Dysfunctional intracellular calcium cycling in cardiac alternans

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Summary

1. Cardiac alternans refers to a condition in which there is a periodic beat-to-beat oscillation in electrical activity and the strength of cardiac muscle contraction at a constant heart rate. Clinically, cardiac alternans occurs in settings that are typical for cardiac arrhythmias and has been causally linked to these conditions.

2. At the cellular level, alternans is defined as beat-to-beat alternations in contraction amplitude (mechanical alternans), action potential duration (APD; electrical or APD alternans), and Ca^{2+} transient amplitude (Ca^{2+} alternans).

3. The cause of alternans is multifactorial, however alternans always originate from disturbances of the bi-directional coupling between membrane voltage (V_m) and intracellular calcium ($[Ca^{2+}]_i$). Bi-directional coupling refers to the fact that in cardiac cells, V_m depolarization and the generation of action potentials cause the elevation of $[Ca^{2+}]_i$ that is required for contraction (a process referred to as excitation-contraction coupling). The changes of $[Ca^{2+}]_i$ on the other hand control V_m because important membrane currents are Ca^{2+}-dependent.

4. Evidence is mounting that alternans is ultimately caused by disturbances of cellular Ca^{2+} homeostasis. Here we review how two key factors of cardiac cellular Ca^{2+} signaling - the release of Ca^{2+} from internal stores and the capability of clearing the cytosol from Ca^{2+} after each beat - determine the conditions under which alternans occurs. The contributions from key Ca^{2+} handling proteins - surface membrane channels, ion pumps and transporters, and internal Ca^{2+} release channels - are discussed.

Introduction

Cardiac alternans refers to a condition characterized by a periodic beat-to-beat oscillation in electrical activity and the strength of cardiac muscle contraction at a constant heart rate. The clinical manifestations of alternans occur in many settings in which arrhythmias are also common; however, its origin can be followed to the cellular and subcellular level. Here, we will review the alternans field from the perspective of the cellular disturbances of electrical and calcium signaling which lead to the proarrhythmic condition of alternans.

Excitation-contraction coupling in cardiac muscle

Each heartbeat requires a coordinated activation of cardiac muscle cells to sustain the pump function of the heart. Excitation-contraction coupling describes the process that converts electrical activation into mechanical activity and muscle contraction. The sequence of events begins with depolarization of the surface membrane potential (V_m) by an action potential, followed by the entry of extracellular calcium through voltage-gated sarcolemmal L-type Ca^{2+} channels (also referred to as dihydropyridine receptors, DHPRs). Ca^{2+} influx triggers intracellular Ca^{2+} release by activating Ca^{2+}-sensitive Ca^{2+} release channels (ryanodine receptors, RyRs) in the sarcoplasmic reticulum (SR) membrane by a mechanism termed Ca^{2+}-induced Ca^{2+} release (CICR).[1] The amplified Ca^{2+} release from the SR raises intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) which activates the contractile apparatus and force is produced. Relaxation of cardiac cells is dependent upon mechanisms that lower $[Ca^{2+}]_i$ through reuptake into the SR by the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) and extrusion from the cell primarily *via* sarcolemmal sodium-calcium exchange (NCX). Reuptake of Ca^{2+} provides the necessary filling of the SR to allow sufficient Ca^{2+} for release during the next heartbeat.

Ventricular myocytes typically have a well-developed transverse (t) tubular system. The t-tubular system consists of invagination of the surface membrane that extends as a 3-dimensional network of narrow transverse and longitudinal tubules throughout the entire cell.[2] Approximately 30-50% of the sarcolemma exists as the t-tubular system and forms a well-connected membrane network within the cell, but contiguous with the extracellular space. DHPRs together with many other ion channels and transporters are located in the t-tubular membrane. Clusters of RyRs on the terminal cisternae of the SR membrane appose DHPRs separated only by a narrow (a few nanometers) cleft, forming a dyad of two adjacent membranes.[3] The dyad is the functional unit of SR Ca^{2+} release, termed SR Ca^{2+} release unit (CRU)[4] or couplon.[5] Ca^{2+} sparks are considered the elementary events of Ca^{2+} signaling in cardiac cells[6] arising from CICR at individual CRUs, and according to the 'local control' model of cardiac excitation-contraction coupling[7] are recruited independently and spatially summate to produce a Ca^{2+} transient.[8,9] The well developed t-tubular network in ventricular myocytes ensures simultaneous activation of SR Ca^{2+} release throughout the entire ventricular myocyte during an action potential, resulting in spatially separate rather homogeneous Ca^{2+} transients (Figure 1A).

The fundamental process of excitation-contraction coupling in atrial and ventricular cells shows similarities, but also important structural and functional differences. The t-tubular system in atrial cells is significantly less developed or even entirely absent,[10,11] although there are species differences. For example, rudimentary t-tubular structures are found in rat,[12] sheep[13] and dog.[14] The spatial vicinity to the surface membrane defines two types of SR, termed

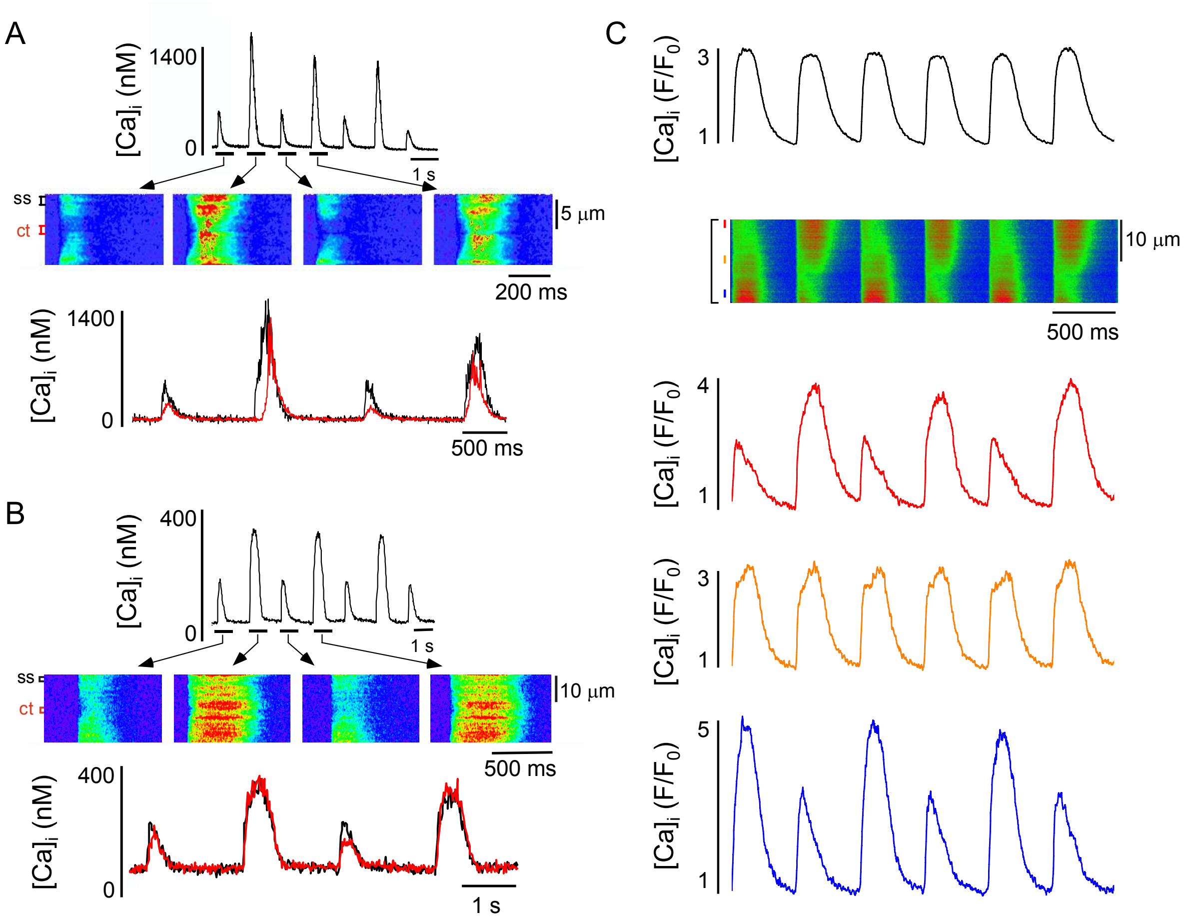

Figure 1. Cellular and subcellular Ca^{2+} alternans in cardiac myocytes. A,B: *Spatiotemporal characteristics of Ca^{2+} transients during alternans in a atrial (**A**) and ventricular (**B**) myocyte. From top: whole cell Ca^{2+} transients, transverse confocal line scan images and subcellular $[Ca^{2+}]_i$ profiles recorded from subsarcolemmal (ss, black; corresponding to j-SR Ca^{2+} release) and central (ct, red; corresponding to nj-SR Ca^{2+} release) regions of the myocyte. Panels A and B modified from Hüser* et al.*[10] with permission. **C:** Spatiotemporal characteristics of Ca^{2+} transients during alternans in an atrial myocyte where subcellular discordant or 'out-of-phase' alternans are present. The global $[Ca^{2+}]_i$ profile suggests no Ca^{2+} alternans, however spatially restricted profiles identify subcellular regions with no alternans coexisting with regions alternating out-of-phase.*

junctional (j-SR) and non-junctional (nj-SR) SR. Because of the absence of t-tubules in atrial myocytes j-SR is restricted to the cell periphery. Both j-SR and nj-SR express RyRs, and - compared to ventricular myocytes - have a higher density of IP_3 receptors.[15,16] In atrial cells, peripheral j-SR and the more centrally located nj-SR are capable of active and robust SR Ca^{2+} release, however the mechanism of activation differs. Action potential-induced membrane depolarization activates Ca^{2+} entry through L-type Ca^{2+} channels which triggers CICR from RyRs of the j-SR. Elevation of peripheral $[Ca^{2+}]_i$ propagates then *via* CICR in a Ca^{2+} wave-like fashion in centripetal direction by a diffusion-reaction process or a 'fire-diffuse-fire' mechanism (Figure 1B). As a characteristic consequence of this mode of activation and ultrastructural arrangements, Ca^{2+} release is spatially inhomogeneous[16-18] with complex subcellular $[Ca^{2+}]_i$ gradients (Figures 1B and 1C). These structural and functional differences are important for the susceptibility to spontaneous pro-arrhythmic Ca^{2+} release events (Ca^{2+} waves) and the propensity to develop cardiac alternans as will be discussed below.

Cardiac alternans

In 1872, for the first time a very interesting phenomenon, consisting of beat-to-beat oscillations in arterial pressure that occurred while the heart rate remained constant, was reported by Traube.[19] This observation, called '*pulsus alternans*' would ultimately be known as mechanical alternans. With the arrival of the electrocardiogram (ECG) similar beat-to-beat alternations of electrical activity of the heart (electrical alternans) were recorded in laboratory animals[20] and humans,[21] and are typically referred to as repolarization or T-wave alternans. It was recognized early on that conditions of *pulsus alternans* were associated with severe cardiac pathologies and poor prognosis.[22] To date, it is well established that cardiac

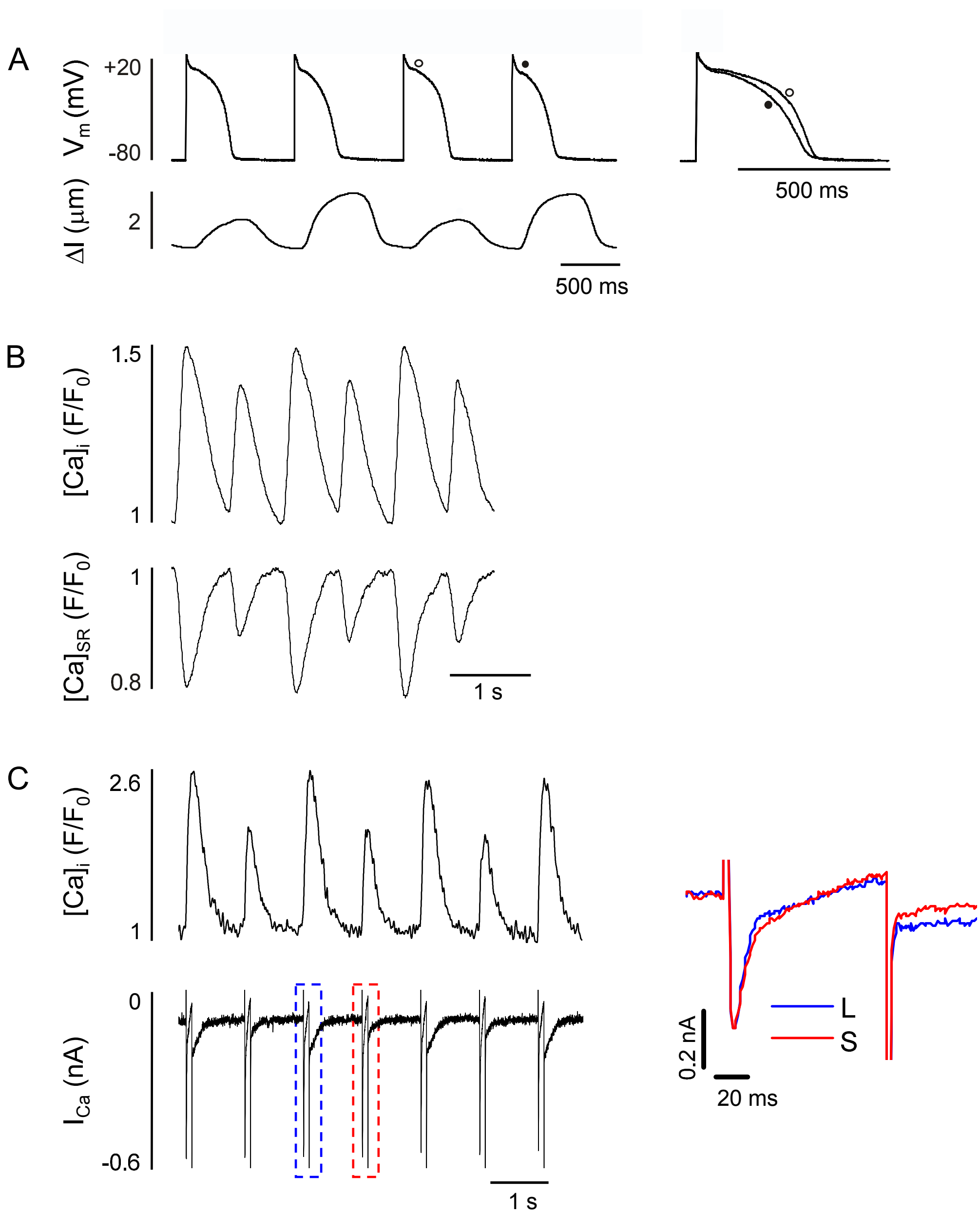

Figure 2. Electrical, mechanical and Ca^{2+} alternans in cardiac myocytes. A: *Simultaneous recordings of action potentials and cell shortening from a single ventricular myocyte revealing discordant electromechanical alternans. To the right, two action potentials recorded during successive small- (open circle) and large-amplitude (filled circle) shortenings are superimposed to illustrate the differences in duration and kinetics. Modified from Hüser* et al.[10] *with permission.* ***B:*** *Simultaneous recordings of cytosolic ($[Ca^{2+}]_i$; top) and intra-SR ($[Ca^{2+}]_{SR}$; bottom) Ca^{2+} alternans from a single ventricular myocyte.* ***C:*** *Simultaneous recordings of $[Ca^{2+}]_i$ (top) and I_{Ca} (bottom) in voltage-clamped atrial myocytes. To the right, an overlay of I_{Ca} measured during a large-amplitude Ca^{2+} transient (L; blue trace) and a small-amplitude Ca^{2+} transient (S; red trace) shows that Ca^{2+} alternans are not accompanied by alternating peak I_{Ca}. Modified from Shrkyl* et al.[6] *with permission.*

alternans is linked to increased risk for atrial and ventricular arrhythmias and sudden cardiac death across a wide range of pathophysiological conditions, including ischemia and myocardial infarction.[16,23-29] T-wave alternans in the ECG and microvolt electrical alternans testing have become a prognostic tool for arrhythmia risk stratification and antiarrhythmic therapy.[30-32]

At the cellular level, cardiac alternans is defined by cyclic, beat-to-beat alternations in contraction amplitude (mechanical alternans), action potential duration (APD; electrical or APD alternans), and Ca^{2+} transient amplitude (Ca^{2+} alternans) at constant stimulation frequency (Figure 2). Alternans is induced typically by rapid heart rates, however, the pacing threshold required to initiate it is influenced by a wide variety of factors and conditions[33-36] and varies among different mammalian species.[37,38]

Conditions that lower the pacing threshold include hypothermia,[38-42] interference with cellular energy metabolism through inhibition of glycolysis,[10,43-45] hypocalcaemia,[38,41,46,47] disturbance of mitochondrial functions,[44,48,49] hypercapnic acidosis,[50,51] ischemia,[52-56] hypertrophy,[57] IP_3 receptor-dependent Ca^{2+} release,[58,59] and heart failure.[60-62] A shift to a higher pacing threshold for alternans has been reported in conditions of hypercalcaemia,[38,46] pharmacological sensitization of the SR Ca^{2+} release channels,[63] and calcium channel antagonists.[42,54] Interestingly, β-adrenergic stimulation, while generally having positive inotropic effects, can either enhance[64] or suppress[10,44,48] alternans (*cf.* discussion below).

Mechanism of cardiac alternans: bi-directional coupling between V_m and $[Ca^{2+}]_i$

The plethora of studies on cardiac alternans clearly document that this proarrhythmic condition is multifactorial. Nonetheless, it is generally agreed that instabilities of the bi-directional coupling of V_m and $[Ca^{2+}]_i$ are a cucial factor for the generation of alternans. 'Bi-directional' coupling refers to the fact that membrane depolarization in form of an action potential is required to initiate Ca^{2+} release and to elevate $[Ca^{2+}]_i$, however the ensuing dynamics of $[Ca^{2+}]_i$ affect V_m through the Ca^{2+}-dependence of numerous membrane conductances as outlined below.[65] Consequently, the question arises as to whether alternans are either V_m or $[Ca^{2+}]_i$ driven.[35,36,66,67] As such, a classic 'chicken or egg conundrum' exists in the literature relating to the fact that the mechanisms responsible for alternans remain incompletely understood.[68-70]

$V_m \rightarrow [Ca^{2+}]_i$ coupling

V_m-driven alternans is determined by a single parameter - APD restitution. The key concept behind the paradigm of V_m-driven alternans is that APD restitution is a time-dependent process resulting from the fact that recovery from inactivation of ion currents underlying the action potential requires time (thus resulting in absolute and relative refractoriness of excitability). APD restitution is defined as the relationship between APD and diastolic interval (DI). The heart rate is inversely related to cycle length (CL), which is calculated as CL = APD + DI. When heart rate increases, the APD shortens to preserve the diastolic interval for ventricular filling. Therefore, electrical alternans is critically dependent on beat-to-beat changes in diastolic interval. $V_m \rightarrow [Ca^{2+}]_i$ coupling is generally believed to be positive, *i.e.*, a long APD is paralleled by a strong contraction and large amplitude Ca^{2+} transient. Positive coupling between APD and Ca^{2+} transient or contraction amplitude is also referred to as 'in-phase' or 'concordant' at the cellular level. 'Negative' $V_m \leftrightarrow [Ca^{2+}]_i$ coupling results in 'discordant' or 'out-of-phase' alternans at the single cell level (Figure 2A). The term 'discordant' is also used at the multicellular tissue level where it refers to different regions of the myocardium alternating asynchronously or 'out-of-phase'. Such regions are separated by nodal lines[71] which mark areas of highest $[Ca^{2+}]_i$ and APD gradients and become sites of origin for arrhythmias. The terminology discordant/concordant is also used at the subcellular level and describes alternans pattern of subcellular regions within a single cell (Figure 1C).[43,72,73] Alternations of the diastolic interval is critical for the availability of the L-type Ca^{2+} channel current ($I_{Ca,L}$) at a given heartbeat. A longer diastolic interval allows more time for recovery of $I_{Ca,L}$, leading to enhanced $I_{Ca,L}$, larger Ca^{2+} release and longer APD during the following beat. Now the longer APD is followed by a shorter diastolic interval, leading to less recovery of $I_{Ca,L}$ with less Ca^{2+} release and shorter APD during the next beat, thus sustaining alternans.

$[Ca^{2+}]_i \rightarrow V_m$ coupling

$[Ca^{2+}]_i \rightarrow V_m$ coupling is determined by the fact that $[Ca^{2+}]_i$ feeds back on V_m. This occurs through the Ca^{2+}-dependence of ion channels and transporters, *i.e.* membrane conductances that in turn also control $[Ca^{2+}]_i$ cycling. With respect to cardiac alternans, $I_{Ca,L}$ and I_{NCX} are most important.[35,36] $[Ca^{2+}]_i \rightarrow V_m$ coupling can be positive or negative depending on which of the Ca^{2+}-dependent ion currents or transporters dominates. For example, a positive $[Ca^{2+}]_i \rightarrow V_m$ coupling occurs when the large Ca^{2+} transient causes a prolongation of APD by potentiating the inward I_{NCX} (1 Ca^{2+} ion extruded in exchange to 3 Na^+ ions) to a greater extent than reducing $I_{Ca,L}$ through Ca^{2+}-dependent inactivation.

Negative $[Ca^{2+}]_i \rightarrow V_m$ coupling occurs when reduction of $I_{Ca,L}$ dominates over increased I_{NCX} which ultimately results in APD shortening. Other Ca^{2+}-sensitive currents (non-selective cation current, Cl^- current) may modulate $[Ca^{2+}]_i \rightarrow V_m$ coupling, but appear to be quantitatively less important.

Two key parameters relevant to the generation of $[Ca^{2+}]_i$-driven alternans at the cellular level are i) fractional Ca^{2+} release from the SR and SR Ca^{2+} load, and ii) the efficiency of beat-to-beat cytosolic Ca^{2+} sequestration.[35,36] Fractional release of Ca^{2+} refers to the nonlinear relationship between the end-diastolic SR Ca^{2+} content and the amount of Ca^{2+} (or % of SR Ca^{2+} content) released by CICR with each heartbeat (*i.e.*, a larger fraction of Ca^{2+} is released at a higher SR Ca^{2+} content).[74] Ca^{2+} sequestration is a phenomenological parameter and refers to the net efficiency of cytosolic Ca^{2+} removal. It is dependent on i) the activity of SERCA to reload the SR, ii) Na^+/Ca^{2+} exchange and plasmalemmal Ca^{2+}-ATPase activity to extrude Ca^{2+} from the cell, iii) cytosolic buffering (including mitochondrial Ca^{2+} uptake), and iv) diastolic SR Ca^{2+} leak. Therefore, alternans can occur at modest SR loads and small fractional releases under conditions where Ca^{2+} sequestration is low. Alternatively at high sequestration rates, higher Ca^{2+} loads and fractional release are required to induce alternans. In general, factors increasing fractional release promote, and factors increasing Ca^{2+} sequestration efficiency protect against alternans. To illustrate, in heart failure where SERCA expression is reduced and Ca^{2+} release from the SR is increased, or during acute cardiac ischemia (where SR Ca^{2+} load is initially unaffected, but SERCA activity is diminished due to reduced ATP levels), the heart is pushed into instability due to diminished Ca^{2+} sequestration. On the other hand, under β-adrenergic stimulation SERCA activity and consequently SR Ca^{2+} uptake and load are increased, leading to enhanced fractional release that tends to promote alternans. Increased SERCA activity, however, also increases the efficiency of Ca^{2+} sequestration, resulting in protection against alternans. Whether β-adrenergic stimulation favors[64] or protects[10,44,48] against alternans and alternans-related arrhythmias depends on which β-adrenergic effects predominate.

Recently, an overarching conceptual model for cardiac alternans has been forwarded, termed '3R theory'.[34,75,76] 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. Ca^{2+} alternans occurs due to instabilities of the relationship of 3 critical spark properties (the '3 Rs'): 1) **R**andomness of Ca^{2+} sparks, 2) **R**ecruitment of sparks by neighboring CRUs, and 3) **R**efractoriness of a CRU. An individual CRU can be in 3 different states: recovered (*i.e.* ready to fire), firing or refractory. The theory predicts (by numerical computations) that alternans occurs when the probability of a spontaneous primary spark is intermediate (intermediate randomness), coupling among CRUs is strong (high probability of a primary spark triggering a secondary spark from a neighboring CRU; high degree of recruitment), and a high degree of refractoriness is prevalent (*i.e.* the probability of a CRU *not* being recovered from previous release is high). This unifying theoretical framework predicts how Ca^{2+} cycling proteins and organelles (L-type Ca^{2+} channels, RyR, SERCA, NCX, Ca^{2+} buffers and mitochondria) affect the 3 Rs and SR Ca^{2+} load, and thus the prevalence of Ca^{2+} alternans. Interestingly, in the 3R framework SR Ca^{2+} load is not an explicit parameter which is consistent with our observation that Ca^{2+} alternans are not dependent on alternating end-diastolic $[Ca^{2+}]_{SR}$.[10,63,77] Nonetheless, SR Ca^{2+} load is a critical factor for Ca^{2+} alternans since load determines the efficiency of the L-type Ca^{2+} current to trigger release; it controls RyR function through its luminal Ca^{2+} sensitivity and influences refractoriness of release. In the next section we will summarize the specific contributions of the major Ca^{2+} signaling proteins and organelles to alternans.

As mentioned earlier, alternans is a recognized risk factor for ventricular and atrial arrhythmias.[70,78,79] $[Ca^{2+}]_i \rightarrow V_m$ coupling can be positive or negative, *i.e.*, result in both concordant and discordant alternans. At the level of the heart, spatially discordant alternans favor re-entry, triggering ectopic beats and facilitating the onset of lethal arrhythmic events[80,81] whereas concordant alternans is considered less arrhythmogenic.[82] At the cellular level atrial myocytes are particularly susceptible to Ca^{2+} alternans induced by pacing or metabolic inhibition. In atrial myocytes alternans is typically subcellularly inhomogeneous (Figures 1B and 1C). Subcellular inhomogeneities consist of subcellular transverse and longitudinal gradients of the degree of Ca^{2+} alternans, and subcellular regions alternating out-of-phase.[10,16,43,45,59] These $[Ca^{2+}]_i$ gradients and inhomogeneities result from the unique structural and functional features of atrial excitation-contraction coupling and are consistent with simulation studies on the relationship between the lack of t-tubules and generation of alternans.[83] We demonstrated that the complex subcellular $[Ca^{2+}]_i$ inhomogeneities of atrial alternans generates a substrate for spontaneous (*i.e.*, not electrically triggered) proarrhythmic Ca^{2+} release and represents a mechanistic link to atrial arrhythmia at the cellular level.[43] Of particular interest is the observation of subcellular 'discordant' Ca^{2+} alternans where subcellular regions alternate out-of-phase (Figure 1C). These subcellular areas are typically separated by regions where spontaneous Ca^{2+} waves originate with high probability, reminiscent of the nodal lines observed at tissue level.[71]

Thus, it appears that spatially discordant alternans phenomena at tissue level can be recapitulated at the cellular level.

Ca^{2+} handling proteins and organelles and their role in cardiac alternans

Although clearly a multifactorial phenomenon, consensus is emerging that electromechanical and Ca^{2+} alternans are ultimately linked to impaired $[Ca^{2+}]_i$ regulation, and $[Ca^{2+}]_i \rightarrow V_m$ coupling dominates the mechanisms that are responsible for the occurrence of alternans.[68,84-86] In the following paragraphs we will address the contributions of L-type Ca^{2+} channels, SR and Ca^{2+} load, the SR Ca^{2+} release machinery (RyRs) and mitochondria to alternans.

L-type Ca^{2+} channels

Considering that $I_{Ca,L}$ is the critical trigger for CICR during excitation-contraction coupling and SR Ca^{2+} release is graded with the magnitude of the current,[87,88] beat-to-beat alternation of $I_{Ca,L}$ has been considered a candidate to cause alternans. A potential mechanism entails incomplete time-dependent recovery from inactivation of $I_{Ca,L}$ which could lead to Ca^{2+} alternans.[89-91] This hypothesis, however would have to reconcile the observation in both atrial (Figure 2C) and ventricular myocytes that alternans can occur while peak $I_{Ca,L}$ remains unchanged from one beat to the next.[10,63,77,92-94] Furthermore, mechanical and Ca^{2+} alternans can occur in the absence of APD alternans (confirmed in patch-clamp experiments) and with constant $I_{Ca,L}$.[10,63,92,95,96] Ca^{2+} alternans is observed even in myocytes stimulated at a high frequency during action potential voltage clamp in the absence of APD alternans.[95] Together these data suggest that $I_{Ca,L}$ is unlikely paramount in the onset of alternans.

SERCA and SR Ca^{2+} load

It can be speculated that at higher pacing frequencies, limitations of SR Ca^{2+} uptake kinetics preclude adequate refilling of Ca^{2+} stores, particularly after a large Ca^{2+} transient. Consequently, the reduced filling only permits a small Ca^{2+} transient during the next beat thus resulting in Ca^{2+} alternans. This led to the suggestion that beat-to-beat alternations in end-diastolic SR Ca^{2+} load is a prerequisite for alternans,[93] possibly due to an instability in the feedback control of SR Ca^{2+} content.[97] However, direct and dynamic measurements of intra-SR [Ca^{2+}] have shown (Figure 2B) that alternans do not require beat-to-beat alternations in SR Ca^{2+} content.[10,63,77] The role of Ca^{2+} reuptake into the SR and reestablishing Ca^{2+} load has been further investigated by enhancing SERCA activity.[98-100] Indeed, using genetic approaches to up-regulate SERCA2a (cardiac isoform) resulted in suppression of alternans.[83,100-102]

RyR and restitution of SR Ca^{2+} release

The magnitude of a Ca^{2+} transient is determined by the recovery of the trigger of CICR ($I_{Ca,L}$ restitution), SR Ca^{2+} load and the release mechanism itself (RyRs and associated regulatory proteins) from the preceding heartbeat. If recovery of any of these parameters is incomplete, the subsequent Ca^{2+} transient is expected to be reduced, thus facilitating the onset of alternans. Ca^{2+} release is unavailable immediately after release due to RyR inactivation. Recovery of elementary Ca^{2+} sparks and whole-cell Ca^{2+} transients after a preceding release requires several hundred milliseconds to reach full availability.[103-108] Incomplete RyR recovery from inactivation may contribute to instabilities of Ca^{2+} release and vulnerability to alternans and arrhythmias, particularly when pacing frequencies overlap with the time scale of RyR and Ca^{2+} release restitution.[109] Thus, refractoriness of release and its time-dependent recovery can become the critical factor for the occurrence of Ca^{2+} alternans, as has been shown experimentally[110] and in computational studies.[111] In a comprehensive investigation we recently demonstrated refractoriness of SR Ca^{2+} release as the key causative factor for alternans in atrial tissue. Restitution properties and refractoriness of Ca^{2+} release during alternans were evaluated by four different approaches: 1) latency of spontaneous global Ca^{2+} releases (Ca^{2+} waves) and 2) Ca^{2+} spark frequency during rest after a large and a small alternans Ca^{2+} transient, 3) premature action potential-induced Ca^{2+} transients after a large and a small beat, and 4) the efficacy of a photolytically induced Ca^{2+} signal to trigger additional Ca^{2+} release during alternans. The results showed that restitution of SR Ca^{2+} release was significantly delayed after the large Ca^{2+} transient, leading to the conclusion that beat-to-beat alternation of the time-dependent restitution properties and refractory kinetics of SR Ca^{2+} release represents a key mechanism underlying alternans.[63]

Mitochondria

Mitochondria contribute to cardiac Ca^{2+} cycling and excitation-contraction coupling at different levels: as a major source of ATP (energetics) that provides the fuel for the contractile apparatus, sustains ion pumps and alters the activity of Ca^{2+} handling proteins, for example through phosphorylation or acting as a direct modulator (*e.g.*, modulation of RyR activity by MgATP). Mitochondria shape cytosolic Ca^{2+} signals directly through Ca^{2+} sequestration. Furthermore, mitochondria can be a major source of reactive oxygen species (ROS), thus determine the cellular redox environment which profoundly affects cardiac excitability and the activity of Ca^{2+} handling proteins, including the RyR and SERCA (for review, see Zima & Blatter, 2006[112]). The pivotal role of mitochondria for Ca^{2+} signaling and excitation-contraction coupling is further underscored by the fact that these organelles occupy approximately 35% of the cell volume. Despite the undisputed importance of mitochondria for cardiac Ca^{2+} signaling and excitation-contraction coupling, it is rather surprising that mitochondria have been rarely the topic of studies on alternans mechanism.[113] In two recent studies we demonstrated that impairment of mitochondrial functions enhanced alternans.[44,48] In these studies the application of pharmacological blockers targeted to the various mitochondrial functions all enhanced the degree of Ca^{2+} alternans induced by pacing. This could be achieved by dissipation of mitochondrial membrane potential, as well as by inhibition of mitochondrial F_i/F_o-ATP synthase, inhibition of electron transport chain and Ca-dependent dehydrogenases, and by blockage of mitochondrial Ca^{2+} uptake or extrusion. These results are in agreement with other studies that confirmed that mitochondrial uncoupling facilitates alternans,[49] and demonstrated that an altered redox environment can generate conditions that favor alternans.[94] Thus, with all likelihood mitochondria will emerge as a critical factor for the development of alternans.

Concluding remarks

Cardiac alternans is an intriguing phenomenon with clinical implications to a range of cardiac pathologies, while also providing insights into the intricacies of cellular Ca^{2+} cycling in heart muscle. Although clearly a multifactorial process, the experimental, theoretical and computational data exploring electrical, Ca^{2+} and mechanical alternans indicate that dysfunctional Ca^{2+} cycling appears to be the crucial mechanistic link between the contractile dysfunction and electrical instabilities seen at the cellular level, as well as clinically in patients. Despite the complexity of cardiac Ca^{2+} signaling, recent years have seen remarkable progress towards the understanding of the phenomenon of cardiac alternans. Growing theoretical and experimental evidence emphasizes that cellular Ca^{2+} signaling - and particularly the key proteins responsible for beat-to-beat Ca^{2+} release - are at the 'heart' of the problem of cardiac alternans. The recognition of the central role of the cardiac Ca^{2+} release machinery for alternans will pave the way, by pharmacologically or genetically targeting these Ca^{2+} handling proteins, to develop novel therapeutic strategies for the suppression of cardiac arrhythmias.

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