# The intricacies of atrial calcium cycling during excitation-contraction coupling

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### Introduction

In the heart, excitation-contraction coupling (ECC) is a sequence of events that begins with membrane depolarization by an action potential (AP), followed by activation of voltage-gated Ca channels in the surface membrane, initiation of Ca release from the SR Ca store by CICR, and the resulting contraction. Most of our knowledge of the mechanistic details of ECC originates from studies of ventricular muscle. However, seminal studies on atrial ECC and Ca signaling, while documenting important similarities between atrial and ventricular tissues, have revealed critical differences. A striking difference between atrial and ventricular myocytes is the presence of an extensive system of transverse tubules (t-tubules), which are surface membrane invaginations that extend throughout the entire ventricular myocyte and are organized in a sarcomeric pattern. The t-tubule system is an integral part of the surface membrane that allows the placement of voltage-gated L-type Ca channels (LCCs) in close vicinity to clusters of RyR Ca release channels in the SR membrane. Each of these Ca release units (CRUs) therefore has its own source of activator Ca in form of a small number of adjacent LCCs. As a consequence of these structural arrangements, Ca release during ventricular ECC is spatially homogeneous throughout the cell. In contrast, atrial myocytes either lack t-tubules or have only a sparse and irregular system (Hüser et al., 1996; Mackenzie et al., 2001; Bootman et al., 2006; Smyrnias et al., 2010), creating important ramifications for atrial Ca dynamics during ECC. In the following, we focus on atrium-specific features of the initiation of ECC and SR Ca release before turning to a discussion of SR Ca depletion and release termination.

### Initiation of SR Ca release

In contrast to ventricular myocytes, AP-induced Ca release in atrial cells is spatially inhomogeneous (Berlin, 1995; Hüser et al., 1996; Blatter et al., 2003; Bootman

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et al., 2011; Shkryl and Blatter, 2013). Typically, the elevation of cytosolic [Ca] ([Ca]<sub>i</sub>) starts in the cell periphery, where the opening of LCCs provides the required Ca to induce CICR from the most peripheral SR Ca release sites (Kockskämper et al., 2001; Shkryl and Blatter, 2013). This generates Ca gradients that are large enough to overcome endogenous cytosolic Ca buffering (Sheehan and Blatter, 2003) and allow centripetal Ca diffusion and activation of CICR from SR release sites in progressively more central regions of the cell. Thus, CICR propagates from the periphery to the center in a Ca wave-like fashion by a diffusion-reaction process or a "fire-diffuse-fire" mechanism (Keizer et al., 1998; Shkryl and Blatter, 2013), with the inherent consequences of complex [Ca]<sub>i</sub> inhomogeneities and subcellular Ca gradients during ECC (Fig. 1). A putative role of an intracellular axial membrane structure recently described in certain species further enhances the complexity of atrial Ca signals during ECC (Brandenburg et al., 2016).

The extent of t-tubule endowment of atrial myocytes shows considerable species differences (for reference, see Richards et al., 2011; Trafford et al., 2013). In cat and rabbit atrial cells, for example, the t-tubular system is entirely absent (Hüser et al., 1996; Maxwell and Blatter, 2017). These species differences notwithstanding, the absence of t-tubules essentially divides the atrial SR Ca store into two types of SR on the basis of their location relative to the surface membrane: junctional SR (j-SR) and the much more abundant nonjunctional SR (nj-SR). RyR Ca release channels are abundant in the membranes of both j-SR and nj-SR and participate in normal ECC (Carl et al., 1995; Kockskämper et al., 2001; Mackenzie et al., 2001, 2004; Woo et al., 2003; Bootman et al., 2006; Smyrnias et al., 2010). RyRs are organized in a 3D array of channel clusters (Hüser et al., 1996; Kockskämper et al., 2001; Sheehan and Blatter, 2003; Shkryl and Blatter, 2013). The j-SR forms close physical associations with the adjacent sarcolemmal that are termed peripheral couplings. In these peripheral couplings, the surface membrane hosts voltage-gated LCCs

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Abbreviations used: AP, action potential; CASQ, calsequestrin; CaT, Ca transient; CRU, Ca release unit; ECC, excitation-contraction coupling; FDUF, fire-diffuse-up-take-fire; IICR, IP<sub>3</sub> receptor-induced Ca release; j-SR, junctional SR; LCC, L-type Ca channel; nj-SR, nonjunctional SR; SERCA, sarcoplasmic/endoplasmic Ca ATPase; t-tubule, transverse tubule.

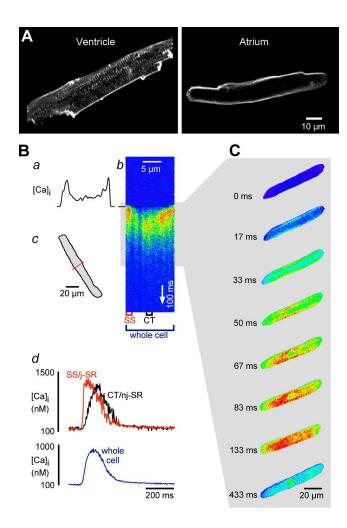


Figure 1. Ca signaling during ECC in atrial myocytes. (A) Transverse tubule staining in ventricular and atrial myocytes with the membrane-bound fluorescent dye Di-8-ANEPPS. (B) Transverse (c) confocal line scan image of an atrial AP-induced CaT. Electrical stimulation triggered a "U"-shaped CaT (b), indicating that [Ca]; increased first at the cell periphery (a) before propagating toward the center of the myocyte. (d) Local CaTs (top) originating from subsarcolemmal (SS) j-SR and central (CT) nj-SR regions and the Ca signal averaged over the entire width of the cell (bottom). (C) Spatiotemporal pattern of an AP-induced atrial CaT visualized by two-dimensional confocal microscopy. Reproduced from Blatter et al. (2003).

that are facing clusters of RyRs in the SR membrane across a narrow intermembrane cleft, reminiscent of dyads in ventricular myocytes (McNutt and Fawcett, 1969; Kockskämper et al., 2001). Thus, the CRUs (Franzini-Armstrong and Jorgensen, 1994) of the j-SR are functionally organized like a "classical" couplon (Stern et al., 1999; Scriven et al., 2013). Ca entry through LCCs in response to an AP raises [Ca]<sub>i</sub> in the cleft fast enough and to sufficiently high levels to activate CICR from j-SR RyRs. In contrast, the quantitatively much more abundant nj-SR is located in central regions of the atrial myocyte and does not associate with the surface membrane.

This raises the conceptual question of how RyRs of the nj-SR are activated in the first place. This conundrum is anchored in the fact that the cardiac-specific isoform of the RyR (RyR type 2, or RyR2) has, under physiological conditions (particularly with respect to cytosolic Mg and ATP concentrations), low Ca sensitivity (Meissner and Henderson, 1987; Cannell and Soeller, 1997; Zima et al., 2003; Qin et al., 2009; Chen et al., 2013) that in principle would preclude CICR considering that bulk cytosolic Ca transient (CaT) amplitude barely exceeds 1 μM, and thus the RyRs of the nj-SR apparently do not experience high enough [Ca]<sub>i</sub> levels required for triggering channel opening. This is particularly baffling because the vast majority of RyRs and CRUs reside in the nj-SR, which nonetheless participate in ECC on a beatto-beat basis and are responsible for the bulk Ca supply for atrial contraction.

As mentioned above, the centripetal propagation of activation from the cell periphery to the center during physiological atrial ECC is reminiscent of a propagating Ca wave that can be observed in atrial and ventricular cells under pathological conditions, especially SR Ca overload (in this context, see also the discussion of store overload-induced Ca release by Jones et al. in this issue). It was therefore our earlier observations on (pathological) Ca waves in ventricular myocytes that fertilized a novel paradigm of atrial ECC. We observed that in ventricular myocytes, cell-wide propagation of spontaneous Ca waves depends on an intra-SR Ca "sensitization" wave (Maxwell and Blatter, 2012). During wave propagation, the elevation of [Ca]i at the wave front leads to local Ca uptake by the sarcoplasmic/endoplasmic Ca ATPase (SERCA), which results in a local increase of [Ca]<sub>SR</sub> that sensitizes the RyR to cytosolic CICR via its luminal Ca dependence (Györke and Györke, 1998; Fill and Copello, 2002; and see Györke et al. in this issue). By this mechanism, the cytosolic Ca sensitivity of the RyR is shifted to lower levels and brings the threshold for CICR into the range of the amplitude of a propagating cytosolic Ca wave. A mechanism of wave propagation involving regulation of cytosolic Ca sensitivity of the RyR by luminal Ca during wave propagation has been proposed on the basis of indirect experimental conclusions (Keller et al., 2007) and theoretical considerations (Ramay et al., 2010; see discussion by Sobie et al. in this issue), but our study demonstrated such a mechanism experimentally with direct simultaneous measurements of [Ca]<sub>i</sub> and [Ca]<sub>SR</sub> (Maxwell and Blatter, 2012).

Subsequently we extended the concept of an intra-SR Ca sensitization signal to atrial muscle and tested a novel hypothesis of atrial SR Ca release during physiological ECC (Maxwell and Blatter, 2017). Experimentally, we measured simultaneously [Ca]<sub>i</sub> and [Ca]<sub>SR</sub> with a high-affinity cytosolic (rhod-2) and a low-affinity intra-SR Ca dye (fluo-5N) with high-resolution fluorescence confocal imaging. Cytosolic CaTs and [Ca]

SR depletion signals were recorded simultaneously from individual CRUs with transverse confocal line scan imaging of rabbit ventricular (Fig. 2 A) and atrial (Fig. 2 B) myocytes during AP-induced Ca release. In ventricular myocytes, the presence of t-tubules ensures the simultaneous activation of essentially all CRUs. Thus, the onset of the cytosolic CaT was identical in the cell periphery (Fig. 2 A, a) and in the cell center (Fig. 2 A, b), and the rise of [Ca]<sub>i</sub> coincided strictly with the onset of the decline of [Ca]<sub>SR</sub>; that is, rise of [Ca]<sub>i</sub> and depletion of [Ca]<sub>SR</sub> are tightly coupled and spatially homogeneous across the cell. Rabbit atrial myocytes, which lack ttubules entirely, exhibited spatially inhomogeneous cytosolic and SR Ca signals. In the cell periphery (j-SR) where release initiates, the features of CICR resembled ventricular ECC: the rise of [Ca]<sub>i</sub> and decline of [Ca]<sub>SR</sub> occurred simultaneously (Fig. 2 B, a). This is explained by the fact that the atrial peripheral couplings of the j-SR have all the key attributes of a (ventricular) couplon: LCCs face RyR clusters across a narrow cleft and Ca influx via LCCs rabidly rises to trigger robust CICR from j-SR. Release from central regions (Fig. 2 B, b), however, is delayed because of the time required for activation to propagate to the center of the cell and results in a slower rise of [Ca]i that peaks at a lower level compared with the cell periphery. Furthermore, the cell center revealed a temporal dispersion between onset of the cytosolic CaT and the decline of [Ca]<sub>SR</sub>. We defined the time interval between the rise of peripheral subsarcolemmal [Ca]i and the beginning of decline of central [Ca]<sub>SR</sub> as latency (Δt between dashed vertical lines 1 and 3 in Fig. 2 B). Along the transverse axis, the latency steadily increased with increasing distance from the cell periphery. Most important, during the latency period, the [Ca]<sub>SR</sub> signal revealed a surprising feature. Instead of the expected decline, a rise of [Ca]<sub>SR</sub> was observed that peaked immediately before [Ca]<sub>SR</sub> began to decrease. This transient increase of [Ca]<sub>SR</sub> was highly reproducible in amplitude and kinetics and could be observed reliably from beat to beat at the same subcellular locations. Guided by our earlier observation of an intra-SR Ca sensitization wave (Maxwell and Blatter, 2012) that drives spontaneous Ca wave propagation in ventricular myocytes, we hypothesize that this rise of [Ca]<sub>SR</sub> during the latency period was brought upon by Ca uptake by SERCA at the propagation front and serves as an intra-SR Ca sensitization signal that via luminal action lowers the activation threshold of the RyR to cytosolic CICR. The higher luminal [Ca]<sub>SR</sub> also lengthens RyR open time (Chen et al., 2013, 2014) and increases RyR unitary Ca flux. Together, these luminal Ca actions promote RyR activation and inter-RyR CICR and are favorable to robust propagation of activation by a mechanism we called "tandem activation" of the nj-SR RyRs by cytosolic and luminal Ca. Consistent with a central role of SERCA in this process, interventions

that targeted SERCA activity affected the atrial intra-SR Ca sensitization signal. We used the β-adrenergic agonist isoproterenol to increase SERCA activity, which occurs through phosphorylation of the inhibitory protein phospholamban (Luo et al., 1994). In the presence of isoproterenol, the amplitude of the Ca sensitization signal increased together with its duration and the latency. In contrast, in the presence of the SERCA blocker cyclopiazonic acid, no Ca sensitization signal was observed. These observations are consistent with the hypothesis that increased SERCA activity enhances SR Ca uptake at the front of propagating activation, which generates the Ca sensitization signal but also prolongs the time until a decline of [Ca]<sub>SR</sub>, and thus SR Ca depletion can be observed. Interestingly, compared with ventricular tissue, expression levels of the endogenous SERCA inhibitor phospholamban are lower in atrial myocytes (Koss et al., 1995; Lüss et al., 1999), which would logically favor the generation of the SR Ca sensitization signal because of overall higher SERCA activity, and the transverse spacing of CRUs was estimated to be smaller (Chen-Izu et al., 2006), which would be favorable for centripetal propagation of activation.

On the basis of these experimental findings, we proposed a novel paradigm of atrial ECC that we termed the "fire-diffuse-uptake-fire" (FDUF) mechanism (Fig. 3). To summarize, the sequence of events defining atrial ECC starts as membrane depolarization by an AP and activation of LCCs that trigger CICR from peripheral couplings of the j-SR. Ca release from peripheral j-SR establishes a robust Ca gradient that initiates centripetal Ca movement that subsequently triggers CICR from the first array of nj-SR CRUs, which further initiates CICR from progressively more centrally located nj-SR CRUs. The process of propagation of CICR through the nj-SR network is sustained by the FDUF mechanism and the aforementioned tandem activation of nj-SR RyRs. Propagating CICR ultimately results in a cell-wide elevation of [Ca]<sub>i</sub> that initiates and sustains contraction.

### Termination of SR Ca release

The beat-to-beat pump function of the heart requires fine-tuned termination of SR Ca release and removal of cytosolic Ca to ready the ECC machinery for the next cycle. Restoration of diastolic [Ca]<sub>i</sub> and [Ca]<sub>SR</sub> levels occurs via Ca reuptake by SERCA and extrusion across the sarcolemma by Na/Ca exchange (and minor beat-to-beat contributions by mitochondrial Ca sequestration and extrusion by the plasmalemmal Ca ATPase). The detailed mechanism of release termination in cardiac myocytes has remained a matter of debate (see also the contribution by Cannell and Kong in this issue). Agreement exists about the existence of four principle modes of termination, but their relative contributions under what specific conditions (including their remodeling in disease) is at the heart of the debate. Of the four modes

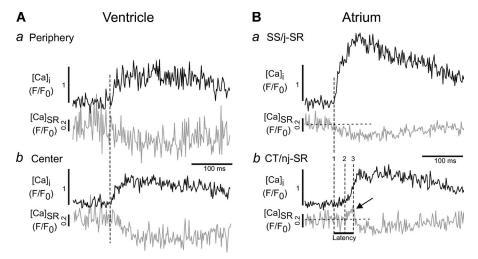


Figure 2. Intra-SR Ca sensitization signal during atrial ECC. (A) [Ca]; (rhod-2) and [Ca]<sub>SR</sub> (fluo-5N) fluorescence signals (F/F<sub>0</sub>; averaged over 0.6-µm-wide regions of interest) elicited by AP depolarization and recorded from individual peripheral (a) and central (b) release sites in a ventricular myocyte. The dashed vertical line marks the simultaneous onset of the cytosolic CaT and SR depletion in both subcellular regions. (B) [Ca]<sub>i</sub> and [Ca]<sub>SR</sub> signals recorded from an atrial cell. In the cell periphery (a), the rise of [Ca]; and [Ca]<sub>SR</sub> decline is simultaneous (vertical dashed line 1). In the cell center (b), the rise of [Ca]; is delayed, and [Ca]<sub>SR</sub> depletion lags behind the rise of [Ca]<sub>i</sub>. The decline of [Ca]<sub>SR</sub> is preceded by the Ca sensitization signal, a transient elevation of [Ca]<sub>SR</sub> (arrow). Vertical dashed lines 2 and 3 mark the duration of the Ca sensitization signal, and the time interval 1-3 is defined as latency. Reproduced from Maxwell and Blatter (2017).

of termination, two are cytosol resident, while the other two are SR resident. Termination by cytosol-resident mechanisms can occur by pernicious attrition (also referred to as induction decay; Laver et al., 2013), where termination occurs by a decrease of RyR Ca flux and subsequent failure of inter-RyR CICR (Gillespie and Fill, 2013), and Ca-dependent inactivation where RyRs

close subsequent to Ca binding to a low-affinity binding site on the RyR (Liu et al., 2010). In contrast, SR-resident mechanisms terminate Ca release as a result of falling [Ca]<sub>SR</sub> either by direct control of RyR gating by luminal Ca (Qin et al., 2008; MacLennan and Chen, 2009; Chen et al., 2014) or via RyR interaction with the SR Ca buffer calsequestrin (CASQ; Terentyev et al.,

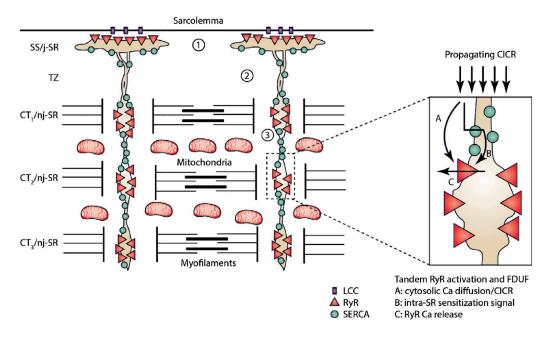


Figure 3. Atrial ECC: tandem RyR activation and FDUF mechanism. AP-induced Ca release from j-SR by LCC activation (1), followed by propagation through mitochondria-free transition zone (TZ; 2) and activation of centripetal propagating CICR (3) from central (CT) nj-SR CRUs ( $CT_1 \rightarrow CT_2 \rightarrow CT_3 \rightarrow ...$ ). Inset: FDUF mechanism. Tandem RyR activation by cytosolic CICR (A) and luminal RyR sensitization (B) by elevated [Ca]<sub>SR</sub> (SR Ca sensitization signal) resulting in Ca release (C). SR Ca sensitization signal is generated by Ca uptake at the activation front by SERCA. Reproduced from Maxwell and Blatter (2017).

2003; Györke et al., 2009). A common denominator of these four modes of termination is the fact RyR closing in every case ultimately depends on  $[Ca]_{SR}$ . Lower SR Ca promotes termination by pernicious attrition and SR-resident mechanisms but limits termination by Ca-dependent inactivation.

Recent experimental findings with respect to SR Ca depletion during physiological ECC and AP-induced CaTs raise interesting questions regarding the mechanism(s) of termination in atrial muscle. We compared cytosolic CaTs and corresponding SR Ca depletion signals originating from j-SR and nj-SR in atrial cells (Fig. 4). Consistent with earlier results (Sheehan and Blatter, 2003), central cytosolic CaT amplitudes are smaller than subsarcolemmal CaTs (Fig. 4 A). Surprising, however, was the consistent observation of significantly larger [Ca]<sub>SR</sub> depletion amplitudes in regions of the nj-SR, despite a smaller amplitude of the cytosolic signal (Fig. 4 B). A closer look at the [Ca]<sub>i</sub> and [Ca]<sub>SR</sub> signals at individual CRUs along a transverse axis during ECC (Fig. 4 C) revealed the largest cytosolic CaT amplitude in the cell periphery reflecting the initial AP-induced release of Ca from the j-SR. Activation then propagates through the ~1-µm-wide transition zone between j-SR and the first row of nj-SR CRUs with no decrement in amplitude. However, as activation reaches the first nj-SR CRU (CT<sub>1</sub>) the cytosolic CaT amplitude decreased to less than half, with further small progressive decline along the centripetal direction of propagation. In contrast, the depletion signal was smallest in the cell periphery (j-SR), showed little change in the transition zone (reflecting SR depletion in non-CRU regions of the SR; Zima et al., 2008; Picht et al., 2011), but was significantly larger at nj-SR CRUs and constant along the direction of propagation. We also investigated the properties of spontaneous elementary cytosolic Ca release (Ca sparks) and corresponding Ca depletion (Ca blinks) events measured simultaneously from individual CRUs (Figs. 4, D and E). Spontaneous Ca sparks originating from j-SR have a larger amplitude than nj-SR sparks, consistent with earlier findings (Sheehan et al., 2006), but the nadir of nj-SR Ca blinks reached a lower [Ca]<sub>SR</sub> level than j-SR blinks, and the depletion amplitude was larger. Thus, spontaneous elementary Ca release and depletion events from individual CRUs mirror the differential properties of AP-induced CaTs originating from j-SR and nj-SR (Fig. 4 A).

The lower depletion levels of the nj-SR during physiological ECC and Ca release suggest more effective CICR at nj-SR CRUs and/or differences in release termination between j-SR and nj-SR. The ability of a smaller cytosolic Ca signal to trigger a larger depletion is advantageous for centripetal propagation of CICR. It indicates that the requirement for the magnitude of the cytosolic trigger Ca signal is less stringent in the nj-SR, and fractional Ca release (i.e., the relationship between magnitude of

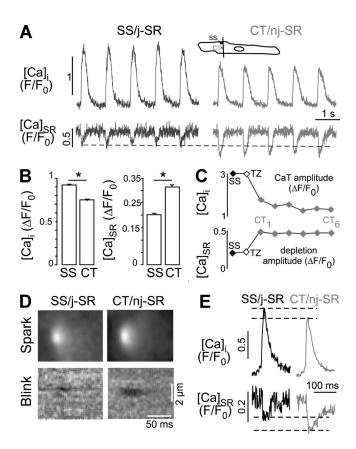


Figure 4. Cytosolic and Ca depletion signals from j-SR and nj-SR. (A) [Ca]<sub>i</sub> and [Ca]<sub>SR</sub> changes elicited by AP depolarization from subsarcolemmal (SS) j-SR and central (CT) nj-SR in an atrial myocyte (inset). (B) Mean cytosolic CaT and SR Ca depletion amplitudes of AP-induced Ca release from j-SR and nj-SR. \*, P < 0.001. (C) Cytosolic CaT and SR Ca depletion amplitudes along a transverse line showing Ca signals in the SS and transition zone (TZ) between j-SR and nj-SR regions, as well as from CT nj-SR release sites at increasing cell depth (CT1...CT6). Distance between measurements is 1  $\mu$ m. (D) Averaged confocal lines scan images (F/F0) of Ca sparks and corresponding Ca blinks originating from SS j-SR and CT nj-SR. (E) Mean Ca spark and blink profiles (F/F0) from images in D. Reproduced from Maxwell and Blatter (2017).

trigger and amount of released Ca) appears to be larger in the nj-SR. Several potential mechanisms can be envisioned for the more pronounced depletion of nj-SR release sites. One possibility is the pool size of releasable Ca of an individual CRU that could differ between j-SR and nj-SR. Although the higher cytosolic Ca signal in the cell periphery could be the result of a larger amount of Ca release from a j-SR CRU, geometric and structural factors may contribute. In the cell periphery, Ca is released into the narrow cleft of the j-SR peripheral couplings. [Ca]<sub>i</sub> in this confined space reaches high levels rapidly, while the same amount of Ca release from a source that is not confined by surrounding membranes likely dissipates more rapidly and will not reach comparable peak levels. Indeed, in the cell periphery, spontaneous Ca sparks, the quintessential measure of Ca

release of an individual CRU, are spatially asymmetrical. They are elongated in longitudinal direction by  $\sim 1.7$ compared with the transverse dimension (Shkryl and Blatter, 2013), whereas Ca sparks from nj-SR are symmetrical. After surface membrane permeabilization, the asymmetry and the amplitude of j-SR sparks are reduced (presumably by disrupting the physical integrity of the narrow cleft of the peripheral couplings), but the spatial dimensions (Shkryl and Blatter, 2008) and amplitude (Sheehan et al., 2006) of nj-SR sparks are unaffected. Thus, the geometry of the narrow cleft of the peripheral couplings of the j-SR is essential for shaping the local Ca signal and contributes to the larger amplitude of peripheral Ca release. Furthermore, Ca entry through LCCs makes a measurable contribution to cleft [Ca] (Kockskämper et al., 2001), a source of Ca that is absent in the cell center, and different Ca removal pathways (only the j-SR is in the vicinity of plasmalemmal Na/Ca exchange) contribute to shaping peripheral and central CaTs (Hohendanner et al., 2016).

SR Ca depletion levels and release termination is dependent on intra-SR Ca buffering that is provided by the endogenous Ca buffer CASQ. CASQ has a dual role in SR Ca handling. On one hand, CASQ buffers SR Ca in a [Ca]<sub>SR</sub>-dependent fashion and thereby determines Ca storage capacity of the SR and the functional size of the Ca store (Terentyev et al., 2003); on the other hand, CASQ is involved in luminal regulation of RyR gating (Györke and Terentyev, 2008; Györke et al., 2009; Knollmann, 2009). CASQ-dependent RyR regulation occurs by regulating free [Ca]<sub>SR</sub> and/or through conformational regulation in conjunction with the auxiliary SR proteins junctin, triadin, and histidine-rich Ca binding protein. Recent findings document a key role of the CASQ polymerization state for its buffer and RyR regulatory function (Manno et al., 2017). Interestingly, high-resolution studies have shown subcellular differences in CASQ endowment in atrial cells. RyR and CASQ show a lower degree of colocalization and less CASQ staining in the nj-SR (Schulson et al., 2011), suggesting less CASQ-mediated RyR inhibition and higher RyR excitability in the interior of atrial cells (i.e., RyRs of the nj-SR) and facilitation of the spread of excitation from the periphery to the center. Furthermore, lower CASQ levels and thus reduced Ca buffering would allow depletion to lower [Ca]<sub>SR</sub> levels, consistent with our observations during CaTs (Fig. 4 A) and during spontaneous Ca sparks and blinks (Fig. 4 E).

Related to release termination is the topic of refractoriness or restitution of the release machinery. Ion channels participating in ECC have time-dependent characteristics of recovery from inactivation, typically referred to as restitution properties. "Restitution" refers to the time interval required for the SR Ca release to overcome refractoriness and to become fully available again after a previous release event. In atrial myocytes,

restitution of Ca release from nj-SR is slower than from j-SR, reflecting another important difference in Ca handling between j-SR and nj-SR (Shkryl et al., 2012). (The topic of refractoriness is also addressed by Györke et al. [2017] in this issue.)

# j-SR and nj-SR: Two functional entities or a single Ca store?

The multitude of differences in Ca-handling properties of j-SR and nj-SR in atrial myocytes presented above begs the question whether j-SR and nj-SR are components of a single functional Ca store or actually represent two different entities. Collectively, the observation of significant differences in cytosolic and intra-SR Ca signals during ECC, elementary release and depletion signals of individual CRUs, SR Ca release mechanisms (FDUF mechanism of the nj-SR), CASQ endowment, proximity to the sarcolemma, and Ca release restitution properties could indeed suggest the possibility that j-SR and nj-SR should be viewed as separate functional and structural entities. On the other hand, there are experimental observations that argue more in favor of a single, albeit highly complex, Ca storage compartment that encompasses both j-SR and nj-SR. Despite the fact of lower [Ca]<sub>SR</sub> depletion levels of the nj-SR at the peak of electrically evoked CaTs, no intra-SR Ca gradients between j-SR and nj-SR were observed at the end of diastole or at rest. This is consistent with our earlier observation that intra-SR Ca diffusion in cardiac myocytes can be relatively fast (Picht et al., 2011). We determined an intra-SR Ca diffusion coefficient (D<sub>Ca,SR</sub>) of ~60 μm<sup>2</sup> s<sup>-1</sup>, a value that is larger than previous estimates of D<sub>Ca,SR</sub> (<10 μm<sup>2</sup> s<sup>-1</sup>) and higher than estimates for cytosolic Ca diffusion (~15 µm<sup>2</sup> s<sup>-1</sup>; Kushmerick and Podolsky, 1969; Swietach et al., 2008). Furthermore, we demonstrated directly that an activated CRU can drain Ca from a neighboring CRU under conditions of high connectivity of CRUs (Zima et al., 2008). Fluorescence recovery after photobleaching experiments of a fluorescent probe entrapped in the SR also demonstrated high connectivity between j-SR and nj-SR. Thus, the latter arguments favor a concept of a single but highly complex Ca store divided into microdomains with unique Ca-handling attributes and distinct sets of regulatory mechanisms of initiation and termination of Ca release.

### Conclusions and outlook

We propose a novel paradigm of atrial ECC that we termed the "fire-diffuse-uptake-fire" mechanism (Fig. 3). The key elements are a novel central role of SERCA during ECC (i.e., SERCA function is not restricted to removal of cytosolic Ca during relaxation only) that is responsible for the proposed tandem activation of the RyR by cytosolic and luminal Ca. We further demonstrate that not only initiation of SR Ca release but also depletion and termination properties are differentially

regulated in the j-SR and the nj-SR. This differential regulation is important in view of atrial function in health and disease. The atria have important hemodynamic duties. Atrial contraction contributes to ventricular filling, referred to as "atrial kick" or atrial booster function (Rahimtoola et al., 1975; Trafford et al., 2013; Hoit, 2014; Mehrzad et al., 2014). The contribution to ventricular filling covers a wide dynamic range that can span 20%-40% of ventricular filling at the end of ventricular diastole, depending on factors such as heart rate or as a consequence of atrial remodeling in disease. The nj-SR CRUs carry the bulk of Ca release burden of atrial contraction, thus a fine-tuned regulation of nj-SR Ca release is paramount for a dynamic response to hemodynamic demands. The atrial ECC mechanism is well suited for this purpose. As shown above, the nj-SR can deplete to lower levels and thus extend and prolong Ca release to secure ample Ca supply as required by contractile demands. An important auxiliary pathway for the regulation of atrial contractility is the engagement of IP3 receptor-induced Ca release (IICR). IP3 receptors are found in significantly higher density in atrial cells compared with ventricular myocytes (Domeier et al., 2008) and are capable of modulating Ca release during ECC (Kockskämper et al., 2008). IICR has positive inotropic effects in atrial cells but also enhances the propensity of proarrhythmic Ca release (Zima and Blatter, 2004; Li et al., 2005). We have shown previously that in earlier stages of ventricular failure, atrial Ca transients are enhanced (Hohendanner et al., 2015, 2016) and improve atrial contractile function, ventricular filling, and thus ejection fraction and cardiac output. Up-regulation of IICR and recruitment of IICR predominantly in the nj-SR are important contributors to improved hemodynamics in this process. IICR is typically activated by vasoactive agonists (e.g., angiotensin II, endothelin-1) and demonstrates that humoral factors represent an additional layer of regulation of atrial Ca signaling during ECC. Thus, nj-SR Ca release is characterized by a wide dynamic range of regulation, indicative of a broad inotropic reserve to respond to contractile and hemodynamic demands, whereas j-SR release is designed primarily to ensure the initiation of propagating CICR with high fidelity. The FDUF mechanism as the central element of nj-SR Ca release is regulated. Although it remains to be determined to what extent the FDUF mechanism is remodeled and how exactly is regulated in cardiac disease, interventions that manipulate the Ca sensitization signal and the FDUF mechanism can be envisioned as promising potential therapeutic targets with beneficial outcomes to preserve cardiac function.

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