

# Ambiguous interactions between diastolic and SR $\text{Ca}^{2+}$ in the regulation of cardiac $\text{Ca}^{2+}$ release

Eric A. Sobie,<sup>1</sup> George S.B. Williams,<sup>2</sup> and W.J. Lederer<sup>2</sup>

<sup>1</sup>Department of Pharmacological Sciences, Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, NY

<sup>2</sup>BioMET, Center for Biomedical Engineering and Technology, University of Maryland, Baltimore, MD

## Introduction

In all types of muscle cells, contraction is initiated by an increase in intracellular  $\text{Ca}^{2+}$ . Another similarity among skeletal, cardiac, and smooth muscle cells is that much of the  $\text{Ca}^{2+}$  responsible for contraction is released from the SR. Beyond these common features, however, molecular and subcellular differences among muscle types can frequently explain functional differences. For instance, SR  $\text{Ca}^{2+}$  release in cardiac myocytes occurs primarily through the type 2 RyR (RyR2), whereas different release channels are more important in other cell types. Another relevant feature in heart is that cells must contract and relax roughly once per second or faster. Thus, although skeletal and smooth muscle may experience either brief or sustained elevations of intracellular  $[\text{Ca}^{2+}]$ , rhythmic and regular heartbeats in cardiac myocytes result in continuous oscillations in intracellular  $[\text{Ca}^{2+}]$ , accompanied by regular dynamic changes in SR  $[\text{Ca}^{2+}]$ . These concentration changes in the two compartments have been shown in recent years to convey information and regulate the release process. In this Perspective, we aim to review what has been learned about the regulatory importance of cardiac SR  $[\text{Ca}^{2+}]$  and establish some constraints on plausible ranges for changes in SR  $[\text{Ca}^{2+}]$  during release. In doing so, we emphasize how close coupling between experimental studies and numerical simulations has improved our understanding, and we discuss the importance of the interplay between SR  $[\text{Ca}^{2+}]$  and diastolic  $[\text{Ca}^{2+}]$  in the transition between stable and unstable cellular  $\text{Ca}^{2+}$  release. In particular, we argue that increased diastolic  $[\text{Ca}^{2+}]$  can raise RyR2 open probability in a manner that is potentially dangerous when combined with elevated SR  $[\text{Ca}^{2+}]$ .

## Dynamic changes in SR $[\text{Ca}^{2+}]$ during calcium cycling

Conservation of mass ensures that  $\text{Ca}^{2+}$  can neither appear out of thin air nor disappear into the void. Thus, when  $\text{Ca}^{2+}$  is released from the SR, the rise of cytosolic  $[\text{Ca}^{2+}]$  must be accompanied by a corresponding decline

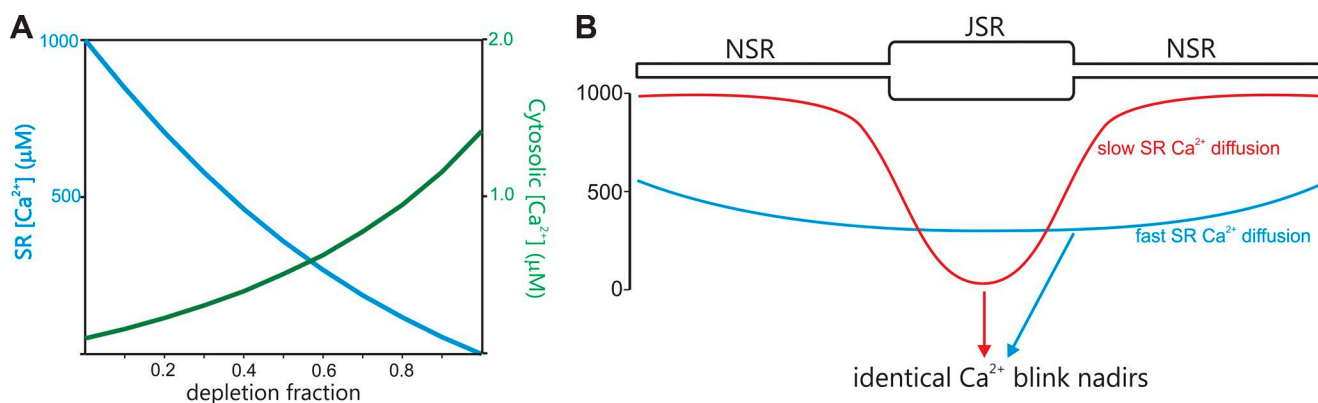
in SR  $[\text{Ca}^{2+}]$ . In skeletal muscle, because SR stores are very large, individual muscle twitches are accompanied by negligible changes in SR  $[\text{Ca}^{2+}]$  (Launikonis et al., 2006), and special experimental conditions such as very long depolarizations are required to observe substantial SR  $\text{Ca}^{2+}$  depletion (Manno et al., 2017). In cardiac myocytes, however, SR  $\text{Ca}^{2+}$  stores are comparatively much smaller. This means that both individual cellular contractions (Shannon et al., 2003) and local release events (Brochet et al., 2005) are accompanied by substantial depletion of SR  $[\text{Ca}^{2+}]$ . Although it is clear that these changes in SR  $[\text{Ca}^{2+}]$  regulate the release process, the mechanisms involved in this regulation and the precise functional importance of changes in SR  $[\text{Ca}^{2+}]$  remain intensively debated.

If the SR penetrated essentially everywhere in the cytoplasm and RyR2s released  $\text{Ca}^{2+}$  from all locations simultaneously, then calculating how increases in cytoplasmic  $[\text{Ca}^{2+}]$  corresponded with decreases in SR  $[\text{Ca}^{2+}]$  would be straightforward. For instance, in the absence of both cytosolic and SR  $\text{Ca}^{2+}$  buffers, the cytosolic  $[\text{Ca}^{2+}]$  increase would be quantitatively related to the SR  $[\text{Ca}^{2+}]$  decrease through the ratio of the two volumes. We can derive some rough estimates if we assume that the cytosol occupies 65% of the total cellular volume and the SR occupies 3% (with the remainder primarily mitochondria). If we also assume that, in a resting cell, diastolic  $[\text{Ca}^{2+}]$  and SR  $[\text{Ca}^{2+}]$  are 100 nM and 1 mM, respectively, then complete depletion of SR  $[\text{Ca}^{2+}]$  will cause cytosolic  $[\text{Ca}^{2+}]$  to increase from 100 nM to 46.3  $\mu\text{M}$ , whereas 50% depletion will cause an increase in cytosolic  $[\text{Ca}^{2+}]$  to 23.1  $\mu\text{M}$ . The fact that cytosolic  $[\text{Ca}^{2+}]$  never reaches such values, even when SR  $[\text{Ca}^{2+}]$  is emptied, indicates that  $\text{Ca}^{2+}$  buffering in the cytosol is strong compared with buffering in the SR. Although the presence of  $\text{Ca}^{2+}$  buffers complicates this analysis such that concentration changes are difficult to calculate with a pencil and paper, buffer powers in

Correspondence to Eric A. Sobie: eric.sobie@mssm.edu

Abbreviations used: JSR, junctional SR; NSR, network SR; SERCA, sarco/endoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase; SOICR, store overload-induced  $\text{Ca}^{2+}$  release.





**Figure 1. Hypothetical changes in SR and cytosolic  $[Ca^{2+}]$  during SR release.** (A) An increase in the fraction of total SR  $Ca^{2+}$  released causes a decrease in SR free  $[Ca^{2+}]$  and a corresponding increase in cytosolic free  $[Ca^{2+}]$ . Curves are nonlinear because of the presence of  $Ca^{2+}$  buffers in each compartment. Calculations assumed: (1) SR  $[Ca^{2+}]$  and cytosolic  $[Ca^{2+}]$  were 1,000  $\mu M$  and 0.1  $\mu M$ , respectively, before release; (2) cytosolic volume was 22.66 times larger than SR volume; (3) cytosolic buffering sites had maximal occupancy of 2,500  $\mu M$  and a  $K_D$  of 630  $\mu M$ ; (4) SR buffering sites had maximal occupancy of 220  $\mu M$  and a  $K_D$  of 0.96  $\mu M$ . (B) Hypothetical profiles of SR  $[Ca^{2+}]$  during release if  $Ca^{2+}$  diffusion within the SR is either extremely fast (blue) or quite slow (red). Because of blurring by confocal microscopes, attempts to record these SR  $[Ca^{2+}]$  profiles are likely to yield similar values for the apparent extent of depletion.

both cytosol and SR have been measured (Berlin et al., 1994; Shannon and Bers, 1997; Trafford et al., 1999) and can be incorporated into the analysis. Fig. 1 A shows how a uniform increase in cytosolic  $[Ca^{2+}]$  depends on the extent of SR  $[Ca^{2+}]$  depletion, assuming realistic cytosolic and SR buffering.

What complicates the calculation more than buffers, however, is the fact that the SR does not release  $Ca^{2+}$  at all locations simultaneously. Instead, RyR2s are clustered, and spatial segregation of these RyR2 clusters is a critical feature responsible for the stability of SR  $Ca^{2+}$  release (Niggli and Lederer, 1990; Stern, 1992; Cannell et al., 1995). This spatial segregation necessarily means that during SR  $Ca^{2+}$  release, cytosolic  $[Ca^{2+}]$  is greater in the immediate vicinity of the RyR2s, and lower further from the channels. By the same logic, SR  $Ca^{2+}$  depletion must be greater right next to the RyR2s compared with farther away. Spatial nonuniformities in cytosolic  $[Ca^{2+}]$  during SR  $Ca^{2+}$  release have long been appreciated, and considerable effort has been devoted to estimating the extent of the heterogeneity. Indeed, several mathematical modeling studies, dating back more than 30 yr, have calculated spatial changes in cytosolic  $[Ca^{2+}]$  (Cannell and Allen, 1984; Langer and Peskoff, 1996; Soeller and Cannell, 1997), and novel experimental methods have been developed to derive better estimates of  $[Ca^{2+}]$  in the immediate vicinity of RyR2s (Despa et al., 2014; Shang et al., 2014). It has been technically more challenging, however, to determine spatial nonuniformity in SR  $[Ca^{2+}]$ , and as a result comparatively less effort has been devoted to understanding the luminal side of the SR membrane, even though the same principles apply.

### Spatial heterogeneity of SR $[Ca^{2+}]$ during local $Ca^{2+}$ release events

During local  $Ca^{2+}$  release, by how much does SR  $Ca^{2+}$  content deplete? Mathematical modeling studies, beginning with ours in 2002 (Sobie et al., 2002), have suggested that during a  $Ca^{2+}$  spark, in which release lasts from 10–20 ms,  $[Ca^{2+}]$  in the junctional SR, or JSR, will deplete by 80–90% (i.e., from a resting level of 1000  $\mu M$  to 100–200  $\mu M$ ). This initial estimate was based on a relatively simple, phenomenological model of local  $Ca^{2+}$  release, but more recent models, which represent geometrical details more explicitly, have obtained nearly identical calculations of the extent of depletion during sparks (Hake et al., 2012; Cannell et al., 2013; Walker et al., 2014). Experimental studies, however, using  $Ca^{2+}$  indicators localized in the SR, such as fluo-5N, have consistently recorded local depletion signals, or  $Ca^{2+}$  blinks, with a nadir of roughly 50–60% of the initial value (Brochet et al., 2005, 2011; Zima et al., 2008b; Picht et al., 2011). To understand this discrepancy, a critical point to note is the limited spatial resolution of the experimental recordings. With a typical confocal microscope, an infinitesimally small source of light will be imaged to the shape of a prolate spheroid. The dimensions of this spheroid, measured as full width at half maximal intensity, will be  $\sim 0.5 \mu m$  in the two directions in the plane of focus and 1  $\mu m$  in the direction perpendicular to the plane of focus, yielding a “recording volume” of roughly 150 aL (atto =  $10^{-18}$ ). In contrast, a typical region of JSR containing RyR2s can be approximated as a disk with diameter  $\sim 300$  nm and a height of  $\sim 35$  nm, yielding a volume of 2.5 aL. This means that, when measuring  $Ca^{2+}$  blinks, the microscope records from a volume that is  $\sim 60$  times larger than the JSR volume that is being interrogated. Given that the

local  $[Ca^{2+}]$  right near the site of release will necessarily be lower than the concentration away the site of release, it is clear that a typical blink will represent a weighted mean of more extensive depletion near the RyR2s and less extensive depletion further away from the channels.

When trying to relate the experimental blink signal to the true extent of  $Ca^{2+}$  depletion immediately near the RyR2s, a critical factor is the spatial scale over which  $[Ca^{2+}]$  varies within the SR, and this in turn depends on the speed of  $Ca^{2+}$  movement in the SR. Here, references to SR  $Ca^{2+}$  movement include both diffusion within the network SR (NSR) and  $Ca^{2+}$  transfer from NSR to JSR. Two hypothetical possibilities are illustrated in Fig. 1 B. If  $Ca^{2+}$  movement is extremely fast,  $[Ca^{2+}]$  will quickly adjust to a relatively constant value throughout the SR during release (Fig. 1 B, blue curve). On the other hand, if movement is slow, in particular if a “bottleneck” exists between the JSR and the NSR, then JSR concentration during release will be dramatically lower than the concentration in the surrounding NSR (Fig. 1 B, red curve). The important point is that because of blurring by the confocal microscope, both scenarios will produce identical  $Ca^{2+}$  blink nadirs.

We believe the latter scenario is more likely than the former, for several reasons. First, as noted above, several mathematical models, developed by us and other groups (Sobie et al., 2002; Hake et al., 2012; Cannell et al., 2013; Walker et al., 2014), have predicted that JSR  $[Ca^{2+}]$  depletes to 10–20% of its initial value during  $Ca^{2+}$  sparks. Beyond predicting the extent of  $Ca^{2+}$  depletion in the immediate vicinity of the RyR2s, modeling studies have also demonstrated that an apparent depletion of 50% estimated from experimental blink measurements is consistent with a true depletion of 80–90% near RyR2s (Williams et al., 2011; Hake et al., 2012; Kong et al., 2013). Second, related computational studies have shown that extremely fast SR  $Ca^{2+}$  diffusion may lead to unrealistic behavior. When developing a model of the  $Ca^{2+}$  spark, a critical parameter that must be chosen involves the speed of  $Ca^{2+}$  movement from NSR to JSR. In our studies, we have constrained these parameters on the basis of data showing that, after an initial  $Ca^{2+}$  spark, the amplitude of a second spark from the same site recovers with a time constant of 70–90 ms (Sobie et al., 2005; Ramay et al., 2011; Guo et al., 2012b; Poláková et al., 2015). This recovery of  $Ca^{2+}$  spark amplitude is broadly consistent with the wide range of  $Ca^{2+}$  blink recovery time constants, 30–150 ms, that have been measured (Brochet et al., 2005; Zima et al., 2008b; Picht et al., 2011). In general, we would expect spark amplitude to be proportional to total JSR  $[Ca^{2+}]$  (free  $Ca^{2+}$  plus  $Ca^{2+}$  bound to buffers), a quantity that should recover more quickly than the free JSR  $[Ca^{2+}]$  that is detected in blink measurements. When NSR-to-JSR transfer rates are chosen to match these experimental data,  $Ca^{2+}$  sparks terminate and recover normally (Ramay et al.,

2011; Cannell et al., 2013; Stern et al., 2013). However, when the rate of  $Ca^{2+}$  transfer from NSR to JSR is made dramatically faster,  $Ca^{2+}$  sparks can fail to terminate, leading to the emergence of so-called metastable sparks (Stern et al., 2013; Sato et al., 2016; Song et al., 2016).

Third, as discussed in more detail below, solid evidence indicates that during regenerative  $Ca^{2+}$  waves, in which  $Ca^{2+}$  sparks trigger additional sparks in a chain reaction that moves through the cell, SR  $[Ca^{2+}]$  seems to transiently increase at sites that have not yet been triggered (Maxwell and Blatter, 2012, 2017). We would only expect such behavior if  $Ca^{2+}$  movement between JSR and NSR were relatively slow; extremely fast diffusion would create a flat profile of  $[Ca^{2+}]$  within the SR and make it impossible for  $Ca^{2+}$  to accumulate at untriggered sites. This group of observations, collected under different experimental conditions by several different groups, supports the idea that within the SR lumen, each release unit is mostly disconnected from its neighbors.

### The physiological importance of local SR depletion

Why should we care about local decreases in SR  $[Ca^{2+}]$ ? The short answer is that compelling evidence, gathered over roughly the past two decades, indicates that local reductions in SR  $[Ca^{2+}]$  have important regulatory roles under normal and pathological conditions. In 2002, a stochastic mathematical model of the  $Ca^{2+}$  spark (Sobie et al., 2002) and an experimental study published soon afterward (Terentyev et al., 2002) independently postulated that substantial local depletion of SR  $[Ca^{2+}]$  is required for release termination. In the years following, important confirmatory evidence was gathered by several groups. Many of these studies are described more extensively in the Perspectives by Cannell and Kong and Györke et al. in this issue and are therefore not discussed in detail here. In brief, results obtained in these studies included the following: (1) changes in SR  $Ca^{2+}$  buffering due to overexpression or knockdown of calsequestrin affect the duration of sparks (Terentyev et al., 2003); (2) partial blockade of RyR2s can dramatically prolong  $Ca^{2+}$  sparks (Zima et al., 2008a,b), presumably by slowing the rate at which the JSR depletes; and (3) refilling of SR  $[Ca^{2+}]$  controls the time course of  $Ca^{2+}$  release refractoriness at both the  $Ca^{2+}$  spark (Sobie et al., 2005; Ramay et al., 2011) and cellular (Szentesi et al., 2004; Kornyevev et al., 2012) levels.

Despite the results that clearly indicated an important role for SR  $[Ca^{2+}]$  depletion in release termination, some weaknesses of the early mathematical model (Sobie et al., 2002) should be mentioned. In particular, the limited experimental data available in 2002 required our group to make assumptions that have subsequently been questioned. First, on the basis of two compelling studies investigating RyR gating (Marx et al., 1998, 2001), we assumed that the opening and closing of each RyR2 influenced the behavior of its neighbors through allosteric

interactions, or so-called coupled gating. This feature, however, has not been confirmed in experiments from other groups (Xiao et al., 2007) and is virtually impossible to either prove or refute in intact cells. Consequently, the potential importance of coupled gating remains indeterminate. Second, in 2002 we assumed that changes in SR  $[Ca^{2+}]$  had a relatively large effect on RyR2 gating, on the basis of somewhat limited data (Györke and Györke, 1998; Ching et al., 2000). Data gathered subsequently, which paint a more complete picture of how SR  $[Ca^{2+}]$  influences RyR2 gating, seem to indicate that the effect is not as strong as originally implemented (Qin et al., 2008), and descendants of the 2002 model have updated formulations to reflect these new data (Ramay et al., 2011; Williams et al., 2011; Wescott et al., 2016).

In addition, mathematical modeling results have demonstrated that RyR2 gating does not need to depend on SR  $[Ca^{2+}]$  in order for  $Ca^{2+}$  sparks to terminate robustly (Cannell et al., 2013; Laver et al., 2013; Stern et al., 2013; Walker et al., 2014). In this scenario, the important feature is the fact that when  $[Ca^{2+}]$  in the JSR decreases, the  $Ca^{2+}$  current flowing through each open RyR2 will decline, and each RyR2 opening will be less likely to reopen any neighbors that stochastically close, until the spark terminates when all RyR2s are closed simultaneously (Cannell and Kong, 2017). This termination mechanism has been named “induction decay” (Cannell et al., 2013; Laver et al., 2013) and, in a less quantitative presentation of the same notion, “pernicious attrition” (Gillespie and Fill, 2013). These recent studies are important because they have demonstrated that regulation of RyR2 gating by SR  $[Ca^{2+}]$  is not essential, and they have brought renewed attention to the importance of the  $Ca^{2+}$  current flowing through each open RyR2 (Guo et al., 2012a). It is important to note, however, that the ability of an RyR2 to trigger its neighbors, and the dependence of this on SR  $[Ca^{2+}]$ , has been implicitly included in all previous models that simulate realistic depletion of JSR  $[Ca^{2+}]$ .

The exact physiological role for changes in SR  $[Ca^{2+}]$  to modulate RyR2 gating, therefore, remains somewhat in question, given that this mechanism is not required for  $Ca^{2+}$  spark termination. Recent modeling studies have proposed reasonable hypotheses, specifically that SR  $[Ca^{2+}]$  regulation of RyR2 gating may enable a narrow distribution of  $Ca^{2+}$  spark durations (Stern et al., 2013) and that this feature contributes substantially to the nonlinear relationship between SR  $[Ca^{2+}]$  and release triggering (Walker et al., 2014). Thus, the mathematical modeling has helped to clarify hypotheses, but many of these ideas remain to be conclusively confirmed or refuted.

#### Unstable $Ca^{2+}$ release and the role of SR $[Ca^{2+}]$

Under pathological conditions,  $Ca^{2+}$  release through clusters of RyR2s is not necessarily spatially constrained but instead can propagate between RyR2 clusters such

that spontaneous release occurs throughout the entire cell. In this mechanism, the  $Ca^{2+}$  released from one RyR2 cluster diffuses in the cytosol to raise  $[Ca^{2+}]$  in the vicinity of neighboring clusters. When the diffusing  $Ca^{2+}$  is sufficient to activate the opening of RyR2s in these nearby clusters, a propagating, regenerative  $Ca^{2+}$  wave can result.

It has been well established over many years that these  $Ca^{2+}$  waves tend to occur under conditions of elevated SR  $[Ca^{2+}]$ . In fact, some studies have observed a sharp transition from localized local spontaneous release ( $Ca^{2+}$  sparks) to regenerative waves, such that a “threshold SR  $Ca^{2+}$  content” can be determined (Díaz et al., 1997; Eisner et al., 2009). From these data and related work examining the consequences of RyR2 mutations, some investigators have proposed the concept of SOI CR, or store overload-induced  $Ca^{2+}$  release (Jiang et al., 2004, 2005). However, although it is clear that increases in SR  $[Ca^{2+}]$  contribute to the generation of spontaneous  $Ca^{2+}$  waves, it is also clear that these waves propagate through a mechanism of CICR in the cytosol. This is illustrated most clearly by the observation that cell-wide waves can readily be terminated by adding buffers such as EGTA or BAPTA to the cytosol. Under these conditions, elevated SR  $[Ca^{2+}]$  continues to encourage spontaneous  $Ca^{2+}$  release, but this release occurs only in the form of localized  $Ca^{2+}$  sparks, not in the form of cell-wide  $Ca^{2+}$  waves (Lukyanenko et al., 2001; Loughrey et al., 2002).

To better understand the practical distinction between CICR and a purely SR-mediated SOICR mechanism, we can consider a single cluster of RyR2, with an associated JSR volume. The mechanism of action of CICR is inherently a positive feedback process. When an individual RyR2 within the cluster opens stochastically, the efflux of  $Ca^{2+}$  from the JSR raises local  $[Ca^{2+}]$  on the cytosolic side of the SR membrane. This higher local cytosolic  $[Ca^{2+}]$  increases the likelihood that adjacent RyR2 channels will open. If a second RyR2 opens, the additional  $Ca^{2+}$  efflux from the JSR will increase local cytosolic  $[Ca^{2+}]$  further, and a  $Ca^{2+}$  spark can be produced when sufficient RyR2s are activated. In contrast, SOICR is intrinsically a negative feedback mechanism. In this case, a single RyR2 that opens stochastically because of the high  $[Ca^{2+}]$  on the luminal side of the SR membrane will pass  $Ca^{2+}$  ions that will act to decrease local SR  $[Ca^{2+}]$ . This decrease of SR  $[Ca^{2+}]$  would then make it less likely for a mechanism based on luminal activation to trigger the opening of additional RyR2s within that cluster. Thus SOICR, acting in isolation, would not constitute a self-sustaining, regenerative process that produces  $Ca^{2+}$  sparks and  $Ca^{2+}$  waves. For these reasons, it is more appropriate to characterize increased SR  $[Ca^{2+}]$  as encouraging or enabling spontaneous regenerative  $Ca^{2+}$  release by CICR rather than directly triggering or inducing  $Ca^{2+}$  release.

A question that remains intriguing concerns the spatiotemporal changes in SR  $[Ca^{2+}]$  that occur on a time scale of tens of milliseconds during waves. It is clear that at the local site of  $Ca^{2+}$  release, SR  $[Ca^{2+}]$  must decrease at the same time that local cytosolic  $[Ca^{2+}]$  increases. What remain less clear, however, are the changes that occur at neighboring clusters of RyR2s. Cytosolic  $[Ca^{2+}]$  will increase at these untriggered clusters because of diffusion of  $Ca^{2+}$  from the initial site of release, but what will happen to SR  $[Ca^{2+}]$ ? This quantity could theoretically either decrease if the SR is well-connected between neighboring release units, or it could increase when  $Ca^{2+}$  that has diffused in the cytosol is pumped into the SR by sarco/endoplasmic reticulum  $Ca^{2+}$ -ATPase (SERCA) at locations immediately adjacent to the untriggered cluster. A further complication concerns how these concentration changes might be altered by increased SERCA pump activity, for instance as occurs during  $\beta$ -adrenergic activation. Because an increase in SERCA activity would presumably decrease cytosolic  $[Ca^{2+}]$  at untriggered clusters while potentially increasing SR  $[Ca^{2+}]$  at these locations, the overall effects on  $Ca^{2+}$  wave propagation remain ambiguous.

A decade ago, an important experimental study observed that sudden inhibition of the SERCA pump led to an immediate decrease in the velocity of propagating  $Ca^{2+}$  waves (Keller et al., 2007). On the basis of this finding, the authors speculated that SERCA pumps take up  $Ca^{2+}$  near untriggered sites, and the resulting localized increase in SR  $[Ca^{2+}]$  sensitizes the RyR2s ahead of a propagating  $Ca^{2+}$  wave. A subsequent mathematical modeling investigation showed that this hypothesis is feasible, but only if  $Ca^{2+}$  diffusion in the SR is relatively slow compared with  $Ca^{2+}$  diffusion in the cytosol (Ramay et al., 2010). Soon thereafter, an experimental study showed that localized increases in SR  $[Ca^{2+}]$  do in fact occur during  $Ca^{2+}$  waves in ventricular cells (Maxwell and Blatter, 2012), and more recent work suggests that this sensitization mechanism is critical to the propagation of SR  $Ca^{2+}$  release in atrial myocytes (Maxwell and Blatter, 2017).

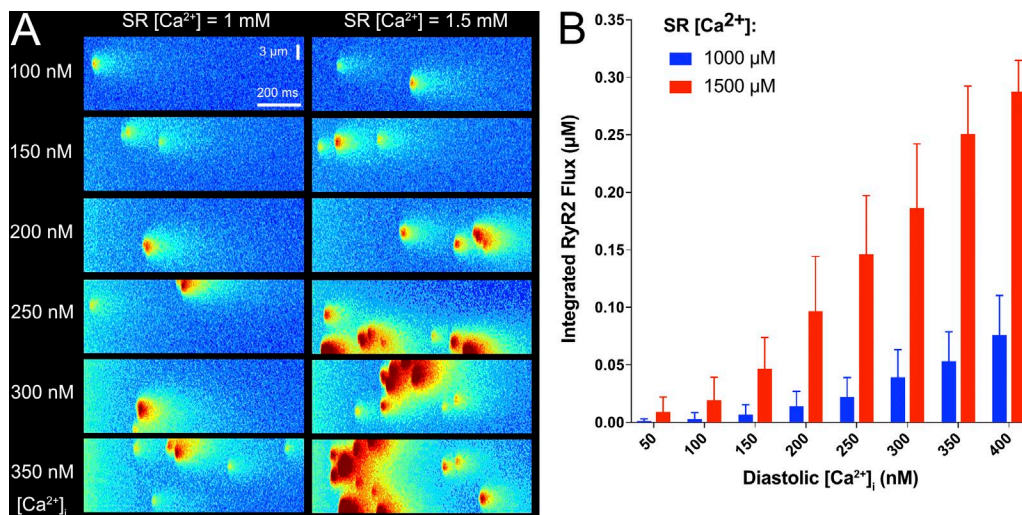
This particular issue is notable for a couple of reasons. First, the timeline illustrates the usefulness of close interplay between experiments and numerical simulations, even when the studies are performed by different groups. A hypothesis was proposed on the basis of indirect evidence, as a way to explain surprising results (Keller et al., 2007), numerical simulations demonstrated the feasibility of the hypothesis, as long as certain conditions hold (Ramay et al., 2010), then subsequent experiments provided direct support (Maxwell and Blatter, 2012). Second, the changes in SR  $[Ca^{2+}]$  that are possible during waves relate closely to the nonuniformity of SR  $[Ca^{2+}]$  during blinks and in turn to how well the experimental blink signal reflects the true extent of SR depletion. In this case, simulations

showed that increases in SR  $[Ca^{2+}]$  ahead of  $Ca^{2+}$  waves are possible only when SR  $Ca^{2+}$  diffusion is rather slow. From this inference, it follows that JSR units in cardiac myocytes must be relatively isolated from one another and, in turn, that SR  $[Ca^{2+}]$  during local release must be quite nonuniform (e.g., closer to the red than the blue profile in Fig. 1 B).

### The semi-neglected factor of diastolic $[Ca^{2+}]$

With considerable recent research energy focused on determining the regulatory importance of changes in SR  $[Ca^{2+}]$ , an additional important factor has been relatively neglected, namely, diastolic  $[Ca^{2+}]$ , that is, the minimum cytosolic  $[Ca^{2+}]$  reached in myocytes between contractions. Given that CICR is the commonly accepted mechanism discussed in textbooks, it seems surprising that the importance of cytosolic  $[Ca^{2+}]$  could be somewhat overlooked. However, small changes in diastolic  $[Ca^{2+}]$  can be challenging to track in experiments, and investigators naturally focus on the more interesting dynamic time courses rather than on changes in the baseline level. Examining diastolic  $[Ca^{2+}]$ , however, is important because of the factors that modify it. Conditions that lead to increased SR  $[Ca^{2+}]$ , such as rapid pacing and  $\beta$ -adrenergic stimulation, are frequently accompanied by an increase in diastolic  $[Ca^{2+}]$ . When this occurs, the rate at which RyR2s spontaneously open at rest should be increased in a nonlinear manner. Mathematical models based on planar lipid bilayer data generally assume that the RyR2 opening rate depends on local cytosolic  $[Ca^{2+}]$  raised to a power between 2 and 4 (Ramay et al., 2011; Sato and Bers, 2011; Williams et al., 2011; Walker et al., 2014; Wescott et al., 2016). Thus a 50% increase in diastolic  $[Ca^{2+}]$  will not cause a 50% increase in the spontaneous RyR2 opening rate but instead will increase the opening rate up to five-fold. These more frequent RyR2 openings will not only increase the rate of  $Ca^{2+}$  sparks and the “leak” that is seen in resting cells (Bovo et al., 2011) but will also produce many more potential triggers for regenerative  $Ca^{2+}$  waves. Under such conditions, elevated diastolic and SR  $[Ca^{2+}]$  can synergize in a potentially dangerous way. Diastolic  $[Ca^{2+}]$  will increase the number of  $Ca^{2+}$  sparks that can potentially initiate waves, and the elevated SR  $[Ca^{2+}]$  will increase the probability that an individual event will trigger release from neighboring RyR2 clusters. Together these effects can greatly increase the risk of unstable  $Ca^{2+}$  release.

To illustrate this idea, we performed simulations using a recently published stochastic mathematical model of a cell containing multiple, spatially distributed clusters of RyR2s (Wescott et al., 2016). By using the model, we are able to independently vary the initial values of SR  $[Ca^{2+}]$  and diastolic  $[Ca^{2+}]$ , an intervention that is challenging to perform in experiments. Fig. 2 A shows sample results from these stochastic simulations. In these



**Figure 2. Contributions of increased SR and diastolic  $[\text{Ca}^{2+}]$  to unstable  $\text{Ca}^{2+}$  release.** (A) Simulated line-scan images of typical stochastic simulations of  $\text{Ca}^{2+}$  sparks and waves. Simulations were performed with a mathematical model of  $\text{Ca}^{2+}$  release, as recently described (Wescott et al., 2016). The horizontal dimension represents time (1 s), and the vertical dimension represents transverse distance (14  $\mu\text{m}$ ). Labels indicate initial values of cytosolic and SR  $[\text{Ca}^{2+}]$ . Each SR  $\text{Ca}^{2+}$  release unit contains a cluster of 50 stochastically gating RyR2s (see Wescott et al. [2016] for more details), and RyR2 clusters are randomly distributed, with a mean intersite distance of 700 nm. Large SR  $\text{Ca}^{2+}$  release events involving multiple RyR2 clusters are frequently observed when both SR  $[\text{Ca}^{2+}]$  and diastolic  $[\text{Ca}^{2+}]$  are increased (bottom right images). (B) Integrated RyR2  $\text{Ca}^{2+}$  release flux as a function of initial diastolic  $[\text{Ca}^{2+}]_i$  when initial SR  $[\text{Ca}^{2+}]$  is either 1 mM (blue) or 1.5 mM (red). Flux is integrated over each 1-s simulation and averaged over 50 trials. Error bars show standard deviation.

spatiotemporal images, analogous to those that would be obtained experimentally by a confocal microscope in line-scan mode, we observe both  $\text{Ca}^{2+}$  sparks, reflecting release from an individual RyR2 cluster, and larger events reflecting release from multiple clusters. The results show that an increase in SR  $[\text{Ca}^{2+}]$  can, by itself, increase the probability that individual  $\text{Ca}^{2+}$  sparks will trigger neighboring sites and produce larger release events. However, when SR  $[\text{Ca}^{2+}]$  is increased and diastolic  $[\text{Ca}^{2+}]$  remains low, these multisite events remain relatively uncommon. In contrast, when diastolic  $[\text{Ca}^{2+}]$  and SR  $[\text{Ca}^{2+}]$  are simultaneously elevated, both the frequency of  $\text{Ca}^{2+}$  release events and their spatial extent are substantially increased. These effects are quantified in Fig. 2 B, which plots the integrated  $\text{Ca}^{2+}$  release flux, or  $\text{Ca}^{2+}$  release “mass,” as a function of SR and diastolic  $[\text{Ca}^{2+}]$ . The potentially dangerous interplay between these two factors can be readily seen. Thus, experimental and mathematical modeling studies exploring the transition between stable and unstable  $\text{Ca}^{2+}$  release need to carefully consider the effects of both diastolic and SR  $[\text{Ca}^{2+}]$ .

Although the above discussion focuses primarily on the role of  $\text{Ca}^{2+}$  release in ventricular myocytes, we should note that diastolic and SR  $[\text{Ca}^{2+}]$  can also potentially synergize in other cell types, such as sinoatrial nodal cells, atrial myocytes (Blatter in this issue), and Purkinje cells. In sinoatrial nodal cells, considerable recent evidence suggests that spontaneous  $\text{Ca}^{2+}$  release events, in the form of sparks triggering additional

sparks, drives the late phase of diastolic depolarization when released  $\text{Ca}^{2+}$  is extruded from the cell through  $\text{Na}^+\text{-Ca}^{2+}$  exchange (Stern et al., 2014; Yaniv et al., 2015). In atrial and Purkinje cells,  $\text{Ca}^{2+}$  release is initiated only at the cell periphery and propagates into the cell center under some conditions (Blatter et al., 2003; Lee et al., 2011). Both of these phenomena are likely to depend on diastolic as well as SR  $[\text{Ca}^{2+}]$ , emphasizing the importance of considering the interplay between these two factors.

### Conclusions and outlook

It is clear that numerous studies performed over the past two decades have yielded substantial insight into the importance of SR  $[\text{Ca}^{2+}]$  in regulating  $\text{Ca}^{2+}$  release. When considering the published literature as a whole, a common theme that emerges is the difficult-to-predict interplay among overall SR  $[\text{Ca}^{2+}]$  content, local changes in free SR  $[\text{Ca}^{2+}]$ , the potential regulation of RyR2 gating by SR  $[\text{Ca}^{2+}]$ , and the changes in diastolic  $[\text{Ca}^{2+}]$  that frequently accompany altered SR  $[\text{Ca}^{2+}]$ . The fact that these factors sometimes work in opposite directions highlights the need to integrate experimental studies with mechanistic mathematical modeling, and, indeed, many of the advances described above came from such efforts.

Going forward, experimental and computational advances are likely to help to address the remaining unresolved questions and provide additional insight. First, technical advances such as knock-in mice, local-

ized  $\text{Ca}^{2+}$  probes, and super-resolution microscopy will presumably help to fully resolve issues such as the spatial nonuniformity of SR  $[\text{Ca}^{2+}]$  during release and the importance of SR  $[\text{Ca}^{2+}]$  in regulating RyR2 gating. Second, even though most experimental studies focus on the average behavior of events such as sparks and blinks, and most models are developed on the basis of repeating, uniform units, it is clear that considerable heterogeneity exists both within a cell and between cells. For instance, both structural parameters such as the number of RyR2 per cluster (Baddeley et al., 2009) and physiological variables such as blink time constants (Zima et al., 2008b) exhibit wide distributions. Modeling studies have shown how heterogeneity between RyR2 clusters can influence both  $\text{Ca}^{2+}$  spark properties (Lee et al., 2013) and the emergence of intracellular  $\text{Ca}^{2+}$  waves (Nivala et al., 2013), but more work clearly needs to be done to understand the consequences of heterogeneity. However, with recently developed tools in hand to address these unresolved issues, it is clear that the next several years will bring significant additional insight into the roles of SR and diastolic  $[\text{Ca}^{2+}]$  in regulating cardiac myocyte function.

## ACKNOWLEDGMENTS

Research in E.A. Sobie's laboratory is supported by the National Institutes of Health (U54 HG008098 and U01 HL136297), the National Science Foundation (MCB 1615677), and the American Heart Association (15GRNT25490006). W.J. Lederer is supported by National Institutes of Health grants R01 HL106056, R01 HL105239, and U01 HL116321. G.S.B. Williams is supported by National Institutes of Health award K25 HL125762.

The authors declare no competing financial interests.

Eduardo Ríos served as editor.

Submitted: 19 April 2017

Accepted: 21 July 2017

## REFERENCES

- Baddeley, D., I.D. Jayasinghe, L. Lam, S. Rossberger, M.B. Cannell, and C. Soeller. 2009. Optical single-channel resolution imaging of the ryanodine receptor distribution in rat cardiac myocytes. *Proc. Natl. Acad. Sci. USA*. 106:22275–22280. <http://dx.doi.org/10.1073/pnas.0908971106>
- Berlin, J.R., J.W. Bassani, and D.M. Bers. 1994. Intrinsic cytosolic calcium buffering properties of single rat cardiac myocytes. *Biophys. J.* 67:1775–1787. [http://dx.doi.org/10.1016/S0006-3495\(94\)80652-6](http://dx.doi.org/10.1016/S0006-3495(94)80652-6)
- Blatter, L.A. 2017. The intricacies of atrial calcium cycling during excitation–contraction coupling. *J. Gen. Physiol.* 149. <http://dx.doi.org/10.1085/jgp.201711809>
- Blatter, L.A., J. Kocksämper, K.A. Sheehan, A.V. Zima, J. Hüser, and S.L. Lipsius. 2003. Local calcium gradients during excitation–contraction coupling and alternans in atrial myocytes. *J. Physiol.* 546:19–31. <http://dx.doi.org/10.1113/jphysiol.2002.025239>
- Bovo, E., S.R. Mazurek, L.A. Blatter, and A.V. Zima. 2011. Regulation of sarcoplasmic reticulum  $\text{Ca}^{2+}$  leak by cytosolic  $\text{Ca}^{2+}$  in rabbit ventricular myocytes. *J. Physiol.* 589:6039–6050. <http://dx.doi.org/10.1113/jphysiol.2011.214171>
- Brochet, D.X., D. Yang, A. Di Maio, W.J. Lederer, C. Franzini-Armstrong, and H. Cheng. 2005.  $\text{Ca}^{2+}$  blinks: Rapid nanoscopic store calcium signaling. *Proc. Natl. Acad. Sci. USA*. 102:3099–3104. <http://dx.doi.org/10.1073/pnas.0500059102>
- Brochet, D.X., W. Xie, D. Yang, H. Cheng, and W.J. Lederer. 2011. Quarky calcium release in the heart. *Circ. Res.* 108:210–218. <http://dx.doi.org/10.1161/CIRCRESAHA.110.231258>
- Cannell, M.B., and D.G. Allen. 1984. Model of calcium movements during activation in the sarcomere of frog skeletal muscle. *Biophys. J.* 45:913–925. [http://dx.doi.org/10.1016/S0006-3495\(84\)84238-1](http://dx.doi.org/10.1016/S0006-3495(84)84238-1)
- Cannell, M.B., and C.H.T. Kong. 2017. Quenching the spark: Termination of CICR in the submicroscopic space of the dyad. *J. Gen. Physiol.* 149. <http://dx.doi.org/10.1085/jgp.201711807>
- Cannell, M.B., H. Cheng, and W.J. Lederer. 1995. The control of calcium release in heart muscle. *Science*. 268:1045–1049. <http://dx.doi.org/10.1126/science.7754384>
- Cannell, M.B., C.H. Kong, M.S. Imtiaz, and D.R. Laver. 2013. Control of sarcoplasmic reticulum  $\text{Ca}^{2+}$  release by stochastic RyR gating within a 3D model of the cardiac dyad and importance of induction decay for CICR termination. *Biophys. J.* 104:2149–2159. <http://dx.doi.org/10.1016/j.bpj.2013.03.058>
- Ching, L.L., A.J. Williams, and R. Sitsapesan. 2000. Evidence for  $\text{Ca}^{2+}$  activation and inactivation sites on the luminal side of the cardiac ryanodine receptor complex. *Circ. Res.* 87:201–206. <http://dx.doi.org/10.1161/01.RES.87.3.201>
- Despa, S., B. Shui, J. Bossuyt, D. Lang, M.I. Kotlikoff, and D.M. Bers. 2014. Junctional cleft  $[\text{Ca}^{2+}]_i$  measurements using novel cleft-targeted  $\text{Ca}^{2+}$  sensors. *Circ. Res.* 115:339–347. <http://dx.doi.org/10.1161/CIRCRESAHA.115.303582>
- Díaz, M.E., A.W. Trafford, S.C. O'Neill, and D.A. Eisner. 1997. Measurement of sarcoplasmic reticulum  $\text{Ca}^{2+}$  content and sarcolemmal  $\text{Ca}^{2+}$  fluxes in isolated rat ventricular myocytes during spontaneous  $\text{Ca}^{2+}$  release. *J. Physiol.* 501:3–16. <http://dx.doi.org/10.1111/j.1469-7793.1997.003bo.x>
- Eisner, D.A., T. Kashimura, S.C. O'Neill, L.A. Venetucci, and A.W. Trafford. 2009. What role does modulation of the ryanodine receptor play in cardiac inotropy and arrhythmogenesis? *J. Mol. Cell. Cardiol.* 46:474–481. <http://dx.doi.org/10.1016/j.yjmcc.2008.12.005>
- Gillespie, D., and M. Fill. 2013. Pernicious attrition and inter-RyR2 CICR current control in cardiac muscle. *J. Mol. Cell. Cardiol.* 58:53–58. <http://dx.doi.org/10.1016/j.yjmcc.2013.01.011>
- Guo, T., D. Gillespie, and M. Fill. 2012a. Ryanodine receptor current amplitude controls  $\text{Ca}^{2+}$  sparks in cardiac muscle. *Circ. Res.* 111:28–36. <http://dx.doi.org/10.1161/CIRCRESAHA.112.265652>
- Guo, T., T. Zhang, K.S. Ginsburg, S. Mishra, J.H. Brown, and D.M. Bers. 2012b. CaMKII $\delta$ C slows  $[\text{Ca}]_i$  decline in cardiac myocytes by promoting Ca sparks. *Biophys. J.* 102:2461–2470. <http://dx.doi.org/10.1016/j.bpj.2012.04.015>
- Györke, I., and S. Györke. 1998. Regulation of the cardiac ryanodine receptor channel by luminal  $\text{Ca}^{2+}$  involves luminal  $\text{Ca}^{2+}$  sensing sites. *Biophys. J.* 75:2801–2810. [http://dx.doi.org/10.1016/S0006-3495\(98\)77723-9](http://dx.doi.org/10.1016/S0006-3495(98)77723-9)
- Györke, S., A.E. Belevych, B. Liu, I.V. Kubasov, C.A. Carnes, and P.B. Radwański. 2017. The role of luminal Ca regulation in Ca signaling refractoriness and cardiac arrhythmogenesis. *J. Gen. Physiol.* 149. <http://dx.doi.org/10.1085/jgp.201711808>
- Hake, J., A.G. Edwards, Z. Yu, P.M. Keken-Huskey, A.P. Michailova, J.A. McCammon, M.J. Holst, M. Hoshijima, and A.D. McCulloch. 2012. Modelling cardiac calcium sparks in a three-dimensional

- reconstruction of a calcium release unit. *J. Physiol.* 590:4403–4422. <http://dx.doi.org/10.1113/jphysiol.2012.227926>
- Jiang, D., B. Xiao, D. Yang, R. Wang, P. Choi, L. Zhang, H. Cheng, and S.R. Chen. 2004. RyR2 mutations linked to ventricular tachycardia and sudden death reduce the threshold for store-overload-induced  $\text{Ca}^{2+}$  release (SOICR). *Proc. Natl. Acad. Sci. USA.* 101:13062–13067. <http://dx.doi.org/10.1073/pnas.0402388101>
- Jiang, D., R. Wang, B. Xiao, H. Kong, D.J. Hunt, P. Choi, L. Zhang, and S.R. Chen. 2005. Enhanced store overload-induced  $\text{Ca}^{2+}$  release and channel sensitivity to luminal  $\text{Ca}^{2+}$  activation are common defects of RyR2 mutations linked to ventricular tachycardia and sudden death. *Circ. Res.* 97:1173–1181. <http://dx.doi.org/10.1161/01.RES.0000192146.85173.4b>
- Keller, M., J.P.Y. Kao, M. Egger, and E. Niggli. 2007. Calcium waves driven by “sensitization” wave-fronts. *Cardiovasc. Res.* 74:39–45. <http://dx.doi.org/10.1016/j.cardiores.2007.02.006>
- Kong, C.H., D.R. Laver, and M.B. Cannell. 2013. Extraction of sub-microscopic Ca fluxes from blurred and noisy fluorescent indicator images with a detailed model fitting approach. *PLOS Comput. Biol.* 9:e1002931. <http://dx.doi.org/10.1371/journal.pcbi.1002931>
- Korneyev, D., A.D. Petrosky, B. Zepeda, M. Ferreira, B. Knollmann, and A.L. Escobar. 2012. Calsequestrin 2 deletion shortens the refractoriness of  $\text{Ca}^{2+}$  release and reduces rate-dependent  $\text{Ca}^{2+}$ -alternans in intact mouse hearts. *J. Mol. Cell. Cardiol.* 52:21–31. <http://dx.doi.org/10.1016/j.yjmcc.2011.09.020>
- Langer, G.A., and A. Peskoff. 1996. Calcium concentration and movement in the diadic cleft space of the cardiac ventricular cell. *Biophys. J.* 70:1169–1182. [http://dx.doi.org/10.1016/S0006-3495\(96\)79677-7](http://dx.doi.org/10.1016/S0006-3495(96)79677-7)
- Launikonis, B.S., J. Zhou, L. Royer, T.R. Shannon, G. Brum, and E. Ríos. 2006. Depletion “scraps” and dynamic buffering inside the cellular calcium store. *Proc. Natl. Acad. Sci. USA.* 103:2982–2987. <http://dx.doi.org/10.1073/pnas.0511252103>
- Laver, D.R., C.H. Kong, M.S. Imtiaz, and M.B. Cannell. 2013. Termination of calcium-induced calcium release by induction decay: An emergent property of stochastic channel gating and molecular scale architecture. *J. Mol. Cell. Cardiol.* 54:98–100. <http://dx.doi.org/10.1016/j.yjmcc.2012.10.009>
- Lee, Y.S., W. Dun, P.A. Boyden, and E.A. Sobie. 2011. Complex and rate-dependent beat-to-beat variations in  $\text{Ca}^{2+}$  transients of canine Purkinje cells. *J. Mol. Cell. Cardiol.* 50:662–669. <http://dx.doi.org/10.1016/j.yjmcc.2010.12.023>
- Lee, Y.S., O.Z. Liu, H.S. Hwang, B.C. Knollmann, and E.A. Sobie. 2013. Parameter sensitivity analysis of stochastic models provides insights into cardiac calcium sparks. *Biophys. J.* 104:1142–1150. <http://dx.doi.org/10.1016/j.bpj.2012.12.055>
- Loughrey, C.M., K.E. MacEachern, P. Neary, and G.L. Smith. 2002. The relationship between intracellular  $[\text{Ca}^{2+}]$  and  $\text{Ca}^{2+}$  wave characteristics in permeabilised cardiomyocytes from the rabbit. *J. Physiol.* 543:859–870. <http://dx.doi.org/10.1113/jphysiol.2002.021519>
- Lukyanenko, V., S. Viatchenko-Karpinski, A. Smirnov, T.F. Wiesner, and S. Györke. 2001. Dynamic regulation of sarcoplasmic reticulum  $\text{Ca}^{2+}$  content and release by luminal  $\text{Ca}^{2+}$ -sensitive leak in rat ventricular myocytes. *Biophys. J.* 81:785–798. [http://dx.doi.org/10.1016/S0006-3495\(01\)75741-4](http://dx.doi.org/10.1016/S0006-3495(01)75741-4)
- Manno, C., L.C. Figueroa, D. Gillespie, R. Fitts, C. Kang, C. Franzini-Armstrong, and E. Ríos. 2017. Calsequestrin depolymerizes when calcium is depleted in the sarcoplasmic reticulum of working muscle. *Proc. Natl. Acad. Sci. USA.* 114:E638–E647. <http://dx.doi.org/10.1073/pnas.1620265114>
- Marx, S.O., K. Ondrias, and A.R. Marks. 1998. Coupled gating between individual skeletal muscle  $\text{Ca}^{2+}$  release channels (ryanodine receptors). *Science.* 281:818–821. <http://dx.doi.org/10.1126/science.281.5378.818>
- Marx, S.O., J. Gaburjakova, M. Gaburjakova, C. Henrikson, K. Ondrias, and A.R. Marks. 2001. Coupled gating between cardiac calcium release channels (ryanodine receptors). *Circ. Res.* 88:1151–1158. <http://dx.doi.org/10.1161/hh1101.091268>
- Maxwell, J.T., and L.A. Blatter. 2012. Facilitation of cytosolic calcium wave propagation by local calcium uptake into the sarcoplasmic reticulum in cardiac myocytes. *J. Physiol.* 590:6037–6045. <http://dx.doi.org/10.1113/jphysiol.2012.239434>
- Maxwell, J.T., and L.A. Blatter. 2017. A novel mechanism of tandem activation of ryanodine receptors by cytosolic and SR luminal  $\text{Ca}^{2+}$  during excitation-contraction coupling in atrial myocytes. *J. Physiol.* 595:3835–3845. <http://dx.doi.org/10.1113/JP273611>
- Niggli, E., and W.J. Lederer. 1990. Voltage-independent calcium release in heart muscle. *Science.* 250:565–568. <http://dx.doi.org/10.1126/science.2173135>
- Nivala, M., C.Y. Ko, M. Nivala, J.N. Weiss, and Z. Qu. 2013. The emergence of subcellular pacemaker sites for calcium waves and oscillations. *J. Physiol.* 591:5305–5320. <http://dx.doi.org/10.1113/jphysiol.2013.259960>
- Picht, E., A.V. Zima, T.R. Shannon, A.M. Duncan, L.A. Blatter, and D.M. Bers. 2011. Dynamic calcium movement inside cardiac sarcoplasmic reticulum during release. *Circ. Res.* 108:847–856. <http://dx.doi.org/10.1161/CIRCRESAHA.111.240234>
- Poláková, E., A. Illaste, E. Niggli, and E.A. Sobie. 2015. Maximal acceleration of  $\text{Ca}^{2+}$  release refractoriness by  $\beta$ -adrenergic stimulation requires dual activation of kinases PKA and CaMKII in mouse ventricular myocytes. *J. Physiol.* 593:1495–1507. <http://dx.doi.org/10.1113/jphysiol.2014.278051>
- Qin, J., G. Valle, A. Nani, A. Nori, N. Rizzi, S.G. Priori, P. Volpe, and M. Fill. 2008. Luminal  $\text{Ca}^{2+}$  regulation of single cardiac ryanodine receptors: Insights provided by calsequestrin and its mutants. *J. Gen. Physiol.* 131:325–334. <http://dx.doi.org/10.1085/jgp.200709907>
- Ramay, H.R., M.S. Jafri, W.J. Lederer, and E.A. Sobie. 2010. Predicting local SR  $\text{Ca}^{2+}$  dynamics during  $\text{Ca}^{2+}$  wave propagation in ventricular myocytes. *Biophys. J.* 98:2515–2523. <http://dx.doi.org/10.1016/j.bpj.2010.02.038>
- Ramay, H.R., O.Z. Liu, and E.A. Sobie. 2011. Recovery of cardiac calcium release is controlled by sarcoplasmic reticulum refilling and ryanodine receptor sensitivity. *Cardiovasc. Res.* 91:598–605. <http://dx.doi.org/10.1093/cvr/cvr143>
- Sato, D., and D.M. Bers. 2011. How does stochastic ryanodine receptor-mediated Ca leak fail to initiate a Ca spark? *Biophys. J.* 101:2370–2379. <http://dx.doi.org/10.1016/j.bpj.2011.10.017>
- Sato, D., T.R. Shannon, and D.M. Bers. 2016. Sarcoplasmic reticulum structure and functional properties that promote long-lasting calcium sparks. *Biophys. J.* 110:382–390. <http://dx.doi.org/10.1016/j.bpj.2015.12.009>
- Shang, W., F. Lu, T. Sun, J. Xu, L.L. Li, Y. Wang, G. Wang, L. Chen, X. Wang, M.B. Cannell, et al. 2014. Imaging  $\text{Ca}^{2+}$  nanosparks in heart with a new targeted biosensor. *Circ. Res.* 114:412–420. <http://dx.doi.org/10.1161/CIRCRESAHA.114.302938>
- Shannon, T.R., and D.M. Bers. 1997. Assessment of intra-SR free  $[\text{Ca}]$  and buffering in rat heart. *Biophys. J.* 73:1524–1531. [http://dx.doi.org/10.1016/S0006-3495\(97\)78184-0](http://dx.doi.org/10.1016/S0006-3495(97)78184-0)
- Shannon, T.R., T. Guo, and D.M. Bers. 2003.  $\text{Ca}^{2+}$  scraps: Local depletions of free  $[\text{Ca}^{2+}]$  in cardiac sarcoplasmic reticulum during contractions leave substantial  $\text{Ca}^{2+}$  reserve. *Circ. Res.* 93:40–45. <http://dx.doi.org/10.1161/01.RES.0000079967.11815.19>
- Sobie, E.A., K.W. Dilly, J. dos Santos Cruz, W.J. Lederer, and M.S. Jafri. 2002. Termination of cardiac  $\text{Ca}^{2+}$  sparks: an investigative mathematical model of calcium-induced calcium release. *Biophys. J.* 83:59–78. [http://dx.doi.org/10.1016/S0006-3495\(02\)75149-7](http://dx.doi.org/10.1016/S0006-3495(02)75149-7)

- Sobie, E.A., L.S. Song, and W.J. Lederer. 2005. Local recovery of  $\text{Ca}^{2+}$  release in rat ventricular myocytes. *J. Physiol.* 565:441–447. <http://dx.doi.org/10.1113/jphysiol.2005.086496>
- Soeller, C., and M.B. Cannell. 1997. Numerical simulation of local calcium movements during L-type calcium channel gating in the cardiac diad. *Biophys. J.* 73:97–111. [http://dx.doi.org/10.1016/S0006-3495\(97\)78051-2](http://dx.doi.org/10.1016/S0006-3495(97)78051-2)
- Song, Z., A. Karma, J.N. Weiss, and Z. Qu. 2016. Long-lasting sparks: multi-metastability and release competition in the calcium release unit network. *PLOS Comput. Biol.* 12:e1004671. <http://dx.doi.org/10.1371/journal.pcbi.1004671>
- Stern, M.D. 1992. Theory of excitation-contraction coupling in cardiac muscle. *Biophys. J.* 63:497–517. [http://dx.doi.org/10.1016/S0006-3495\(92\)81615-6](http://dx.doi.org/10.1016/S0006-3495(92)81615-6)
- Stern, M.D., E. Ríos, and V.A. Maltsev. 2013. Life and death of a cardiac calcium spark. *J. Gen. Physiol.* 142:257–274. <http://dx.doi.org/10.1085/jgp.201311034>
- Stern, M.D., L.A. Maltseva, M. Juhaszova, S.J. Sollott, E.G. Lakatta, and V.A. Maltsev. 2014. Hierarchical clustering of ryanodine receptors enables emergence of a calcium clock in sinoatrial node cells. *J. Gen. Physiol.* 143:577–604. <http://dx.doi.org/10.1085/jgp.201311123>
- Szentosi, P., C. Pignier, M. Egger, E.G. Kranias, and E. Niggli. 2004. Sarcoplasmic reticulum  $\text{Ca}^{2+}$  refilling controls recovery from  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release refractoriness in heart muscle. *Circ. Res.* 95:807–813. <http://dx.doi.org/10.1161/01.RES.0000146029.80463.7d>
- Terentyev, D., S. Viatchenko-Karpinski, H.H. Valdivia, A.L. Escobar, and S. Györke. 2002. Luminal  $\text{Ca}^{2+}$  controls termination and refractory behavior of  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release in cardiac myocytes. *Circ. Res.* 91:414–420. <http://dx.doi.org/10.1161/01.RES.0000032490.04207.BD>
- Terentyev, D., S. Viatchenko-Karpinski, I. Györke, P. Volpe, S.C. Williams, and S. Györke. 2003. Calsequestrin determines the functional size and stability of cardiac intracellular calcium stores: mechanism for hereditary arrhythmia. *Proc. Natl. Acad. Sci. USA.* 100:11759–11764. <http://dx.doi.org/10.1073/pnas.1932318100>
- T Trafford, A.W., M.E. Díaz, and D.A. Eisner. 1999. A novel, rapid and reversible method to measure Ca buffering and time-course of total sarcoplasmic reticulum Ca content in cardiac ventricular myocytes. *Pflugers Arch.* 437:501–503. <http://dx.doi.org/10.1007/s004240050808>
- Walker, M.A., G.S. Williams, T. Kohl, S.E. Lehnart, M.S. Jafri, J.L. Greenstein, W.J. Lederer, and R.L. Winslow. 2014. Superresolution modeling of calcium release in the heart. *Biophys. J.* 107:3018–3029. <http://dx.doi.org/10.1016/j.bpj.2014.11.003>
- Wescott, A.P., M.S. Jafri, W.J. Lederer, and G.S. Williams. 2016. Ryanodine receptor sensitivity governs the stability and synchrony of local calcium release during cardiac excitation-contraction coupling. *J. Mol. Cell. Cardiol.* 92:82–92. <http://dx.doi.org/10.1016/j.yjmcc.2016.01.024>
- Williams, G.S., A.C. Chikando, H.T. Tuan, E.A. Sobie, W.J. Lederer, and M.S. Jafri. 2011. Dynamics of calcium sparks and calcium leak in the heart. *Biophys. J.* 101:1287–1296. <http://dx.doi.org/10.1016/j.bpj.2011.07.021>
- Xiao, J., X. Tian, P.P. Jones, J. Bolstad, H. Kong, R. Wang, L. Zhang, H.J. Duff, A.M. Gillis, S. Fleischer, et al. 2007. Removal of FKBP12.6 does not alter the conductance and activation of the cardiac ryanodine receptor or the susceptibility to stress-induced ventricular arrhythmias. *J. Biol. Chem.* 282:34828–34838. <http://dx.doi.org/10.1074/jbc.M707423200>
- Yaniv, Y., E.G. Lakatta, and V.A. Maltsev. 2015. From two competing oscillators to one coupled-clock pacemaker cell system. *Front. Physiol.* 6:28. <http://dx.doi.org/10.3389/fphys.2015.00028>
- Zima, A.V., E. Picht, D.M. Bers, and L.A. Blatter. 2008a. 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.* 94:1867–1879. <http://dx.doi.org/10.1529/biophysj.107.114694>
- Zima, A.V., E. Picht, D.M. Bers, and L.A. Blatter. 2008b. Termination of cardiac  $\text{Ca}^{2+}$  sparks: role of intra-SR  $[\text{Ca}^{2+}]$ , release flux, and intra-SR  $\text{Ca}^{2+}$  diffusion. *Circ. Res.* 103:e105–e115. <http://dx.doi.org/10.1161/CIRCRESAHA.107.183236>