons in skeletal and cardiac muscles. *Biophys J.* 1999; 77:1528–39. [PubMed: 10465763]
5. Cheng H, Lederer WJ, Cannell MB. Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science.* 1993; 262:740–4. [PubMed: 8235594]
6. Cannell MB, Cheng H, Lederer WJ. The control of calcium release in heart muscle. *Science.* 1995; 268:1045–9. [PubMed: 7754384]
7. Lopez-Lopez JR, Shacklock PS, Balke CW, Wier WG. Local calcium transients triggered by single L-type calcium channel currents in cardiac cells. *Science.* 1995; 268:1042–5. [PubMed: 7754383]
8. Sobie EA, Cannell MB, Bridge JHB. Allosteric activation of Na^{+} - Ca^{2+} exchange by L-type Ca^{2+} current augments the trigger flux for SR Ca^{2+} release in ventricular myocytes. *Biophys J.* 2008; 94:L54–L56. [PubMed: 18223001]

9. Torres NS, Larbig R, Rock A, Goldhaber JI, Bridge JH. Na^+ currents are required for efficient excitation-contraction coupling in rabbit ventricular myocytes: a possible contribution of neuronal Na^+ channels. *J Physiol*. 2010; 588:4249–60. [PubMed: 20837647]
10. Cheng H, Lederer MR, Lederer WJ, Cannell MB. Calcium sparks and $[\text{Ca}^{2+}]_i$ waves in cardiac myocytes. *Am J Physiol*. 1996; 270:C148–C159. [PubMed: 8772440]
11. Rovetti R, Cui X, Garfinkel A, Weiss JN, Qu Z. Spark-induced sparks as a mechanism of intracellular calcium alternans in cardiac myocytes. *Circ Res*. 2010; 106:1582–91. [PubMed: 20378857]
12. Heinzel FR, Bito V, Biesmans L, Wu M, Detre E, von Wegner F, et al. Remodeling of T-tubules and reduced synchrony of Ca^{2+} release in myocytes from chronically ischemic myocardium. *Circ Res*. 2008; 102:338–46. [PubMed: 18079411]
13. Louch WE, Bito V, Heinzel FR, Macianskiene R, Vanhaecke J, Flameng W, et al. Reduced synchrony of Ca^{2+} release with loss of T-tubules—a comparison to Ca^{2+} release in human failing cardiomyocytes. *Cardiovasc Res*. 2004; 62:63–73. [PubMed: 15023553]
14. Louch WE, Mork HK, Sexton J, Stromme TA, Laake P, Sjaastad I, et al. T-tubule disorganization and reduced synchrony of Ca^{2+} release in murine cardiomyocytes following myocardial infarction. *Journal of Physiology-London*. 2006; 574:519–33.
15. Song LS, Sobie EA, McCulle S, Lederer WJ, Balke CW, Cheng H. Orphaned ryanodine receptors in the failing heart. *Proc Natl Acad Sci U S A*. 2006; 103:4305–10. [PubMed: 16537526]
16. Heinzel FR, MacQuaide N, Biesmans L, Sipido K. Dyssynchrony of Ca^{2+} release from the sarcoplasmic reticulum as subcellular mechanism of cardiac contractile dysfunction. *J Mol Cell Cardiol*. 2011; 50:390–400. [PubMed: 21075114]
17. Blatter LA, Kocksamper J, Sheehan KA, Zima AV, Huser J, Lipsius SL. Local calcium gradients during excitation-contraction coupling and alternans in atrial myocytes. *J Physiol*. 2003; 546:19–31. [PubMed: 12509476]
18. Bootman MD, Higazi DR, Coombes S, Roderick HL. Calcium signalling during excitation-contraction coupling in mammalian atrial myocytes. *J Cell Sci*. 2006; 119:3915–25. [PubMed: 16988026]
19. Cordeiro JM, Spitzer KW, Giles WR, Ershler PE, Cannell MB, Bridge JH. Location of the initiation site of calcium transients and sparks in rabbit heart Purkinje cells. *J Physiol*. 2001; 531:301–14. [PubMed: 11310434]
20. Lee YS, Dun W, Boyden PA, Sobie EA. Complex and rate-dependent beat-to-beat variations in Ca^{2+} transients of canine Purkinje cells. *J Mol Cell Cardiol*. 2011; 50:662–9. [PubMed: 21232541]
21. Shannon TR, Ginsburg KS, Bers DM. Potentiation of fractional sarcoplasmic reticulum calcium release by total and free intra-sarcoplasmic reticulum calcium concentration. *Biophys J*. 2000; 78:334–43. [PubMed: 10620297]
22. Trafford AW, Diaz ME, Sibbring GC, Eisner DA. Modulation of CICR has no maintained effect on systolic Ca^{2+} : simultaneous measurements of sarcoplasmic reticulum and sarcolemmal Ca^{2+} fluxes in rat ventricular myocytes. *J Physiol*. 2000; 522(Pt 2):259–70. [PubMed: 10639102]
23. Diaz ME, Trafford AW, O'Neill SC, Eisner DA. Measurement of sarcoplasmic reticulum Ca^{2+} content and sarcolemmal Ca^{2+} fluxes in isolated rat ventricular myocytes during spontaneous Ca^{2+} release. *J Physiol (Lond)*. 1997; 501(Pt 1):3–16. [PubMed: 9174989]
24. Eisner DA, Kashimura T, O'Neill SC, Venetucci LA, Trafford AW. What role does modulation of the ryanodine receptor play in cardiac inotropy and arrhythmogenesis? *J Mol Cell Cardiol*. 2009; 46:474–81. [PubMed: 19150449]
25. Shannon TR, Ginsburg KS, Bers DM. Quantitative assessment of the SR Ca^{2+} leak-load relationship. *Circ Res*. 2002; 91:594–600. [PubMed: 12364387]
26. MacQuaide N, Dempster J, Smith GL. Measurement and modeling of Ca^{2+} waves in isolated rabbit ventricular cardiomyocytes. *Biophys J*. 2007; 93:2581–95. [PubMed: 17545234]
27. Diaz ME, O'Neill SC, Eisner DA. Sarcoplasmic reticulum calcium content fluctuation is the key to cardiac alternans. *Circ Res*. 2004; 94:650–6. [PubMed: 14752033]
28. Xie LH, Sato D, Garfinkel A, Qu Z, Weiss JN. Intracellular Ca alternans: coordinated regulation by sarcoplasmic reticulum release, uptake, and leak. *Biophys J*. 2008; 95:3100–10. [PubMed: 18539635]

29. Picht E, DeSantiago J, Blatter LA, Bers DM. Cardiac alternans do not rely on diastolic sarcoplasmic reticulum calcium content fluctuations. *Circ Res.* 2006; 99:740–8. [PubMed: 16946134]
30. Restrepo JG, Weiss JN, Karma A. Calsequestrin-mediated mechanism for cellular calcium transient alternans. *Biophys J.* 2008; 95:3767–89. [PubMed: 18676655]
31. Weiss JN, Nivala M, Garfinkel A, Qu Z. Alternans and arrhythmias: from cell to heart. *Circ Res.* 2011; 108:98–112. [PubMed: 21212392]
32. Laurita KR, Rosenbaum DS. Cellular mechanisms of arrhythmogenic cardiac alternans. *Prog Biophys Mol Biol.* 2008; 97:332–47. [PubMed: 18395246]
33. Stern MD, Cheng HP. Putting out the fire: what terminates calcium-induced calcium release in cardiac muscle? *Cell Calcium.* 2004; 35:591–601. [PubMed: 15110149]
34. Bers DM. Cardiac excitation-contraction coupling. *Nature.* 2002; 415:198–205. [PubMed: 11805843]
35. Wier WG, Balke CW. Ca^{2+} release mechanisms, Ca^{2+} sparks, and local control of excitation-contraction coupling in normal heart muscle. *Circ Res.* 1999; 85:770–6. [PubMed: 10532944]
36. DelPrincipe F, Egger M, Niggli E. Calcium signalling in cardiac muscle: refractoriness revealed by coherent activation. *Nat Cell Biol.* 1999; 1:323–9. [PubMed: 10559957]
37. Bassani JWM, Yuan W, Bers DM. Fractional SR Ca release is regulated by trigger Ca and SR Ca content in cardiac myocytes. *Am J Physiol.* 1995; 268:C1313–C1319. [PubMed: 7762626]
38. Ching LL, Williams AJ, Sitsapesan R. Evidence for Ca^{2+} activation and inactivation sites on the luminal side of the cardiac ryanodine receptor complex. *Circ Res.* 2000; 87:201–6. [PubMed: 10926870]
39. Gyorke I, Gyorke S. Regulation of the cardiac ryanodine receptor channel by luminal Ca^{2+} involves luminal Ca^{2+} sensing sites. *Biophys J.* 1998; 75:2801–10. [PubMed: 9826602]
40. Sobie EA, Dilly KW, Dos Santos CJ, Lederer WJ, Jafri MS. Termination of cardiac Ca^{2+} sparks: an investigative mathematical model of calcium-induced calcium release. *Biophys J.* 2002; 83:59–78. [PubMed: 12080100]
41. Terentyev D, Viatchenko-Karpinski S, Valdivia HH, Escobar AL, Gyorke S. Luminal Ca^{2+} controls termination and refractory behavior of Ca^{2+} -induced Ca^{2+} release in cardiac myocytes. *Circ Res.* 2002; 91:414–20. [PubMed: 12215490]
42. Kabbara AA, Allen DG. The use of the indicator fluo-5N to measure sarcoplasmic reticulum calcium in single muscle fibres of the cane toad. *J Physiol.* 2001; 534:87–97. [PubMed: 11432994]
43. Shannon TR, Guo T, Bers DM. Ca^{2+} Scraps. Local Depletions of Free $[\text{Ca}^{2+}]$ in Cardiac Sarcoplasmic Reticulum During Contractions Leave Substantial Ca^{2+} Reserve. *Circ Res.* 2003; 93:40–5. [PubMed: 12791706]
44. Brochet DX, Yang D, Di Maio A, Lederer WJ, Franzini-Armstrong C, Cheng H. Ca^{2+} blinks: rapid nanoscopic store calcium signaling. *Proc Natl Acad Sci U S A.* 2005; 102:3099–104. [PubMed: 15710901]
45. Terentyev D, Viatchenko-Karpinski S, Gyorke I, Volpe P, Williams SC, Gyorke S. Calsequestrin determines the functional size and stability of cardiac intracellular calcium stores: Mechanism for hereditary arrhythmia. *Proc Natl Acad Sci U S A.* 2003; 100:11759–64. [PubMed: 13130076]
46. Terentyev D, Nori A, Santoro M, Viatchenko-Karpinski S, Kubalova Z, Gyorke I, et al. Abnormal interactions of calsequestrin with the ryanodine receptor calcium release channel complex linked to exercise-induced sudden cardiac death. *Circ Res.* 2006; 98:1151–8. [PubMed: 16601229]
47. Terentyev D, Kubalova Z, Valle G, Nori A, Vedamoorthy S, Terentyeva R, et al. Modulation of SR Ca release by luminal Ca and calsequestrin in cardiac myocytes: effects of CASQ2 mutations linked to sudden cardiac death. *Biophys J.* 2008; 95:2037–48. [PubMed: 18469084]
48. Viatchenko-Karpinski S, Terentyev D, Gyorke I, Terentyeva R, Volpe P, Priori SG, et al. Abnormal calcium signaling and sudden cardiac death associated with mutation of calsequestrin. *Circ Res.* 2004; 94:471–7. [PubMed: 14715535]
49. Zima AV, Picht E, Bers DM, Blatter LA. Partial inhibition of sarcoplasmic reticulum Ca release evokes long-lasting Ca release events in ventricular myocytes: Role of luminal Ca in termination of Ca release. *Biophys J.* 2008; 94:1867–79. [PubMed: 18024505]

50. Zima AV, Picht E, Bers DM, Blatter LA. Termination of cardiac Ca^{2+} sparks: role of intra-SR $[\text{Ca}^{2+}]$, release flux, and intra-SR Ca^{2+} diffusion. *Circ Res*. 2008; 103:e105–e115. [PubMed: 18787194]
51. MacQuaide N, Dempster J, Smith GL. Assessment of sarcoplasmic reticulum Ca^{2+} depletion during spontaneous Ca^{2+} waves in isolated permeabilized rabbit ventricular cardiomyocytes. *Biophys J*. 2009; 96:2744–54. [PubMed: 19348757]
52. Stevens SC, Terentyev D, Kalyanasundaram A, Periasamy M, Gyorke S. Intra-sarcoplasmic reticulum Ca^{2+} oscillations are driven by dynamic regulation of ryanodine receptor function by luminal Ca^{2+} in cardiomyocytes. *J Physiol*. 2009; 587:4863–72. [PubMed: 19703963]
53. Szentosi P, Pignier C, Egger M, Kranias EG, Niggli E. Sarcoplasmic reticulum Ca^{2+} refilling controls recovery from Ca^{2+} -induced Ca^{2+} release refractoriness in heart muscle. *Circ Res*. 2004; 95:807–13. [PubMed: 15388639]
54. Sobie EA, Song LS, Lederer WJ. Local recovery of Ca^{2+} release in rat ventricular myocytes. *J Physiol*. 2005; 565:441–7. [PubMed: 15817631]
55. Ramay HR, Liu OZ, Sobie EA. Recovery of cardiac calcium release is controlled by sarcoplasmic reticulum refilling and ryanodine receptor sensitivity. *Cardiovasc Res*. 2011 in press. 10.1093/cvr/cvr143
56. Laver DR. Ca^{2+} stores regulate ryanodine receptor Ca^{2+} release channels via luminal and cytosolic Ca^{2+} sites. *Biophys J*. 2007; 92:3541–55. [PubMed: 17351009]
57. Qin J, Valle G, Nani A, Nori A, Rizzi N, Priori SG, et al. Luminal Ca^{2+} regulation of single cardiac ryanodine receptors: insights provided by calsequestrin and its mutants. *J Gen Physiol*. 2008; 131:325–34. [PubMed: 18347081]
58. Gyorke I, Hester N, Jones LR, Gyorke S. The role of calsequestrin, triadin, and junctin in conferring cardiac ryanodine receptor responsiveness to luminal calcium. *Biophys J*. 2004; 86:2121–8. [PubMed: 15041652]
59. Chopra N, Yang T, Asghari P, Moore ED, Huke S, Akin B, et al. Ablation of triadin causes loss of cardiac Ca^{2+} release units, impaired excitation-contraction coupling, and cardiac arrhythmias. *Proc Natl Acad Sci U S A*. 2009; 106:7636–41. [PubMed: 19383796]
60. Terentyev D, Viatchenko-Karpinski S, Vedamoorthy S, Oduru S, Gyorke I, Williams SC, et al. Protein protein interactions between triadin and calsequestrin are involved in modulation of sarcoplasmic reticulum calcium release in cardiac myocytes. *J Physiol*. 2007; 583:71–80. [PubMed: 17569730]
61. Ramay HR, Jafri MS, Lederer WJ, Sobie EA. Predicting local SR Ca^{2+} dynamics during Ca^{2+} wave propagation in ventricular myocytes. *Biophys J*. 2010; 98:2515–23. [PubMed: 20513395]
62. Yin CC, D'Cruz LG, Lai FA. Ryanodine receptor arrays: not just a pretty pattern? *Trends Cell Biol*. 2008; 18:149–56. [PubMed: 18329877]
63. Trafford AW, Diaz ME, Eisner DA. Coordinated control of cell Ca^{2+} loading and triggered release from the sarcoplasmic reticulum underlies the rapid inotropic response to increased L-type Ca^{2+} current. *Circ Res*. 2001; 88:195–201. [PubMed: 11157672]
64. Bers, DM. Excitation-contraction coupling and cardiac contractile force. Second. Dordrecht: Kluwer Academic Publishers; 2001.
65. Kettlun C, Gonzalez A, Rios E, Fill M. Unitary Ca^{2+} current through mammalian cardiac and amphibian skeletal muscle ryanodine receptor Channels under near-physiological ionic conditions. *J Gen Physiol*. 2003; 122:407–17. [PubMed: 12975450]
66. Jiang D, Xiao B, Yang D, Wang R, Choi P, Zhang L, et al. RyR2 mutations linked to ventricular tachycardia and sudden death reduce the threshold for store-overload-induced Ca^{2+} release (SOICR). *Proc Natl Acad Sci U S A*. 2004; 101:13062–7. [PubMed: 15322274]
67. Jiang D, Wang R, Xiao B, Kong H, Hunt DJ, Choi P, et al. Enhanced store overload-induced Ca^{2+} release and channel sensitivity to luminal Ca^{2+} activation are common defects of RyR2 mutations linked to ventricular tachycardia and sudden death. *Circ Res*. 2005; 97:1173–81. [PubMed: 16239587]
68. Prosser BL, Ward CW, Lederer WJ. Subcellular Ca^{2+} signaling in the heart: the role of ryanodine receptor sensitivity. *J Gen Physiol*. 2010; 136:135–42. [PubMed: 20660656]

69. Wasserstrom JA, Shiferaw Y, Chen W, Ramakrishna S, Patel H, Kelly JE, et al. Variability in timing of spontaneous calcium release in the intact rat heart is determined by the time course of sarcoplasmic reticulum calcium load. *Circ Res.* 2010; 107:1117–26. [PubMed: 20829511]
70. Santana LF, Kranias EG, Lederer WJ. Calcium sparks and excitation-contraction coupling in phospholamban- deficient mouse ventricular myocytes. *J Physiol (Lond).* 1997; 503:21–9. [PubMed: 9288671]
71. Gomez AM, Cheng H, Lederer WJ, Bers DM. Ca^{2+} diffusion and sarcoplasmic reticulum transport both contribute to $[\text{Ca}^{2+}]_i$ decline during Ca^{2+} sparks in rat ventricular myocytes. *J Physiol (Lond).* 1996; 496:575–81. [PubMed: 8910239]
72. Knollmann BC, Chopra N, Hlaing T, Akin B, Yang T, Etensohn K, et al. *Casq2* deletion causes sarcoplasmic reticulum volume increase, premature Ca^{2+} release, and catecholaminergic polymorphic ventricular tachycardia. *Journal of Clinical Investigation.* 2006; 116:2510–20. [PubMed: 16932808]
73. Song L, Alcalai R, Arad M, Wolf CM, Toka O, Conner DA, et al. Calsequestrin 2 (CASQ2) mutations increase expression of calreticulin and ryanodine receptors, causing catecholaminergic polymorphic ventricular tachycardia. *J Clin Invest.* 2007; 117:1814–23. [PubMed: 17607358]
74. Escobar AL, Korniyev D, Hilliard FA, Chopra N, Knollmann BC. Calsequestrin Determines Refractoriness Of Calcium Release From Sarcoplasmic Reticulum Of Cardiac Muscle. *Biophys J.* 2008; 94:898A.
75. Rizzi N, Liu N, Napolitano C, Nori A, Turcato F, Colombi B, et al. Unexpected structural and functional consequences of the R33Q homozygous mutation in cardiac calsequestrin: a complex arrhythmogenic cascade in a knock in mouse model. *Circ Res.* 2008; 103:298–306. [PubMed: 18583715]
76. Keller M, Kao JPY, Egger M, Niggli E. Calcium waves driven by “sensitization” wave-fronts. *Cardiovascular Research.* 2007; 74:39–45. [PubMed: 17336953]
77. Lukyanenko V, Subramanian S, Gyorke I, Wiesner TF, Gyorke S. The role of luminal Ca^{2+} in the generation of Ca^{2+} waves in rat ventricular myocytes. *J Physiol (Lond).* 1999; 518:173–86. [PubMed: 10373699]
78. Swietach P, Spitzer KW, Vaughan-Jones RD. Modeling calcium waves in cardiac myocytes: importance of calcium diffusion. *Front Biosci.* 2010; 15:661–80. [PubMed: 20036839]
79. Thul R, Smith GD, Coombes S. A bidomain threshold model of propagating calcium waves. *J Math Biol.* 2008; 56:435–63. [PubMed: 17786446]
80. Swietach P, Spitzer KW, Vaughan-Jones RD. Ca^{2+} -mobility in the sarcoplasmic reticulum of ventricular myocytes is low. *Biophys J.* 2008; 95:1412–27. [PubMed: 18390622]
81. Wu X, Bers DM. Sarcoplasmic reticulum and nuclear envelope are one highly interconnected Ca^{2+} store throughout cardiac myocyte. *Circ Res.* 2006; 99:283–91. [PubMed: 16794184]
82. Picht E, Zima AV, Shannon TR, Duncan AM, Blatter LA, Bers DM. Dynamic calcium movement inside cardiac sarcoplasmic reticulum during release. *Circ Res.* 2011; 108:847–56. [PubMed: 21311044]
83. Shen JX, Wang S, Song LS, Han T, Cheng H. Polymorphism of Ca^{2+} sparks evoked from in-focus Ca^{2+} release units in cardiac myocytes. *Biophys J.* 2004; 86:182–90. [PubMed: 14695261]
84. Wang SQ, Stern MD, Rios E, Cheng H. The quantal nature of Ca^{2+} sparks and in situ operation of the ryanodine receptor array in cardiac cells. *Proc Natl Acad Sci U S A.* 2004; 101:3979–84. [PubMed: 15004280]
85. Lacampagne A, Ward CW, Klein MG, Schneider MF. Time course of individual Ca^{2+} sparks in frog skeletal muscle recorded at high time resolution. *J Gen Physiol.* 1999; 113:187–98. [PubMed: 9925818]
86. Rios E, Zhou J, Brum G, Launikonis BS, Stern MD. Calcium-dependent inactivation terminates calcium release in skeletal muscle of amphibians. *J Gen Physiol.* 2008; 131:335–48. [PubMed: 18347079]
87. Brochet DX, Xie W, Yang D, Cheng H, Lederer WJ. Quarky calcium release in the heart. *Circ Res.* 2011; 108:210–8. [PubMed: 21148431]

88. Sobie EA, Guatimosim S, Gomez-Viquez L, Song LS, Hartmann H, Saleet JM, et al. The Ca^{2+} leak paradox and rogue ryanodine receptors: SR Ca^{2+} efflux theory and practice. *Prog Biophys Mol Biol.* 2006; 90:172–85. [PubMed: 16326215]
89. Cheng Y, Yu Z, Hoshijima M, Holst MJ, McCulloch AD, McCammon JA, et al. Numerical analysis of Ca^{2+} signaling in rat ventricular myocytes with realistic transverse-axial tubular geometry and inhibited sarcoplasmic reticulum. *PLoS Comput Biol.* 2010; 6:e1000972. [PubMed: 21060856]
90. Izu LT, Means SA, Shadid JN, Chen-Izu Y, Balke CW. Interplay of ryanodine receptor distribution and calcium dynamics. *Biophys J.* 2006; 91:95–112. [PubMed: 16603499]
91. Shannon TR, Wang F, Bers DM. Regulation of cardiac sarcoplasmic reticulum Ca release by luminal [Ca] and altered gating assessed with a mathematical model. *Biophys J.* 2005; 89:4096–110. [PubMed: 16169970]
92. Hashambhoy YL, Greenstein JL, Winslow RL. Role of CaMKII in RyR leak, EC coupling and action potential duration: a computational model. *J Mol Cell Cardiol.* 2010; 49:617–24. [PubMed: 20655925]
93. Greenstein JL, Hinch R, Winslow RL. Mechanisms of excitation-contraction coupling in an integrative model of the cardiac ventricular myocyte. *Biophys J.* 2006; 90:77–91. [PubMed: 16214852]
94. Hartman JM, Sobie EA, Smith GD. Spontaneous Ca^{2+} sparks and Ca^{2+} homeostasis in a minimal model of permeabilized ventricular myocytes. *Am J Physiol Heart Circ Physiol.* 2010; 299:H1996–H2008. [PubMed: 20852058]
95. Williams GS, Huertas MA, Sobie EA, Jafri MS, Smith GD. Moment closure for local control models of calcium-induced calcium release in cardiac myocytes. *Biophys J.* 2008; 95:1689–703. [PubMed: 18487291]
96. Sobie EA. Parameter sensitivity analysis in electrophysiological models using multivariable regression. *Biophys J.* 2009; 96:1264–74. [PubMed: 19217846]
97. Niederer SA, Fink M, Noble D, Smith NP. A meta-analysis of cardiac electrophysiology computational models. *Exp Physiol.* 2009; 94:486–95. [PubMed: 19139063]
98. Sarkar AX, Sobie EA. Regression analysis for constraining free parameters in electrophysiological models of cardiac cells. *PLoS Comput Biol.* 2010; 6:e1000914. [PubMed: 20824123]
99. Sobie EA, Ramay HR. Excitation-contraction coupling gain in ventricular myocytes: insights from a parsimonious model. *J Physiol.* 2009; 587:1293–9. [PubMed: 19153162]

Research highlights

- Changes in sarcoplasmic reticulum (SR) calcium regulate calcium release in heart cells.
- Under many circumstances, regulatory changes in SR calcium are transient and local.
- Studies that established the importance of local changes in SR calcium are described.
- Unresolved and controversial issues requiring further study are discussed.

Figure 1. Mathematical modeling to predict changes in SR [Ca²⁺] during Ca²⁺ waves

A model was implemented that simulates changes in both cytosolic and SR [Ca²⁺] during the evolution of regenerative Ca²⁺ waves [61]. (A) Representative changes in SR [Ca²⁺], displayed as a line scan pseudo-image, illustrate that spatiotemporal changes in SR [Ca²⁺] are complex. At different locations, SR [Ca²⁺] can increase (red) or decrease (blue), relative to the baseline value before the wave (orange). Horizontal white lines represent locations of transverse tubules. (B) Changes in cytosolic (top) and SR (bottom) [Ca²⁺] at unactivated RyR clusters immediately prior to activation. For slow (12 μm²/s) or medium (60 μm²/s) diffusion, SR [Ca²⁺] can increase at the target site, but SR [Ca²⁺] decreases when diffusion is fast (300 μm²/s). Partial inhibition of SERCA causes an increase in cytosolic [Ca²⁺] and a decrease in SR [Ca²⁺] for all values of $D_{Ca,SR}$. Simulations assume spacing between sites of 1 μm. (Modified with permission from [61])

Figure 2. Quarky Ca²⁺ release in rabbit ventricular myocytes

(A) Simultaneous imaging of Ca²⁺ sparks (top; rhod-2 fluorescence) and Ca²⁺ blinks (bottom; fluo-5N fluorescence) allows for detection of small Ca²⁺ release events and corresponding depletion signals (arrow). (B) Ca²⁺ spark decay and Ca²⁺ blink recovery are strongly correlated. (C) Increasing the concentration of EGTA (from 0.5 mM to 2 mM) speeds the rates of both Ca²⁺ sparks decay and Ca²⁺ blink recovery. (Modified with permission from [87])

Figure 3. Tradeoffs in simulations of release, depletion, and refilling

The balance between Ca²⁺ release and SR refilling dictates tradeoffs in the measurable signals. Graphs of Ca²⁺ release flux, JSR [Ca²⁺], and Ca²⁺ spark shape, which are not drawn to scale, illustrate schematically how biological variables influence the measurable signals. (A) A model with fast (< 30 ms) release termination and slow SR diffusion (as in [40]) will predict a realistic Ca²⁺ spark shape and a realistic rate of JSR [Ca²⁺] recovery, but will predict severe (90%) JSR depletion during sparks. (B) A model with fast release termination and fast SR diffusion will produce realistic sparks and less severe (e.g. 50%) depletion, but recovery of JSR [Ca²⁺] will be unrealistically fast. (C) A model with slow release termination and fast SR diffusion (as in [82]) can predict 50% JSR depletion and realistic recovery, but Ca²⁺ sparks generated by such a model are likely to be prolonged. (D) Ca²⁺ sparks were simulated using a published model [40]. During the first 10 ms of release, local SR release fluxes grow towards a peak value of 3 pA with a time constant of 1.5 ms. Release fluxes subsequently decay exponentially with a time constant of either 10 ms (black; left), or 74 ms (red; right). The Ca²⁺ spark generated by the more slowly-decaying release flux is clearly quite prolonged compared with that produced by release flux that decays faster. Times noted on the figure indicate full duration at half maximum, i.e. the total duration the simulated fluorescence is above 50% of its maximum value.