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INVITED REVIEW

Excitation-contraction coupling and calcium release in atrial muscle

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Abstract

In cardiac muscle, the process of excitation-contraction coupling (ECC) describes the chain of events that links action potential induced myocyte membrane depolarization, surface membrane ion channel activation, triggering of Ca^{2+} induced Ca^{2+} release from the sarcoplasmic reticulum (SR) Ca^{2+} store to activation of the contractile machinery that is ultimately responsible for the pump function of the heart. Here we review similarities and differences of structural and functional attributes of ECC between atrial and ventricular tissue. We explore a novel "fire-diffuse-uptake-fire" paradigm of atrial ECC and Ca^{2+} release that assigns a novel role to the SR SERCA pump and involves a concerted "tandem" activation of the ryanodine receptor Ca^{2+} release channel by cytosolic and luminal Ca^{2+}. We discuss the contribution of the inositol 1,4,5-trisphosphate (IP_3) receptor Ca^{2+} release channel as an auxiliary pathway to Ca^{2+} signaling, and we review IP_3 receptor-induced Ca^{2+} release involvement in beat-to-beat ECC, nuclear Ca^{2+} signaling, and arrhythmogenesis. Finally, we explore the topic of electromechanical and Ca^{2+} alternans and its ramifications for atrial arrhythmia.

Keywords Excitation-contraction coupling · Atrium · Ca^{2+} release · IP_3 receptor Ca^{2+} signaling · Alternans · Arrhythmia

Introduction

Beat-to-beat Ca^{2+} signaling in atrial and ventricular muscle shows similarities, but also significant structural and functional differences. Here we review the mechanisms of atrial and ventricular excitation-contraction coupling (ECC) and Ca^{2+} release, the role of a secondary pathway of Ca^{2+} release via IP_3 receptor Ca^{2+} release channels for atrial ECC and atrial function, and finally the manifestations and functional implications of atrial alternans for atrial arrhythmogenesis.

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Atrial excitation-contraction coupling

In the heart, ECC refers to the process that links electrical activation to cardiac contraction. The sequence of events that constitutes ECC initiates with membrane depolarization by an action potential (AP), followed by opening of voltage-gated Ca^{2+} channels in the surface membrane and Ca^{2+} entry, which in turn triggers Ca^{2+} release from the sarcoplasmic reticulum (SR) Ca^{2+} store through ryanodine receptor (RyR) Ca^{2+} release channels by a process known as Ca^{2+}-induced Ca^{2+} release (CICR). The elevation of cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_i$) subsequently activates the contractile elements that results in cardiac muscle contraction. Most of the mechanistic details of ECC known to date were determined in ventricular muscle. While atrial and ventricular ECC clearly have important similarities, there are critical differences. Atrial and ventricular cells have unique sets of ion channels [78, 107] leading to distinctive AP morphologies that in turn affect Ca^{2+} transient (CaT) triggering efficiency and SR Ca^{2+} store loading [48]. In addition, atrial myocytes exhibit lower expression of phospholamban that results in higher activity of the sarco/endoplasmic reticulum ATPase (SERCA) [6]. It is well known that changes in phopholamban expression levels have profound effects on CaTs and ECC. As we have shown

previously, phospholamban ablation caused accelerated decay of CaTs and Ca^{2+} sparks in mouse ventricular myocytes, increased SR Ca^{2+} load, and frequently led to Ca^{2+} waves that were spatially narrower and often aborted after propagating over only a short distance [41]. However, aside from differences in phospholamban levels, one of the most important differences between atrial and ventricular myocytes is the system of transverse- (t) tubules. These surface membrane invaginations extend in a sarcomeric pattern throughout the entire ventricular myocyte, but not in atrial cells (Fig. 1a). The t-tubule system is an integral part of the surface membrane (or the sarcolemma), that allows the placement of voltage-gated L-type Ca^{2+} channels (LCCs) in close vicinity to RyR clusters. RyR clusters are considered SR Ca^{2+} release units (CRUs; [28]) which give rise to elementary intracellular Ca^{2+} release events, also known as Ca^{2+} sparks. AP-induced whole cell CaTs are the spatial and temporal summation of Ca^{2+} sparks from thousands of CRUs. In the presence of a t-tubular system, the vast majority of CRUs has its own source of activator Ca^{2+} in the form of a small number of adjacent LCCs. As a consequence of these structural arrangements, Ca^{2+} release during ventricular ECC is spatially homogeneous throughout the cell. Atrial myocytes, however, have only a rather sparse and irregular t-tubule system or are even entirely lacking any t-tubules [7, 40, 63, 98] with important consequences for atrial Ca^{2+} dynamics during ECC. Because of these structural features, AP induced Ca^{2+} release in atrial cells is characterized by pronounced spatial inhomogeneities [4, 5, 8, 40, 94]. Elevation of $[Ca^{2+}]_i$ starts in the cell periphery where the opening of LCCs provides the required Ca^{2+} to induce CICR from the most peripheral SR Ca^{2+} release sites [55, 94]. This generates Ca^{2+} gradients that are large enough to overcome endogenous cytosolic Ca^{2+} buffering [92] and allows for centripetal Ca^{2+} diffusion and activation of CICR from SR release sites in progressively more central regions of the cell, thus, during atrial ECC $[Ca^{2+}]_i$ rises by propagating

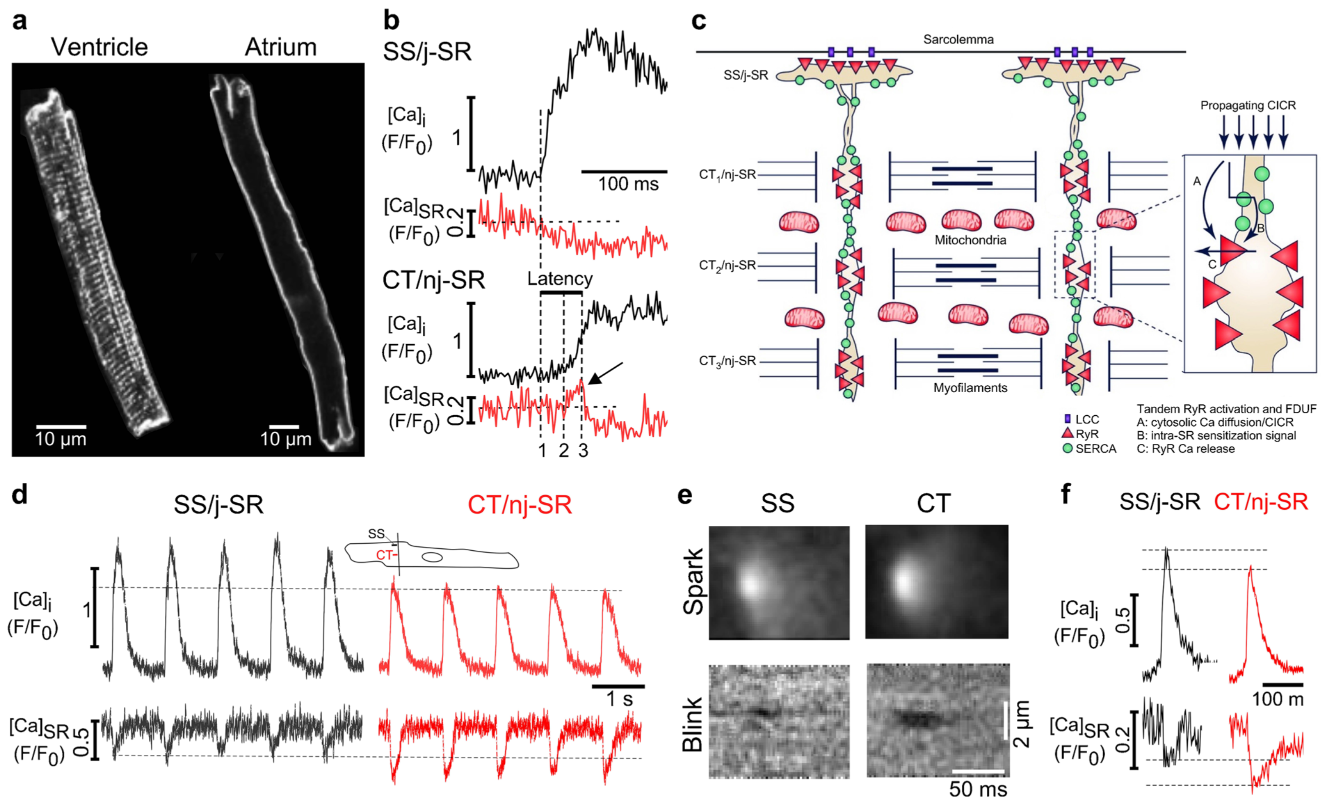


Fig. 1 Ca^{2+} signaling during ECC in atrial myocytes. **a** Membrane staining with the fluorescent probe Di-8-ANEPPS reveals the regular structure of the t-tubule system in ventricular myocytes (left) and the absence of t-tubules in atrial cells (right). **b** Atrial ECC. Subsarcolemmal SS/j-SR (top) and central CT/nj-SR (bottom) $[Ca^{2+}]_i$ and $[Ca^{2+}]_{SR}$ transients from individual CRUs. Intra-SR Ca^{2+} sensitization signal (arrow) and latency of CT/nj-SR Ca^{2+} release. (1), begin SS CaT; (2), begin Ca^{2+} sensitization signal (arrow) and latency of CT/nj-SR Ca^{2+} release are shown. (1), begin SS Ca^{2+} release; (2), begin nj-SR Ca^{2+} sensitization signal; (3), start of nj-SR $[Ca^{2+}]_{SR}$ decline. **c**, Current paradigm of atrial ECC. AP-induced Ca^{2+} release from SS/j-SR by LCC activation starts in the cell periphery followed by centripetally propagating CICR from 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), resulting in Ca^{2+} release (C). **d**, Subcellular SS/j-SR and CT/nj-SR AP-induced $[Ca^{2+}]_i$ and $[Ca^{2+}]_{SR}$ transients. Cytosolic CaT amplitude in central regions are smaller; however, SR depletion levels are lower in the nj-SR. Inset: line scan position and SS and CT regions of interest (1 µm wide). **e**, Averaged confocal line scan images (F/F_0) of Ca^{2+} sparks and corresponding Ca^{2+} blinks originating from SS/j-SR and CT/nj-SR. **f**, Averaged Ca^{2+} spark and Ca^{2+} blink profiles from j-SR and nj-SR CRUs from images in panel **e**. Reproduced and modified from [67].

CICR from the periphery to the center of the cell in a Ca^{2+} wave-like fashion by a diffusion-reaction process or a "fire-diffuse-fire" mechanism [51, 94]. The propagating nature of atrial Ca^{2+} release results in complex $[Ca^{2+}]_i$ inhomogeneities and subcellular $[Ca^{2+}]_i$ gradients.

The extent of t-tubule endowment of atrial myocytes shows considerable species differences (for reference see [85, 104]). A putative role of an intracellular axial membrane structure recently described in certain species further enhances the complexity of atrial Ca^{2+} signals during ECC [9]. However, in cat and rabbit atrial cells for example, the t-tubular system is entirely absent [40, 67]. The absence or paucity of t-tubules divides the atrial SR Ca^{2+} store into two types of SR based on the proximity to the sarcolemma: junctional SR (j-SR) is found in close association with the sarcolemmal membrane, whereas the much more abundant non-junctional SR (nj-SR) is found distant from the sarcolemma in more central regions of the cell. Importantly, RyR Ca^{2+} release channels are abundant in the membranes of both j-SR and nj-SR and participate in physiological ECC [7, 11, 55, 63, 98, 110]. RyRs are organized in a 3-dimensional array of channel clusters or CRUs [40, 55, 92, 94]. The j-SR forms close physical associations with the sarcolemma known as peripheral couplings. Here, the sarcolemma hosts voltage-gated LCCs that are facing clusters of RyRs in the SR membrane across a narrow inter-membrane cleft, similar to dyads in ventricular myocytes [55, 68]. Thus, the CRUs of the j-SR are functionally organized like a "classical couplon" [90, 100]. Ca^{2+} entry through LCCs in response to an AP raises $[Ca^{2+}]_i$ in the cleft fast and high enough to activate CICR from j-SR RyRs. In contrast, the fact that the quantitatively much more abundant nj-SR in central regions does not associate with the sarcolemma 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 Ca^{2+}-sensitivity [70, 82, 114] of the cardiac-specific isoform of the RyR (RyR type-2, or RyR2) is low, and compared to the j-SR, the activating Ca^{2+} signal for the nj-SR CRUs is slower, diffuser, and lower in amplitude. Given the facts that bulk cytosolic CaT amplitude barely exceeds 1 μM and RyR Ca^{2+} sensitivity is low, in principle would preclude activation of nj-SR Ca^{2+} release. Nonetheless, during atrial ECC robust nj-SR Ca^{2+} release indeed occurs and actually provides the bulk Ca^{2+} supply for atrial contraction.

A similar situation is found for Ca^{2+} waves observed in atrial and ventricular cells under pathological conditions, especially during SR Ca^{2+} overload. Waves propagate through the cytosol by CICR in the absence of LCC activation and depend primarily on RyR properties [65], thus raising the same question how, without the LCC Ca^{2+} influx, CICR can be activated efficiently. We observed that in ventricular myocytes cell-wide propagation of spontaneous Ca^{2+} waves depends on an intra-SR Ca^{2+} "sensitization" signal [66]. During wave propagation, the elevation of $[Ca^{2+}]_i$ at the wave front leads to local Ca^{2+} uptake by SERCA which results in a local increase of $[Ca^{2+}]_{SR}$ that sensitizes the RyR to cytosolic CICR via its luminal Ca^{2+}-dependence [32]. By this mechanism, the cytosolic Ca^{2+} 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^{2+} wave. A mechanism of wave propagation involving regulation of cytosolic Ca^{2+} sensitivity of the RyR by luminal Ca^{2+} during wave propagation has been proposed based on indirect experimental conclusions [52] and theoretical considerations [84], and was confirmed empirically with direct simultaneous measurements of $[Ca^{2+}]_i$ and $[Ca^{2+}]_{SR}$ [66].

These observations from ventricular myocytes fertilized a novel paradigm of atrial ECC. We extended the concept of an intra-SR Ca^{2+} sensitization signal to atrial myocytes [67] with the goal to unravel the aforementioned baffling conundrum of atrial ECC. By measuring simultaneously $[Ca^{2+}]_i$ and $[Ca^{2+}]_{SR}$ with high-resolution confocal fluorescence imaging, we determined cytosolic CaTs and $[Ca^{2+}]_{SR}$ depletion signals in atrial myocytes during AP-induced Ca^{2+} release (Fig. 1b). The CaT initiated in the cell periphery through release of Ca^{2+} from j-SR is characterized by coinciding increase of $[Ca^{2+}]_i$ and decline of $[Ca^{2+}]_{SR}$, reminiscent of ventricular cells where the rise of $[Ca^{2+}]_i$ and decline of $[Ca^{2+}]_{SR}$ also occur simultaneously and are highly synchronized throughout the entire myocyte. In stark contrast to ventricular cells, atrial Ca^{2+} release from central regions (nj-SR) lags behind peripheral release due to the time required for the activation to propagate to the center of the cell. The rise of central $[Ca^{2+}]_i$ is slower and peaks at a lower level at a point in time when peripheral $[Ca^{2+}]_i$ is already declining. Furthermore, the cell center revealed a temporal dispersion between onset of the cytosolic CaT and the decline of $[Ca^{2+}]_{SR}$. The time interval between rise of peripheral subsarcolemmal (SS) $[Ca^{2+}]_i$ and beginning of decline of central (CT) $[Ca^{2+}]_{SR}$ was defined as latency (Δt between dashed vertical lines 1 and 3 in Fig. 1b). Along the transverse axis, the latency steadily increased with increasing distance from the cell membrane, and the $[Ca^{2+}]_{SR}$ signal revealed a unique and surprising feature. Instead of an immediate decline, a rise of $[Ca^{2+}]_{SR}$ occurred before $[Ca^{2+}]_{SR}$ began to decrease. This transient increase of $[Ca^{2+}]_{SR}$ was highly reproducible in amplitude and kinetics, and could be observed reliably from beat to beat at the same CRU. Ca^{2+} uptake by SERCA at the propagation front was responsible for this rise of $[Ca^{2+}]_{SR}$ during the latency period and generated—analogous to the previous observation for Ca^{2+} waves—an intra-SR Ca^{2+} sensitization signal that via luminal action lowers the activation threshold of the RyR to cytosolic CICR from nj-SR. The higher luminal $[Ca^{2+}]_{SR}$ also lengthens RyR open time [12] and increases RyR unitary Ca^{2+} flux. Together, these luminal Ca^{2+} actions promote RyR activation and inter-RyR CICR and sustain robust propagating CICR through a mechanism termed "tandem activation" of the nj-

SR RyRs by cytosolic and luminal Ca^{2+}. Additional experiments further confirmed the central role of SERCA in this process. β-adrenergic stimulation with isoproterenol to increase SERCA activity increased amplitude, duration, and latency of the Ca^{2+} sensitization signal. In contrast, SERCA inhibition with cyclopiazonic acid abolished the Ca^{2+} sensitization signal [67].

Based on these experimental findings, we proposed a novel paradigm of atrial ECC termed "fire-diffuse-uptake-fire" or FDUF mechanism (Fig. 1c). In summary, atrial ECC consists of the following sequence of key events: atrial ECC is initiated by AP dependent membrane depolarization leading to LCC activation, Ca^{2+} influx and subsequent CICR from subsarcolemmal j-SR CRUs (or peripheral couplings). The rise of subsarcolemmal $[Ca^{2+}]_i$ establishes a robust Ca^{2+} gradient that drives centripetal Ca^{2+} 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 CRUs. Propagating CICR ultimately results in a cell-wide elevation of $[Ca^{2+}]_i$ that initiates and sustains contraction.

There are additional features unique to atrial ECC and Ca^{2+} release. Comparison of AP-induced cytosolic CaTs and corresponding SR Ca^{2+} depletion signals revealed important differences between j-SR and nj-SR (Fig. 1d). Closer inspection of $[Ca^{2+}]_i$ and $[Ca^{2+}]_{SR}$ signals at individual CRUs revealed the largest cytosolic CaT amplitude in the cell periphery reflecting the initial AP-induced release of Ca^{2+} from the j-SR. Once activation reached the first nj-SR CRU (CT_1), the cytosolic CaT amplitude decreased significantly, with further small progressive decline along the centripetal direction of propagation. In contrast and contrary to expectation, the depletion signal was smallest in the cell periphery (j-SR) and became significantly larger in the nj-SR despite a smaller amplitude of the cytosolic signal. The same pattern was found to apply to spontaneous elementary cytosolic Ca^{2+} release (Ca^{2+} sparks) and corresponding Ca^{2+} depletion events (Ca^{2+} blinks; [10]) measured simultaneously from individual CRUs (Fig. 1e, f). Spontaneous Ca^{2+} sparks originating from j-SR have a larger amplitude than nj-SR sparks consistent with earlier findings [93]; however, nj-SR Ca^{2+} blinks depleted to a lower $[Ca^{2+}]_{SR}$ level than j-SR blinks and the depletion amplitude was larger (Fig. 1f). Thus, spontaneous elementary Ca^{2+} release and depletion events from individual CRUs mirror the differential properties of AP-induced CaTs originating from j-SR and nj-SR.

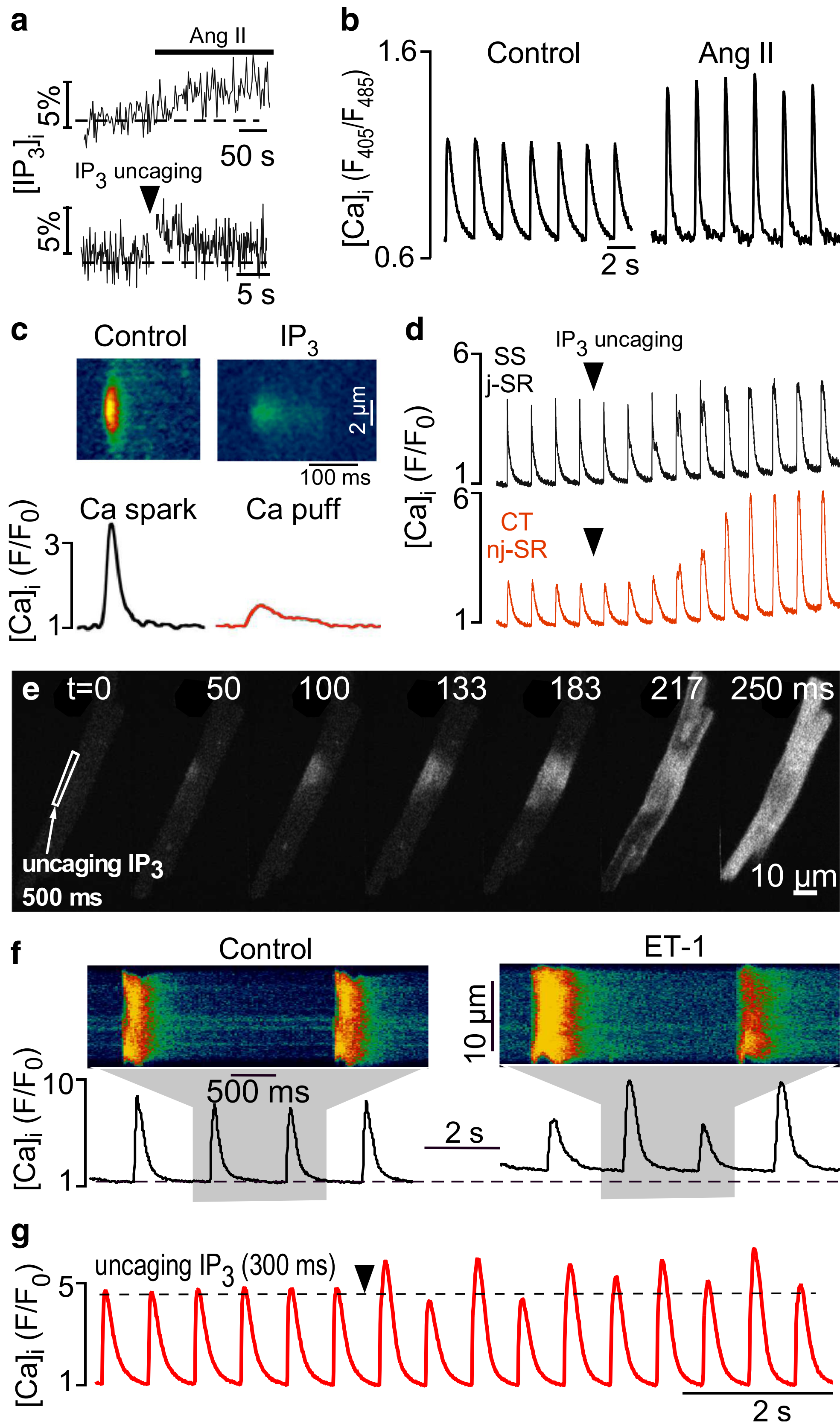
The subcellular differences in cytosolic CaT and SR Ca^{2+} depletion amplitudes raise interesting questions. Does the lower depletion level of the nj-SR suggest more effective CICR at nj-SR CRUs? The ability of a smaller cytosolic Ca^{2+} signal to trigger a larger depletion is advantageous for centripetal propagation of CICR. The requirement for the magnitude of the cytosolic trigger Ca^{2+} signal for nj-SR CICR appears to be less stringent, and fractional Ca^{2+} release, i.e., the relationship between magnitude of trigger and amount of released Ca^{2+}, is larger for the nj-SR. Several potential mechanisms can be envisioned for the more pronounced depletion of nj-SR release sites. One possibility is that the pool size of releasable Ca^{2+} of an individual CRU is different in j-SR and nj-SR. The difference in $[Ca^{2+}]_{SR}$ depletion levels in j-SR and nj-SR might also be related to intra-SR Ca^{2+} buffering. Intra-SR Ca^{2+} buffering is provided by the endogenous Ca^{2+} buffer calsequestrin (CASQ). CASQ buffers SR Ca^{2+} in a $[Ca^{2+}]_{SR}$ dependent fashion and thereby determines Ca^{2+} storage capacity of the SR and the functional size of the Ca^{2+} store [103]. Furthermore, CASQ is also involved in luminal regulation of RyR gating [33, 34, 53]. High-resolution studies revealed subcellular differences in CASQ endowment in atrial cells. RyR and CASQ co-localize to a lower degree and less CASQ staining is detected in the nj-SR [89], suggesting less CASQ-mediated RyR inhibition and higher RyR excitability in the interior of atrial cells that facilitates the spread of excitation from the periphery to the center. Furthermore, lower CASQ levels and less Ca^{2+} buffering allow for depletion to lower $[Ca^{2+}]_{SR}$ levels, consistent with our observations.

Furthermore, geometrical and structural factors contribute to the larger cytosolic Ca^{2+} signal in the cell periphery. In the cell periphery, Ca^{2+} is released into the narrow cleft of the j-SR peripheral couplings. The narrow cleft geometry assures that $[Ca^{2+}]_i$ in this confined space reaches high levels rapidly, while the same amount of Ca^{2+} release from a source that lacks surrounding membranes will dissipate rapidly and fail to reach comparable peak levels. This is indeed reflected in Ca^{2+} spark properties. Ca^{2+} sparks are the quintessential measure of Ca^{2+} release of an individual CRU. In the periphery of atrial cells, Ca^{2+} sparks are spatially asymmetrical and show an elongation in longitudinal direction by ~ 1.7 compared to the transverse dimension [94]. In contrast, Ca^{2+} 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 and amplitude [93] of nj-SR sparks are unaffected. These data clearly show that the geometry of the narrow cleft of the j-SR peripheral couplings shapes the local Ca^{2+} signal. Furthermore, Ca^{2+} entry through LCCs makes a sizable contribution to cleft $[Ca^{2+}]$ [55]. Obviously, this Ca^{2+} source is absent in the center of a cell lacking t-tubules. Finally, the aforementioned lower phospholamban expression level in atrial cells is favorable for the buildup of the intra-SR Ca^{2+} sensitization signal, and differences in Ca^{2+} removal pathways contribute to peripheral and central CaTs [38] since only the j-SR is in the vicinity of plasmalemmal Na^+/Ca^{2+} exchange (NCX), the main Ca^{2+} removal system in cardiac cells.

Restoration of diastolic $[Ca^{2+}]_i$ in central cell regions, however depends predominantly on SR Ca^{2+} reuptake, Ca^{2+} diffusion, and buffering rather than Ca^{2+} extrusion. Further contributions to cardiac ECC come from mitochondria. Mitochondria contribute to ECC by cycling and buffering cytosolic Ca^{2+} which in turn shapes the cytosolic CaT. Mitochondria provide ATP for the contractile apparatus and ion pumps, and alter the activity of Ca^{2+} handling proteins. Mitochondria are also a source of reactive oxygen species (ROS) which determine the redox environment of ECC Ca^{2+} handling proteins [17]. Mitochondria occupy approximately a third of the volume of a cardiac myocytes and have a large capacity to take up and sequester Ca^{2+}. However, it has remained a matter of debate whether and how mitochondria might play a $[Ca^{2+}]_i$ regulatory role on a beat-to-beat basis. An open question is whether the repetitive cytosolic CaTs are mirrored in robust oscillatory changes of mitochondrial Ca^{2+} ($[Ca^{2+}]_{mito}$), or whether the magnitude of mitochondrial Ca uptake on a beat-to-beat basis is small and changes of $[Ca^{2+}]_{mito}$ reflect an integrative signal of cytosolic Ca^{2+} spiking (for discussion see [77]). Mitochondrial Ca^{2+} buffering also profoundly modulates ECC and CaTs in atrial myocytes. Inhibition of mitochondrial Ca^{2+} uptake or collapsing the negative mitochondrial membrane potential significantly accelerates the centripetal SR Ca^{2+} release propagation from the peripheral j-SR through the network of the nj-SR and increases the CaT amplitude of nj-SR release [36]. Furthermore, atrial cells from failing hearts (left-ventricular heart failure) have a reduced mitochondrial density and a decreased capacity to buffer Ca^{2+}, resulting in CaTs of increased amplitude [37]. For efficient atrial ECC, the propagation of CICR from the j-SR to the first array of nj-SR release sites is critical and the mechanism is controversial. In rat atrial myocytes, mitochondria located between j-SR and nj-SR have been suggested to curtail Ca^{2+} passage by sequestering Ca^{2+} and acting as "mitochondrial firewall" [8]; however, in rabbit atrial myocyte, we found that this subcellular region is actually largely devoid of mitochondria and Ca^{2+} movement through this region occurs nearly twice as fast as through the central regions occupied by nj-SR [36].

IP_3 receptor-induced Ca^{2+} release

Atrial myocytes are endowed with a second, less abundant Ca^{2+} release channel, the inositol-1,4,5-trisphosphate receptor (IP_3R). Three isoforms of the IP_3R are known, with the IP_3R type 2 (IP_3R2) being the predominant isoform in heart muscle. IP_3Rs are outnumbered by RyRs ~ 1:50 at the protein level; however, IP_3R expression level in atrial cells is higher than in ventricular cells [19, 57]. IP_3R activation depends on G protein-coupled receptor-mediated activation of phospholipase C (PLC). The subsequent hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) leads to formation of IP_3 (Fig. 2a) and diacylglycerol (DAG), an activator of protein kinase C (PKC). In the atria, increased $[IP_3]$ has been reported after vasoactive agonist stimulation (Fig. 2b), stretch, ischemia/reperfusion, and in dilated cardiomyopathy. Several vasoactive agonists (angiotensin II, endothelin-1, phenylephrine) are capable of IP_3R-induced Ca^{2+} release (IICR) activation, indicative of the fact that atrial Ca^{2+} signaling during ECC is subject to humoral regulation. The role of IICR in the heart has long been debated (reviewed in [57]), and involves participation in ECC but also non-ECC functions (e.g., regulation of nuclear Ca^{2+} [36, 115] and transcription factors [86, 111]). There is strong evidence that the nucleus and especially the nuclear envelope Ca^{2+} store is a distinct IICR signaling domain. Experiments performed in intact and membrane-permeabilized myocytes as well as in isolated nuclei revealed that stimulation of IP_3Rs elicited larger nuclear Ca^{2+} signals than RyR activation, and resting nuclear $[Ca^{2+}]$ increased more and with a different time course than cytosolic $[Ca^{2+}]$. Also, agonist-dependent IICR stimulation increased AP-induced CaTs throughout the entire cell but had the most pronounced effect in the nuclear region, and the highest frequency of Ca^{2+} puffs, the elementary IICR events from IP_3R clusters (Fig. 2c), was found in the nucleus [36, 115]. Compared to RyR-mediated Ca^{2+} sparks, Ca^{2+} puffs have an approximately fivefold smaller amplitude, threefold longer duration, and a two- to threefold slower rise time [37, 113]. In atrial myocytes, IICR participation in ECC results in positive inotropy by increasing the amplitude of the AP-induced CaT (Fig. 2b, d). Enhancement of CaTs occurs through a cytosolic Ca^{2+}-dependent sensitization of the RyR, whereas Ca^{2+} release through IP_3Rs may also add directly to the CaT. But IICR also has proarrhythmic actions [19, 50, 61, 62, 64, 113]. It increases diastolic $[Ca^{2+}]_i$, enhances the propensity of spontaneous Ca^{2+} release, results in spontaneous APs and arrhythmogenic Ca^{2+} waves (Fig. 2e), and leads to Ca^{2+} alternans (Fig. 2f, g). IP_3R is upregulated in cardiac disease [37] and IICR acquires a more prominent role in atrial ECC by enhancing SR Ca^{2+} release from the nj-SR. Enhanced IICR under pathological conditions supports the important hemodynamic duties of the atria. Atrial contraction contributes to ventricular filling, referred to as "atrial kick" or atrial booster function [39, 69, 104]. The contribution to ventricular filling amounts to 20–40% of end-diastolic filling and is subject to atrial remodeling in disease. We have shown previously that in earlier stages of ventricular failure when ventricular CaTs are already deteriorating, atrial CaTs are enhanced [37, 38] and improve atrial contractile function, ventricular filling, and thus ejection fraction and cardiac output. Upregulation of IICR and recruitment of IICR predominantly affect the nj-SR which is


a
Ang II
$[IP_3]_i$
5%
50 s
IP3 uncaging
5%
5 s
b
1.6
0.6
Control
Ang II
$[Ca]_i (F_{405}/F_{485})$
2 s
c
Control
IP3
2 µm
100 ms
Ca spark
Ca puff
$[Ca]_i (F/F_0)$
3
1
d
IP3 uncaging
SS
j-SR
CT
nj-SR
6
1
$[Ca]_i (F/F_0)$
e
t=0
50
100
133
183
217
250 ms
uncaging IP3
500 ms
10 µm
f
Control
ET-1
10 µm
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10
1
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uncaging IP3 (300 ms)
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1
$[Ca]_i (F/F_0)$
2 s

◀ **Fig. 2** IP_3 receptor-induced Ca^{2+} release. **a** $[IP_3]_i$ measurements with the FRET(CFP/YFP)-based IP_3 sensor FIRE in response to angiotensin II (Ang II) and photorelease of caged IP_3 (cag-IP_3 PM). $[IP_3]_i$ expressed as % change of F_{530}/F_{488}. **b** Ang II increased the amplitude of electrically evoked CaTs. **c** Average line scan images (top) and $[Ca^{2+}]_i$ profiles (bottom) of Ca^{2+} sparks (black) and IP_3- (red) mediated Ca^{2+} release events (Ca^{2+} puffs) recorded from membrane permeabilized atrial myocytes. Ca^{2+} puffs were elicited with IP_3 in the presence of tetracaine to eliminate RyR mediated Ca^{2+} sparks. **d** SS/j-SR and CT/nj-SR CaTs after IP_3 uncaging. CaTs originating from nj-SR regions are enhanced to a larger degree by IICR than peripheral SS/j-SR CaTs. **e** IP_3 uncaging in a narrow subsarcolemmal region triggers a propagating Ca^{2+} wave. **f** CaTs and CaT alternans recorded in control and in the presence of endothelin-1 (ET-1). **g** IP_3 uncaging elicits CaT alternans. Arrow head: exposure to 405-nm laser light for photolysis of caged IP_3. Panels **b** and **d** reproduced and modified from [37]. Panels **c** and **f** reproduced and modified from [113]. Panels **e** and **g** reproduced and modified from [95].

hemodynamically favorable since nj-SR Ca^{2+} release is the main Ca^{2+} supplier for atrial contraction.

Atrial electromechanical and Ca^{2+} alternans

As mentioned above, one of the manifestations of Ca signaling disturbances in response to IICR is Ca^{2+} alternans (Fig. 2f, g). Alternans is a recognized risk factor for cardiac arrhythmia—including atrial fibrillation (AF) [1, 14, 27, 74, 105]—and sudden cardiac death [80, 102, 106]. Aside from IICR, a plethora of experimental and pathological conditions can cause cardiac alternans, suggesting a multifactorial process (for reviews see [5, 13, 20, 22, 23, 60, 71, 87, 108, 109]). At the cellular level, cardiac alternans is defined as cyclic, beat-to-beat alternation in contraction amplitude (mechanical alternans), AP duration (APD or electrical alternans), and Ca^{2+} transient amplitude (Ca^{2+} alternans) at constant stimulation frequency (Fig. 3a). APD and CaT are closely linked. This is due to the fact that the regulation of $[Ca^{2+}]_i$ and V_m is bi-directionally coupled ($[Ca^{2+}]_i \leftrightarrow V_m$) and governed by complex feedback pathways. $V_m \rightarrow [Ca^{2+}]_i$ coupling refers to the notion that V_m contributes to $[Ca^{2+}]_i$ regulation and Ca^{2+} signaling through AP attributes and APD restitution properties. The AP and the AP-dependent changes of V_m determine voltage-dependent Ca^{2+} fluxes. APD restitution is a time-dependent process and becomes especially critical at short cycle lengths when beat-to-beat differences in APD restitution and thus AP morphology profoundly affects Ca^{2+} release. $[Ca^{2+}]_i \rightarrow V_m$ coupling is determined by the effect of $[Ca^{2+}]_i$ dynamics, Ca^{2+} fluxes, Ca^{2+}-carrying membrane currents, and Ca^{2+}-dependent ion currents and transporters on V_m and APD. It is generally agreed that this bi-directional coupling represents a key causative factor for alternans. It is however a matter of ongoing debate whether $V_m \rightarrow [Ca^{2+}]_i$ or $[Ca^{2+}]_i \rightarrow V_m$ coupling is the predominant mechanism. While there are arguments and experimental data supporting both directions as the primary cause, recent results (including our own [45, 96]) and computational findings tend to favor the prospect that Ca^{2+} signaling disturbances are the primary causes of alternans (Fig. 3b, c), although the debate is far from settled (for reviews and pertinent experimental work see [5, 13, 22, 23, 30, 60, 87]). Nonetheless, there is also experimental evidence that membrane conductances drive Ca^{2+} alternans, for example, via LCCs [48], Ca^{2+}-activated Cl^- channels [46, 47], and several K^+ channels [49]. AP duration is an important determinant of susceptibility to alternans. AP prolongation is a risk factor for alternans, and AP shortening has been proposed as a therapeutic strategy for alternans protection at the cellular and organ level [49], a strategy that could be especially effective in conditions of long QT syndrome [91].

Two parameters (for review and references see [108, 109]) have emerged as being critically relevant to $[Ca^{2+}]_i \rightarrow V_m$ coupling and the origin of alternans: (1) SR Ca^{2+} load/fractional Ca^{2+} release and (2) the efficiency of cytosolic Ca^{2+} sequestration. The non-linear relationship between Ca^{2+} sequestration and load/fractional release determines the vulnerability to alternans [108]. Here, Ca^{2+} sequestration refers to the net efficiency of clearing the cytosolic compartment of Ca^{2+}. It includes Ca^{2+} reuptake into the SR via SERCA, extrusion via NCX and plasmalemmal Ca^{2+}-ATPase, cytosolic buffering, and mitochondrial uptake, but it also accounts for diastolic SR Ca^{2+} leak (via RyRs, IP_3Rs, and other pathways; see [116]) which counteracts any Ca^{2+} removal pathways. The relationship predicts that factors increasing Ca^{2+} load and fractional release promote, whereas factors increasing Ca^{2+} sequestration protect against alternans. A prominent role in alternans etiology is played by mitochondria [3, 31, 97]. Inhibition of various mitochondrial functions and signaling pathways (dissipation of mitochondrial membrane potential, inhibition of mitochondrial F_1/F_0-ATP synthase, electron transport chain (ETC), Ca^{2+}-dependent dehydrogenases, and mitochondrial Ca^{2+} uptake and extrusion) all enhanced CaT alternans [25, 26], whereas stimulation of Ca^{2+} uptake via the mitochondrial Ca^{2+} uniporter complex improved CaT alternans [79]. The beat-to-beat dynamics of both Ca^{2+} sequestration and load/fractional release are critically dependent on restitution properties and refractory kinetics of the SR Ca^{2+} release mechanism. The amount of Ca^{2+} released during a given heartbeat is determined by the recovery of the trigger for CICR, SR Ca^{2+} load, and the release mechanism itself. APD restitution (including recovery of LCCs) has been recognized as a causative and/or contributing factor to Ca^{2+} alternans and may play a role particularly at high heart rates (reviewed in [108, 109]). The role of SR Ca^{2+} reuptake and re-establishment of Ca^{2+} load have been the subject of numerous investigations [16, 44, 81, 112] together with the controversial question whether cardiac alternans requires beat-to-beat alternations in SR Ca^{2+} content and end-diastolic SR filling [18] or not [42]. While it has been suggested that instability in the

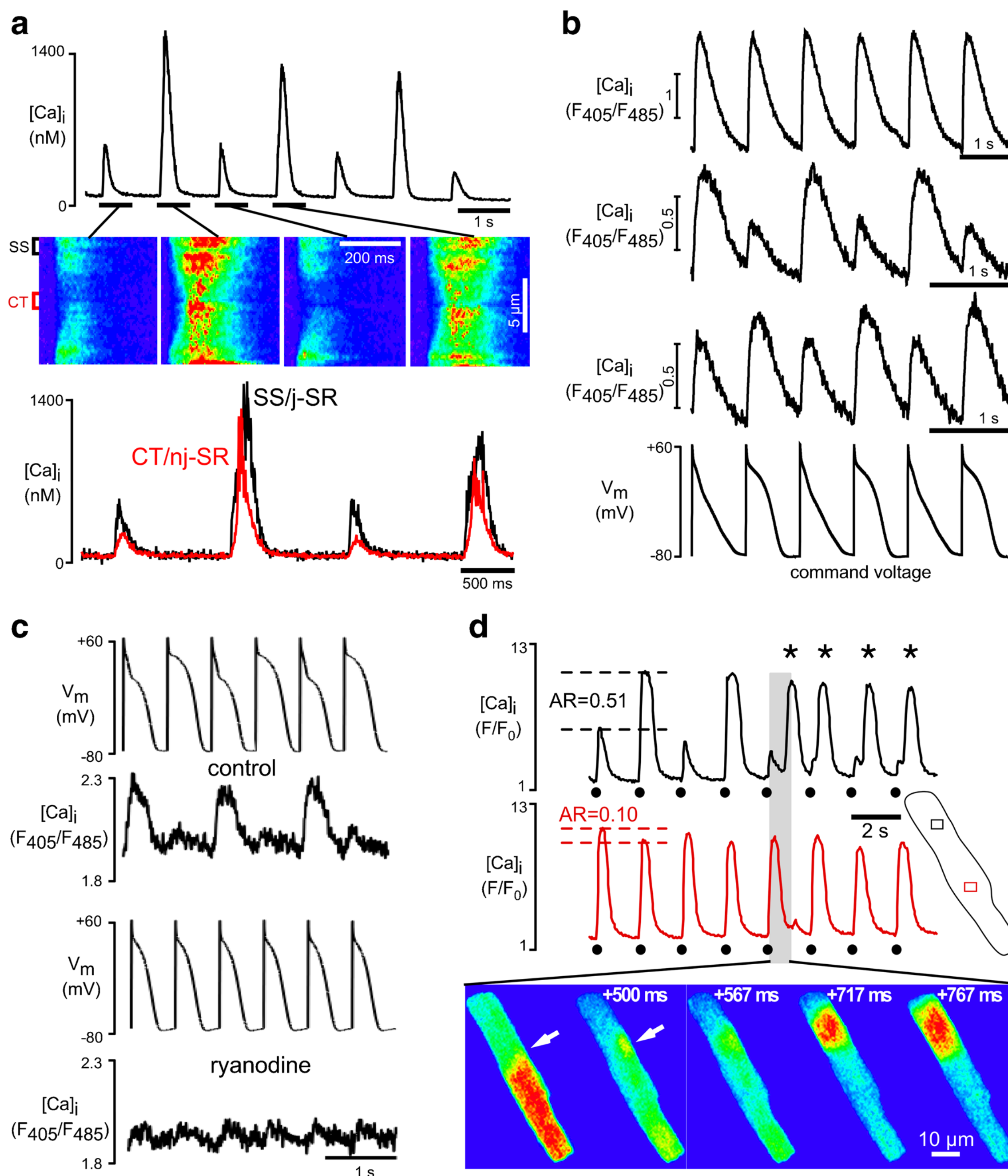

Fig. 3 Electromechanical and Ca^{2+} alternans. **a** Confocal transverse line scan recordings of CaT alternans. Whole cell CaTs (top), selected line scan images (middle), and local (SS, subsarcolemmal, release from j-SR; CT, central, release from nj-SR) subcellular $[Ca^{2+}]_i$ profiles (bottom) during alternans. **b** CaTs recorded from atrial cells under voltage clamp conditions with an AP alternans command voltage protocol. AP alternans voltage clamp elicits no CaT alternans (top), CaT alternans where the large amplitude CaT coincides with the short AP (middle), and CaT alternans where the small amplitude CaT coincides with the short AP (bottom). The data indicate that CaT alternans can develop irrespective of V_m morphology or presence of AP alternans, indicative of a primary disturbance of Ca^{2+} signaling ($[Ca^{2+}]_i \rightarrow V_m$ coupling). **c** Simultaneous recording of $[Ca^{2+}]_i$ and V_m in current clamped atrial myocytes. Pacing induced AP and Ca^{2+} alternans in control (top). Bottom: SR Ca^{2+} release inhibition with ryanodine abolishes AP alternans, indicating that CaT alternans drives electrical alternans. **d** Subcellular out-of-phase Ca^{2+} alternans with longitudinal alternans ratio (AR) gradient. The degree of alternans is quantified by the AR. AR = 1 − CaT_{Small}/CaT_{Large}, where CaT_{Small} and CaT_{Large} are the amplitudes of the small and large CaTs of a pair of alternating CaTs. Top portion of the cell reveals an approximately fivefold higher AR than lower half of the cell. Repetitive propagating Ca^{2+} waves are initiated at the junction of out-of-phase alternating subcellular regions (white arrows). Circles: triggered CaTs. Asterisks: Ca^{2+} waves. Panel **a** reproduced and modified from [42]. Panels **b** and **c** reproduced and modified from [45]. Panel **d** reproduced and modified from [54].

beat-to-beat feedback control of SR content leads to Ca^{2+} alternans [21], we revealed with direct dynamic measurements of $[Ca^{2+}]_{SR}$ that alternans can occur with and without significant end-diastolic $[Ca^{2+}]_{SR}$ fluctuations [20, 81, 96]. Refractoriness or recovery from inactivation of the Ca^{2+} release machinery also plays a key role for alternans. Recovery, when examined at the level of whole-cell CaTs as well as Ca^{2+} sparks [10, 58, 99, 101], occurs on a time scale that overlaps with the stimulation frequencies where Ca^{2+} alternans typically occurs [2, 58]. Ion channels participating in ECC, including the RyR, 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^{2+} release to overcome refractoriness and to become fully available again after a previous release event. In atrial myocytes restitution of Ca^{2+} release from nj-SR is slower than from j-SR, reflecting another important difference in Ca^{2+} handling between j-SR and nj-SR [96], and SR release restitution properties have been demonstrated to play a key role in alternans.

Recently, an overarching conceptual model for cardiac alternans has been forwarded, termed "3R theory" [76, 83, 88]. The 3R theory links Ca^{2+} spark properties (i.e., the properties of Ca^{2+} release from individual CRUs) to whole-cell Ca^{2+} alternans. The 3R theory states Ca^{2+} alternans occurs due to instabilities in the relationship of 3 critical spark attributes: Randomness, Recruitment, and Refractoriness. The theory predicts (based on numerical computations) that alternans occurs when the probability of a spontaneous primary spark is intermediate (intermediate randomness) but coupling between CRUs is strong (high degree of recruitment), and the degree of refractoriness is high. This unifying theoretical framework predicts how ECC Ca^{2+} handling proteins (LCC, RyR, SERCA, NCX, Ca^{2+} buffers) affect the 3 R's and SR Ca^{2+} load, and thus predict Ca^{2+} alternans probability.

Atrial myocytes are particularly susceptible to Ca^{2+} alternans [5, 42, 45, 54, 56]. In contrast to ventricular myocytes, atrial Ca^{2+} alternans is subcellularly inhomogeneous (Fig. 3a) with transverse and longitudinal subcellular gradients in the degree of Ca^{2+} alternans, including subcellular regions alternating out-of-phase (Fig. 3d). This spatio-temporal heterogeneity of Ca^{2+} alternans is unique to atrial myocytes and—together with the unique atrial ECC mechanism—hints that the alternans mechanism is also distinctly different from that in ventricle. Complex subcellular $[Ca^{2+}]_i$ inhomogeneities of atrial alternans generate a substrate for spontaneous (i.e., not electrically triggered) proarrhythmic Ca^{2+} release pointing towards a mechanistic link to atrial arrhythmia at the cellular level [54].

The beat-to-beat alternations in the time course of ventricular AP repolarization are reflected in the ECG as T-wave alternans (TWA). Even subtle TWA in the microvolt range (referred as microvolt TWA) has emerged as a valuable prognostic tool for ventricular arrhythmia risk stratification [72]. The clinical use of atrial AP repolarization alternans as a diagnostic tool, however, is hindered by the fact that in the conventional ECG recordings of the atrial repolarization signal is masked by the ventricular QRS complex. Nevertheless, several experimental [43, 105] and clinical studies [35, 59, 73, 74] using monophasic AP electrodes to monitor atrial repolarization alternans in-vivo have provided convincing evidence that AP alternans in atria may lead directly to atrial fibrillation or its transition from atrial flutter. Taken together, there is strong evidence that AP alternans precedes development of AF and has prognostic value for arrhythmia prediction.

Conclusions

Here we discussed unique structural and functional features of atrial ECC and Ca^{2+} signaling at the cellular level. While there are commonalities between atrial and ventricular ECC, structural differences with respect to arrangement of surface (t-tubules) and internal (SR) membranes, tissue-specific endowment with ion channels, and electrophysiological attributes result in the discussed differential features of atrial and ventricular ECC. We emphasized the FDUF mechanism of tandem activation of SR Ca^{2+} release in atrial cells and the integral role played by the IP_3R and IICR. Furthermore, we reviewed atria-specific manifestations of alternans, its underlying mechanisms, and relationship to atrial arrhythmia, especially AF. AF is the most prevalent manifestation of atrial arrhythmia and a frequent complication of heart failure [24, 29] that carries a poor prognosis. The mechanisms and conditions leading to AF are complex and far from being completely understood [75], and the clinical magnitude of the problem, the high prevalence of the disease [24], and the prospect that the burden will likely only increase as the population ages and AF prevalence rises [15] constitute a grave health problem. Accumulating clinical evidence and a growing number of experimental studies indicate that AF is often related to episodes of atrial electromechanical alternans; however, the alternans-AF link is often just phenomenological, leaving a mechanistic underpinning between alternans and AF elusive. Understanding the cellular mechanisms of electromechanical alternans has the potential to open a window not only towards a better understanding of AF mechanisms, but also novel therapeutic approaches of AF treatment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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