Published in final edited form as:
Circulation. 2012 January 24; 125(3): 539–549. doi:10.1161/CIRCULATIONAHA.111.033563.

The Role of Substrate and Triggers in the Genesis of Cardiac Alternans, from the Myocyte to the Whole Heart: Implications for Therapy

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Keywords

sarcoplasmic reticulum; ryanodine receptor; myocyte; cellular alternans; mechanisms of alternans

Introduction

Electrocardiographic alternans, a phenomenon of beat-to-beat oscillation in electrocardiographic waveforms, was first described by Hering in 1908 [1]. Much of the interest in the alternans phenomenon has focused on alternans during the repolarization phase of the cardiac action potential (AP), also known as repolarization alternans (RA). More specifically, RA has been associated with an increased risk for malignant ventricular arrhythmias and sudden cardiac death (SCD) across a wide range of pathophysiological conditions including both ischemic and non-ischemic congestive heart failure with impaired left ventricle ejection fraction (LVEF) and recent myocardial infarction (MI) [2, 3]. Cardiac alternans can also be produced in structurally normal hearts under conditions of chronotropic stimulation [4, 5] or significant metabolic stress [6].

Given that several comprehensive review papers [7-9, 10] have been published on the mechanisms of RA and the clinical risk stratification aspects of microvolt T-wave alternans (MTWA) testing, in this manuscript, we have attempted to present a novel framework for how an “appropriate” substrate and an “appropriate” trigger event may synergistically contribute to the mechanisms generating cardiac alternans from the cellular to the whole heart level, and propose novel aspects of the use of RA to guide therapy.

Mechanisms of Alternans in Isolated Myocytes

Two major hypotheses have been developed to explain the alternans phenomenon at the cellular level. The first hypothesis suggests that alternation in sarcolemmal currents, membrane voltage and AP morphology leads to beat-to-beat fluctuations in intracellular calcium concentration. In support of this hypothesis, it has recently been shown that the modulation of sarcolemmal Ca^{2+} [11] and K^{+} [12, 13] currents based on changes in AP morphology [14] has a significant effect on the stability of Ca^{2+} handling processes and the

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Conflict of Interest Disclosures None

transition to stable alternans [15,16] (Figure 1A). In contrast, the second major hypothesis suggests that alternation of intracellular calcium concentration ($[Ca^{2+}]_i$) is the primary event which then secondarily leads to alternans of membrane voltage and AP morphology [6, 14, 18-23]. According to the second hypothesis, $[Ca^{2+}]_i$ alternans can result from stress-induced [5, 18] perturbations in any number of Ca^{2+} transport processes including Ca^{2+} entry into the cytoplasm [13], recovery of ryanodine receptors (RyRs) from inactivation, triggering of sarcoplasmic reticulum (SR) Ca^{2+} release [6, 19], SR Ca^{2+} uptake [24], intra-SR Ca^{2+} redistribution [25, 26] and linking of intracellular Ca^{2+} handling to surface membrane voltage [14] alternans [17] (Figure 1B). The mechanisms that give rise to cardiac alternans may reside anywhere along this multi-step process of intracellular calcium cycling. A preponderance of recent data has emerged in support of the second hypothesis, suggesting the primacy of perturbations in Ca^{2+} handling processes as the fundamental event in the genesis of cellular alternans.

Among the many steps involved in calcium cycling, alternation of calcium entry into the cell via incomplete recovery from inactivation of the L-type calcium channel ($I_{Ca,L}$) could theoretically lead to $[Ca^{2+}]_i$ alternans [13, 27]. However, a number of studies have demonstrated that peak $I_{Ca,L}$ is unchanged during alternans [6, 19, 28, 29], and equally importantly, $I_{Ca,L}$ has been shown to be unaltered in myocytes from diseased hearts [30], making this a less likely mechanism for the lower alternans threshold observed in the failing heart. Furthermore, alternans of $[Ca^{2+}]_i$ can be elicited in a high-frequency stimulated myocyte during AP-clamp with similar AP morphology[20], also suggesting that the Ca^{2+} influx trigger of calcium-induced-calcium-release (CICR) is not the primary event in inducing alternans. The use of small depolarizing pulses [28] to induce alternans may account for alternans encountered at very high stimulation frequencies when most of the L-type Ca^{2+} channels are unavailable, and thus provide a plausible explanation for the presence of alternans in the normal heart at unusually high stimulation frequencies [4, 5].

Beat-to-beat fluctuations in sarcoplasmic reticulum Ca^{2+} content have also been implicated as a potential mechanism for alternans. Sarcoplasmic reticulum Ca^{2+} measurements made during alternans using the indirect approach of measurement of the caffeine-evoked Na^+/Ca^{2+} exchanger (NCX) current have suggested that SR Ca^{2+} alternates [17, 28, 31]. However, others have shown that while $[Ca^{2+}]_{SR}$ exerts a major influence on SR Ca^{2+} release, beat-to-beat alternation in $[Ca^{2+}]_{SR}$ is not required for $[Ca^{2+}]_i$ alternans to occur [6, 29].

The rate of recovery of the RyR from a refractory (adapted or inactivated) state is another step in the calcium cycling machinery that may give rise to chronotropically induced alternans. With increased steepness of the released Ca^{2+}-SR Ca^{2+} content relationship, as may occur in diseased hearts [30], small changes in $[Ca^{2+}]_{SR}$ should result in large changes in the beat-to-beat $[Ca^{2+}]_i$, even for a constant $I_{Ca,L}$ trigger [32, 33]. As such, a large $[Ca^{2+}]_i$ would be produced when the $[Ca^{2+}]_{SR}$ is relatively high and a disproportionately small $[Ca^{2+}]_i$ when the $[Ca^{2+}]_{SR}$ content is relatively low. A large $[Ca^{2+}]_i$ would then cause enhanced Ca^{2+} mediated L-type current inactivation, thus suppressing Ca^{2+} entry, as well as enhanced Ca^{2+} extrusion from the myocyte via the NCX [16, 17], all of which results in a lower SR Ca^{2+} content and hence lower $[Ca^{2+}]_i$ on the next beat. The lower $[Ca^{2+}]_i$ then results in decreased Ca^{2+} mediated L-type current inactivation and reduced Ca^{2+} extrusion through the NCX, leading to increased SR Ca^{2+} content and a return to the higher $[Ca^{2+}]_i$ on the following beat (Figure 2). This sequence sets the stage for *concordant cellular* alternans between $[Ca^{2+}]_i$ and membrane voltage/APD such that both oscillate *in-phase* (i.e. large $[Ca^{2+}]_i$ corresponds to a long APD and vice versa).

While the use of small depolarizing pulses to induce alternans [28] may differ significantly from the often encountered chronotropic induction of alternans, the biphasic rise in

$[Ca^{2+}]_i$ [16] has been attributed to an initial steep rise in activation of the RyRs, while the second slower phase has been attributed to wave like propagation. We [16, 17] and others [34] have ascribed this secondary slower phase to secondary RyR openings. In computer simulations, we have shown that in isolated myocytes, elevated SR Ca^{2+} content results in both aberrant SR Ca^{2+} release and $[Ca^{2+}]_i$ alternans, and also gives rise to an inward depolarizing current that results in spontaneous early after-depolarizations (sEADs) and APD prolongation which correlates directly with the magnitude and timing of the aberrant Ca^{2+} release. We have also shown the presence of *discordant cellular* alternans between $[Ca^{2+}]_i$ and APD at the myocyte level and the importance of $[Ca^{2+}]_i$ in defining the in- or out-of-phase relationship between experimentally obtained $[Ca^{2+}]_i$ and AP (Figure 1B).

In aggregate, these findings support the primacy of alternation in $[Ca^{2+}]_i$ in driving APD alternans and also in determining the presence of concordance or discordance between $[Ca^{2+}]_i$ and AP morphology within the individual myocyte. Furthermore, experimental evidence suggests that the same Ca^{2+} cycling perturbations which give rise to cellular alternans also play a fundamental role in the pathogenesis of trigger events (i.e. transient β-stimulation bursts), which in concert create the necessary conditions for the establishment of cellular alternans.

Many studies have suggested that RyRs are more likely to be triggered by cytosolic Ca^{2+} when SR lumenal Ca^{2+} is elevated [35-37] and that increasing SR Ca^{2+} content increases spontaneous SR Ca^{2+} release [38] and delayed after-depolarization (DAD) amplitude towards the threshold to trigger an AP [39-42]. Furthermore, triggered activity arising from DADs in response to high stimulation rates [43] or to catecholamines has been demonstrated in normal ventricular myocytes [44], experimental heart failure preparations [45] and cardiomyopathic human hearts [46]. These studies provide a plausible justification for the hypothesis that SR Ca^{2+} "stabilization" at a sub-maximal value is the primary reason for abolishing alternans in studies in which thapsigargin and ryanodine treatment of myocytes markedly suppressed $[Ca^{2+}]_i$ and prevented APD alternans [18], and ryanodine treatment alone abolished both tension and AP alternans in papillary muscles [25, 26].

In that context, in the normal heart, CICR is manifest by an operational baseline $[Ca^{2+}]_{SR}$ that is lower than the threshold to trigger spontaneous Ca^{2+} release. However, high stimulation frequency or β-adrenergic stimulation may trigger SR Ca^{2+} overload that raises the SR Ca^{2+} baseline level close to or above the threshold at which spontaneous sub-threshold Ca^{2+} release may occur. In the diseased heart, although the baseline SR Ca^{2+} level is decreased, the $[Ca^{2+}]_{SR\text{-threshold}}$ for RyR opening is also decreased. Although β-adrenergic responsiveness is impaired in the diseased heart [47], even a moderate residual or transient β-adrenergic responsiveness [45] may trigger spontaneous sub-threshold Ca^{2+} release at a lower $[Ca^{2+}]_{SR}$. The lower than normal $[Ca^{2+}]_{SR\text{-threshold}}$ for RyR opening in diseased hearts may explain the presence of electrocardiographic alternans at lower heart rates than in normal hearts.

Further justification for the role of SR Ca^{2+} content in the genesis of alternans comes from the recent study by Xie and Weiss [34] demonstrating that under control conditions, myocytes become susceptible to Ca^{2+} overload during rapid pacing and that interactions between spontaneous Ca^{2+} waves and AP-triggered $[Ca^{2+}]_i$ produce *sub-cellular spatially discordant* alternans (SDA) and even more complex sub-cellular $[Ca^{2+}]_i$ patterns. Therefore, the genesis [43, 48] and propagation [49] of Ca^{2+} waves, which are in general associated with increased SR Ca^{2+} content through increased luminal Ca^{2+} sensitization of the RyR to cytosolic Ca^{2+} and perhaps through increased ability of cytosolic Ca^{2+} to activate adjacent RyR sites, may essentially reset local $[Ca^{2+}]_{SR}$ [34] and give rise to sub-cellular alternans. According to this mechanism, a partially propagated Ca^{2+} wave triggers a gradient in SR

refractoriness when the next AP occurs. In the region of the myocyte through which the Ca^{2+} wave has already passed, the affected SR is empty and partially refractory, thus minimizing Ca^{2+} release. In contrast, the region into which the Ca^{2+} wave has not entered causes the release of a normal amount of SR Ca^{2+}, resulting in a spatially non-uniform $[Ca^{2+}]_i$. On the next beat, both $[Ca^{2+}]_{SR}$ content and excitability of the refractory region will have recovered, producing a large release, therefore perpetuating the presence of sub-cellular SDA.

The presence of sub-cellular spatially discordant $[Ca^{2+}]_i$ leads to increased dispersion of sub-cellular electrophysiologic properties and, in the setting of an appropriate trigger, may lead to an arrhythmia at the cellular level. Although sub-cellular SDA is usually preceded by sub-cellular spatially concordant alternans, under certain circumstances sub-cellular SDA may arise spontaneously [34].

In support of the concept of sub-cellular SDA, it is also possible that as SERCA2a, NCX and RyR function is dynamically regulated on a beat-to-beat basis by many metabolic and ionic factors in the microdomain of the SR [50-55], SR Ca^{2+} uptake and release is also dynamically changing, especially in the diseased heart [34, 45, 56], thus creating differential spatial heterogeneity of thresholds for the onset of alternans in different regions of the myocyte [34, 50]. In such cases, small differences of SR Ca^{2+} content in different parts of the myocyte may exist under basal conditions and these differences may be amplified once the steepness of the relationship between Ca^{2+} release and SR Ca^{2+} content begins to rise.

In summary, these data suggest that in the diseased heart, cellular alternans requires a trigger event (such as increased β-stimulation or a Ca^{2+} wave) and an appropriate sub-cellular substrate to develop. Increasing the probability of RyR opening alone does not produce arrhythmogenic Ca^{2+} release due to an accompanying decrease in SR Ca^{2+} content. β-adrenergic stimulation increases SR Ca^{2+} content and thereby allows the increased RyR open probability to produce Ca^{2+} release [56]. A trigger event alone may be sufficient to induce alternans in the normal heart, however, it requires supra-physiologic heart rates in order to create a heterogeneous (fragmented) sub-cellular Ca^{2+} release profile. In the diseased heart, however, perturbations in the intracellular calcium cycling machinery create a sufficiently heterogeneous sub-cellular substrate leading to development of alternans at lower heart rates (Figure 3) and predisposing to arrhythmogenesis [57].

Mechanisms of Alternans in the Intact Heart

In a manner analogous to *sub-cellular* spatially concordant and discordant $[Ca^{2+}]_i$ alternans, APD alternans at the tissue or whole heart level can also be spatially concordant or discordant (for definitions see Table).

Early work has demonstrated significant variation across species in the ability to induce alternans and has also demonstrated that APD alternans is more easily induced at lower temperatures [58], which tend to prolong APD, therefore suggesting a primary role for membrane voltage dynamics in alternans at the tissue level. Subsequently, Weiss et al[9] in computer simulations have shown that at the cellular level, steep APD restitution (the relationship between APD and the previous diastolic interval) slope and $[Ca^{2+}]_i$ cycling dynamics cause the APD and $[Ca^{2+}]_i$ to alternate. They have also demonstrated that at the tissue level, additional factors, such as conduction velocity restitution and ectopic beats, promote spatially discordant alternans. However, despite the demonstration that sustained APD alternans occurs when the APD restitution slope is >1 at a given cycle length, experimental evidence indicates that the onset of APD alternans is primarily attributable to an instability in $[Ca^{2+}]_i$ cycling dynamics rather than steep APD restitution [9, 59]. Voltage clamp experiments in isolated myocytes [20] have demonstrated that $[Ca^{2+}]_i$ exhibits alternans

despite a constant beat-to-beat AP (voltage) waveform, suggesting that APD alternans is typically driven by $[Ca^{2+}]_i$ dynamics and not by voltage dynamics (i.e. steep APD restitution slope). In both isolated ventricular myocytes [18] and intact tissue [60], the onset of APD alternans occurred at a constant cycle length at which APD restitution slope was still considerably <1 and interventions that suppressed $[Ca^{2+}]_{SR}$ cycling eliminated AP alternans irrespective of the APD restitution [18]. As such, studies from both isolated myocytes and intact tissue suggest a primary role for perturbations in calcium cycling processes in the genesis of APD alternans in the whole heart.

However, whether the same mechanisms give rise to APD alternans under all circumstances, and whether the presence of alternans are necessarily always reflective of a proarrhythmic substrate, remains an area of controversy [57]. The presence of discordant alternans and ventricular arrhythmias in a pacing-induced model in the guinea pig [22], a species believed to be highly resistant to alternans [58], suggests that chronotropically induced alternans may generate a non-specific pro-arrhythmic substrate. In contrast to pacing-induced alternans, discordant alternans induced in the setting of acute ischemia [61-63] or heart failure [64] appears to be primarily due to sub-cellular $[Ca^{2+}]$ cycling perturbations and is believed to represent a truly arrhythmogenic substrate. These data further support the hypothesis that the further the diseased state of the heart, the higher the probability of inducing alternans with progressively smaller trigger events (i.e. at lower heart rates resulting from small, transient bursts of β-adrenergic stimulation).

Regardless of the method used to induce alternans, the emergence of discordant APD alternans (reflecting two adjacent areas of the myocardium that oscillate with opposite phase) appears to be a fundamental step in the development of an arrhythmogenic substrate. Studies in normal hearts using optical mapping techniques have shown that discordant AP alternans is associated with a state of marked cardiac electrical instability, as evidenced by the fact that ventricular fibrillation is always preceded by discordant, but never by concordant, APD alternans [5]. This unstable electrical substrate is consistently induced at a critical heart rate threshold and is largely independent of the pacing site [5], suggesting that it is caused by heterogeneities of cellular repolarization properties and not heterogeneous propagation delay. Interestingly, in this study, alternans most commonly involved the slope of the AP plateau and the onset of final repolarization, timing during CICR that coincides with the timing of aberrant RyR release during alternans observed by our group [16] and others [19].

Recently a two-photon confocal imaging study in the intact rat ventricle [65] has shown that the spatial distribution of $[Ca^{2+}]_i$ alternans within the myocyte is time-dependent. Specifically, areas that mark the boundaries between regions of the myocyte that are out of phase during alternans can drift within the myocyte. These phase-mismatched myocyte regions are essentially driven by the myocyte membrane potential, defined by a spatial average potential of all myocytes within the electrotonic space constant, and thus providing a spatial constraint to the region of discordant alternans. Furthermore, the same study [65] has shown that rapid pacing synchronized Ca^{2+} waves in a sufficient mass of neighboring myocytes to cause DADs at the tissue level. In contrast, sporadic Ca^{2+} waves in individual myocytes at slow rates had no effect on membrane potential due to source-sink mismatch. Therefore, sub-cellular heterogeneities in $[Ca^{2+}]$ likely play an important role in the genesis of triggered activity (i.e. EADs and DADs) which may trigger the onset of an arrhythmia in presence of an appropriate substrate [34, 65, 66]. It is also conceivable that if myocytes in a region of tissue synchronously develop Ca^{2+} waves [65], the amplitude and the phase of APD alternans in that region may change relative to the surrounding tissue, thus increasing dispersion of APD and directly contributing to the development of the arrhythmogenic substrate. It should be noted, however, that the precise relationship between discordant sub-

cellular [Ca^{2+}] alternans and APD alternans in the whole heart remains to be fully elucidated and the presence of bidirectional coupling (between $[Ca^{2+}]_i$ and membrane voltage) [57] adds significant complexity to these dynamic interactions.

Building on the premise that sub-cellular [Ca^{2+}] alternans contributes to APD alternans at the tissue level, it is conceivable that following cardiac "injury", during the remodeling phase of the heart, the compensatory increase in β-adrenergic stimulation results in progressively increased SR Ca^{2+} content and a higher probability of inducing alternans. Although in end-stage heart failure the loss of β-adrenergic responsiveness is almost complete [47], in moderate cardiomyopathy, it is likely that residual β-adrenergic responsiveness results in higher $[Ca^{2+}]_{SR}$ content and spontaneous SR Ca^{2+} release [45]. As the heart transitions from the compensatory phase to clinical heart failure, cardiac remodeling progresses to the point that the slope of the released SR Ca^{2+} -SR Ca^{2+} content relationship is steep enough that despite the loss of β-adrenergic responsiveness [47], transient/residual β-adrenergic responsiveness [45] may result in higher $[Ca^{2+}]_{SR}$ content, increased incidence of fractionated and aberrant SR Ca^{2+} release and Ca^{2+} waves, and higher probability of alternans occurrence.

In summary, it appears that AP alternans begins in a localized area in the heart and gives rise to micro-volt level alternans on the surface electrocardiogram [67]. When this region of AP alternans extends to a significant portion of the myocardium (such that it is large enough to overcome the 3-dimensional current sink problem) and becomes sufficiently synchronous, it can then be seen on the surface electrocardiogram as milli-volt level alternans [68]. Localized alternation in APD in turn is associated with delayed recovery on an every other beat basis, resulting in spatial dispersion of recovery, wavebreak and setting the stage for the development of re-entry and arrhythmia on-set (Figure 4) [22, 69, 70].

Clinical Relevance of Repolarization Alternans

Repolarization Alternans and Arrhythmia Susceptibility

The paradigm that repolarization alternans arises from perturbations in calcium cycling within the individual myocyte and the critical role of both substrate and triggers in the pathophysiology of RA, as delineated in the preceding discussion, has important clinical implications. To date, RA has been most commonly encountered in the clinical setting through the use of MTWA testing to predict the risk of ventricular tachyarrhythmic events (VTE) and SCD [71]. A positive MTWA test result has been associated with a significantly heightened risk for SCD during *medium* and *long*-term follow-up across a wide range of clinical settings including ischemic [72] and non-ischemic [73] cardiomopathy and structural heart disease with preserved left ventricle EF [74].

More recently, prospective studies assessing the prognostic utility of MTWA testing in cohorts where a large percentage of patients are implanted with prophylactic implantable cardioverter-defibrillators (ICDs) [75, 76] have suggested that MTWA testing is not as good a predictor of "appropriate" ICD therapy as it is a predictor of VTE/SCD in patients without ICDs. This observation has been attributed to the fact that many "appropriate" ICD therapies treat arrhythmias that would have self-terminated or that ICDs may induce arrhythmias that they subsequently treat [77-79]. To overcome this cofounding factor, we have recently shown that in a pooled cohort of 2883 patients without ICDs, a negative MTWA test in patients with LVEF ≤ 35% predicts a very low annual risk for SCD, while a positive MTWA test predicts a significantly heightened risk of SCD, both in patients with LVEF ≤ or > 35% [80]. If confirmed in prospective studies, these findings may have important implications for refining primary prevention ICD treatment algorithms.

These clinical observations also demonstrate that an increased magnitude of RA is closely associated with the substrate that gives rise to malignant ventricular arrhythmias [9, 59, 81] and that clinical heart failure significantly lowers the heart rate threshold to induce ventricular alternans [64, 82]. Other lines of evidence suggest that RA may also play an important role in the pathogenesis of atrial arrhythmias [83], a setting where the paradigm of substrate and triggers (i.e. pulmonary vein potentials) may have particular relevance. However, these observations do not necessarily prove that RA plays a causative role in the genesis of arrhythmias or that suppressing RA would be a viable therapeutic target. Although differentiating association from causation in the clinical setting can be challenging, several lines of clinical evidence do lend support to a causative role for RA in the genesis of cardiac arrhythmias. Analysis of ambulatory body-surface electrograms (Holter monitors) from patients with various forms of heart disease has demonstrated a sharp upsurge in both alternans and non-alternans periodicities (measured by time-domain techniques) within the minutes prior to spontaneous VTE [84, 85]. These studies demonstrate that non-alternans periodicities such as T-wave lability, a T-wave oscillation pattern that does not follow an alternans-like pattern, may also precede VTEs [86, 87]. However, in contrast to clinical MTWA testing utilizing frequency-domain techniques, the medium and long-term prognostic significance of heightened non-alternans periodicities has not been as well validated.

Analysis of intracardiac electrograms from ICD leads has also demonstrated a sharp increase in RA magnitude immediately prior to spontaneous ventricular arrhythmias [88-90]. However, a similar upsurge in RA has not been observed prior to induced ventricular arrhythmias or preceding inappropriate ICD shocks [88], suggesting that the presence of increased RA magnitude is not just a by-product of a ventricular arrhythmia or a consequence of an ICD shock. Simultaneous measurement of RA from body-surface and intracardiac electrograms by our group [91] and others [92] has shown a high degree of correlation suggesting that these measurements are detecting the same electrical phenomenon.

The mechanism(s) linking RA and arrhythmogenesis have been explored by Kuo et al[93] who have shown that increased dispersion of repolarization (DR) is an important condition for the development of reentrant arrhythmias and Chinushi et al [94, 95] who have shown that increased DR is associated with VTE and concordant or discordant alternans (DR is greater at sites of discordant vs. concordant alternans). Numerous experimental [22, 23, 96, 97] and computational [98-100] studies have demonstrated that APD alternans can provide the substrate for reentry and support the notion that beyond medium and long-term prognosis, heightened RA is also an important *short-term* predictor of arrhythmia susceptibility. Although the presence of discordant APD alternans leading to wavebreak and reentry (also known as the multiple wavelet hypothesis) has emerged as a major model to explain the pathogenesis of VTE, it is important to note that other overlapping models have also been proposed including the focal source hypothesis, in which wavebreak represents a distant epiphenomenon and is not necessarily required to sustain VF. Evidence to support both types of fibrillatory activity may be seen in the same heart and both may be relevant clinically [101] and the extent to which these competing models may have clinical therapeutic implications remains to be defined.

In aggregate, clinical data suggest that the heart either passes through a state of heightened RA on the way to VT/VF or heightened RA occurs in close conjunction with developing VTE [22, 23]. In either scenario, these findings suggest that detecting significantly elevated levels of RA may serve as an important *short*-term predictor of impending arrhythmias and also raise the possibility of using upstream therapies to abort VT/VF prior to arrhythmia onset.

Therapeutic Implications

The ability to detect heightened levels of RA from implantable intracardiac devices opens the door to the possibility of delivering upstream therapy to suppress RA and prevent the development of a favorable substrate for arrhythmogenesis. Upstream therapy also has the important potential benefit of preventing the need for ICD shocks, which have an adverse impact on quality of life and may also have a detrimental effect on heart failure disease progression[102].

The concept of upstream therapy depends on the ability to detect RA with a high degree of sensitivity. Repolarization alternans *in vivo* is known to be a spatially and temporally heterogeneous phenomenon [103] and therefore, any attempt to suppress RA is predicated on the ability to accurately detect alternans regardless of where in the heart it originates. Our group has recently identified a novel lead configuration for the optimal spatio-temporal detection of intra-cardiac repolarization alternans [91]. To examine which intracardiac lead combination is most sensitive for RA detection, in Figure 5 we plot the probability that a far-field bipolar intracardiac lead configuration is positive for RA, given that at least one intracardiac far-field lead is positive, for each of a right-ventricular (RV), coronary sinus (CS), left-ventricular (LV), epicardial (EPI), and triangular RV-CS far-field intracardiac lead configuration. When an intracardiac lead is positive, the probability that a triangular RV-CS lead is positive is 85.5%, greater than any other intracardiac lead, suggesting that this lead configuration may provide an optimal approach for intracardiac RA detection. The use of an RV-CS lead configuration also has important clinical applicability since many currently utilized intracardiac devices already have RV and CS leads (i.e. cardiac resynchronization therapy platforms).

Following detection of heightened RA, electrical therapy has been proposed as a means of suppressing RA and preempting the development of a potentially arrhythmogenic substrate. The use of electrical therapy for this purpose draws on the experience with the use of pace termination of ventricular arrhythmias. Electrical therapy as a means of terminating ventricular arrhythmias [104-113] may result in one of several outcomes: (i) termination of reentry and VTE, (ii) changes in the shape and/or position of the center of the activity and induction of different reentrant waveforms or a focal pattern of repetitive activation, (iii) changes in the “exit” pathway or in the direction of the activity, and (iv) resetting of the activity and persistence of the same reentry.

In an analogous manner, it is conceivable that appropriately delivered pacing stimuli may suppress/terminate RA and abort reentry, thus preventing VT/VF. Although detecting an upsurge in magnitude of RA immediately prior to the onset of ventricular arrhythmias is suggestive of a causative role for RA in arrhythmogenesis, definitive proof of causation requires clear demonstration that suppressing RA prevents arrhythmias. Preclinical studies have demonstrated the feasibility of suppressing RA with dynamic pacing protocols that can be modulated based on real-time measurements of APD[114, 115] with the result of suppressing RA and re-stabilizing the myocardial substrate. However, reproducing these findings in the whole heart has been limited by the inherent spatial and temporal variability of RA as it occurs *in situ*.

Our group has recently developed a method for *in situ* dynamic control of RA in a swine model [116]. This method is based on the premise that adaptive sub-threshold pacing impulses delivered during the absolute refractory period may be capable of controlling RA. In this model, RA is induced via an R-wave triggered pacing protocol which delivers impulses on an every other beat basis and hence leads to a significant rise in RA magnitude as detected by an increase in K_{score} (for a description of the use of the K_{score} to quantify RA magnitude, please see reference [117]). The increase in K_{score} is detectable from both intracardiac (right

ventricle, left ventricle, coronary sinus) and body-surface (lead II) electrodes. Following induction of significant RA, triggered pacing stimuli delivered from a remote location on alternate beats can be used to suppress RA. In this example, RA is induced by pacing from the RV12 electrode on even beats (Figure 6, panel B) and then suppressed by pacing from RV56 on odd beats (Figure 6, panel C). Other permutations of even and odd beat pacing and changes in the polarity of triggered impulses can be used to induce and suppress alternans with a high degree of fidelity (Figure 6, panels D-F).

Extension of these findings raises the possibility of incorporating adaptive pacing protocols into implantable devices such that if the device detects an unstable myocardial substrate (as evidenced by heightened RA magnitude), the adaptive pacing protocol would be activated to deliver electrical therapy to re-stabilize the electrical substrate so that even if a trigger event occurred (i.e. a PVC), that trigger would no longer encounter a vulnerable electrical substrate and the onset of arrhythmia would be prevented. The adaptive pacing protocol could be terminated when the RA magnitude falls below a predetermined threshold.

Beyond adaptive pacing protocols, detection of RA by implantable devices may also be coupled to other forms of suppressive therapy. For instance, there is significant interest in coupling micro-electromechanical systems (MEMS) to implantable devices to facilitate localized delivery of pharmacologic agents for treating various aspects of chronic heart failure (i.e. neurohormonal antagonists, diuretics, anti-arrhythmic agents) [118]. Several classes of pharmacologic agents have been demonstrated to suppress RA and prevent ventricular arrhythmias including β-blockers [119, 120] and certain sodium channel blockers such as ranolazine [121]. It's conceivable that timely and potentially localized delivery of such agents may be capable of suppressing RA and re-stabilizing the electrical substrate. It should be noted, however, that the hypothesis that suppression of RA *in vivo* can be used to preempt arrhythmia on-set remains to be proven. An important proof of concept study recently demonstrated that the use of SERCA2a adenoviral gene transfer in a guinea pig model resulted in a 4-fold reduction in susceptibility to alternans-mediated ventricular arrhythmias [122], and has opened the door to other potential therapeutic approaches for the suppression of RA and prevention of VTEs.

The potential to couple detection of elevated RA magnitude and delivery of therapy within an implantable device offers a real opportunity for developing iterative and closed-loop systems to prevent arrhythmias.

Conclusions

The generally accepted paradigm of requiring both substrate and triggers for the genesis of ventricular arrhythmias [123] lends significant complexity to understanding the underlying mechanisms that give rise to life threatening arrhythmias and SCD. Electrocardiographic alternans-type oscillations represent a response of the ventricle at the first sub-harmonic of the driving frequency (the mean heart rate) which might be viewed as the first bifurcation in the pathway to ventricular fibrillation [124]. This review presents a contemporary view of the mechanisms underlying $[Ca^{2+}]_i$ and AP alternans in the normal and diseased heart.

The prevailing hypothesis of repolarization alternans is that dynamic sub-cellular perturbations in intracellular Ca^{2+} homeostatic mechanisms occurring on a beat-to-beat basis give rise to $[Ca^{2+}]_i$ alternans, which in turn gives rise to APD alternans and electrocardiographic alternans. At the whole heart level, the transition from concordant to discordant APD alternans is associated with a state of significantly heightened cardiac electrical instability due to the fact that discordant APD alternans leads to increased spatial dispersion of refractoriness and wavefront fractionation and eventually to the onset of

reentrant arrhythmias. Enhanced understanding of the pathophysiologic processes which give rise to alternans at the cellular and whole heart level may have important implications for pharmacologic and/or electrical therapeutic approaches to prevent ventricular arrhythmias and sudden cardiac death.

Acknowledgments

Funding Sources The work was supported by a Scientist Development Grant (#0635127N), by National Institute of Aging (NIA) grant 1R21AG035128 and NIH grant 1RO1HL103961. This work was also supported by a Fellowship and a Science Award from the Center for Integration of Medicine and Innovative Technology (CIMIT), the Deane Institute for Integrative Research in Atrial Fibrillation and Stroke and the Cardiovascular Research Society.

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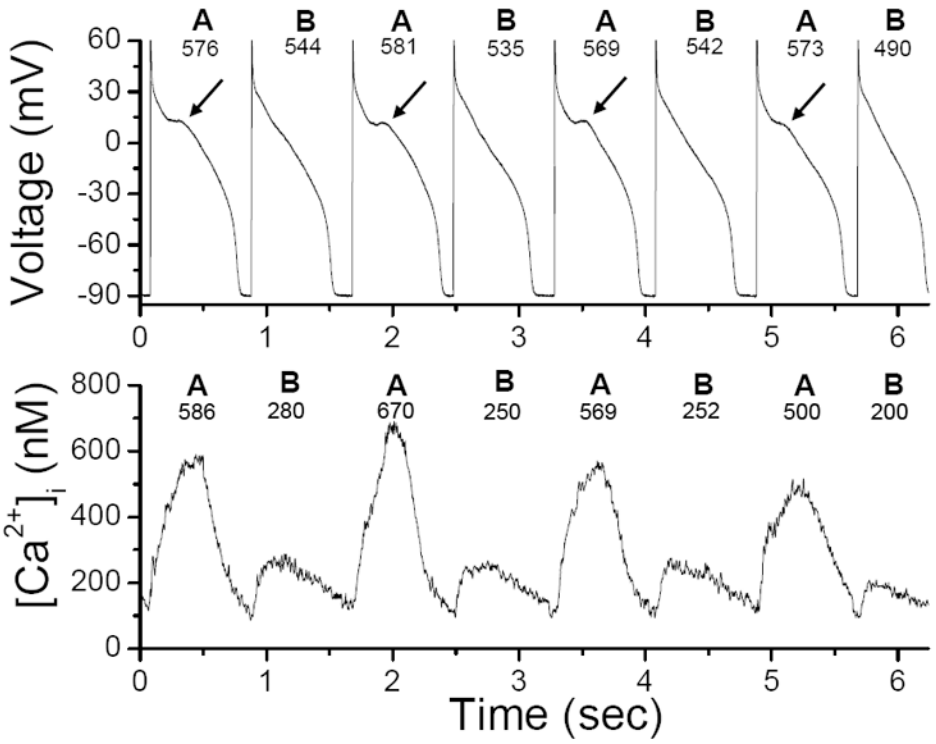

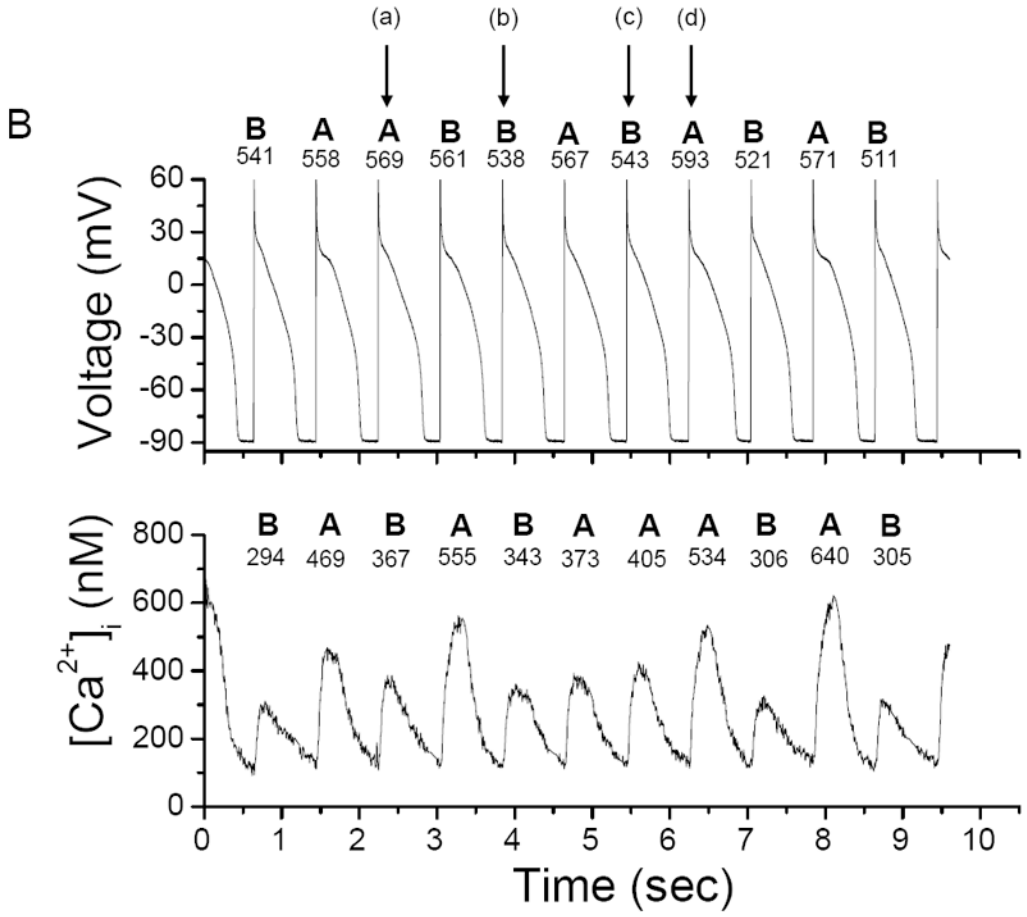

Figure 1. Illustration of $[Ca^{2+}]_i$ and AP alternans [16, 17]

(A) Representative example of concordant $[Ca^{2+}]_i$ and AP alternans in a left ventricular canine myocyte stimulated every 0.8 sec. The arrows indicate a sub-threshold early after-depolarization; (B) representative example of phase transitions (a)-(d) between $[Ca^{2+}]_i$ and AP alternans obtained during the same data record as Figure 1A. In-phase (concordant) $[Ca^{2+}]_i$ and AP alternans leads to out-of-phase (discordant) alternans (a) and (c) and back again to in-phase alternans (b) and (d). "A" and "B" denote large and small $[Ca^{2+}]_i$ or long and short APD respectively; the peak $[Ca^{2+}]_i$ and APD for these beats are also shown.

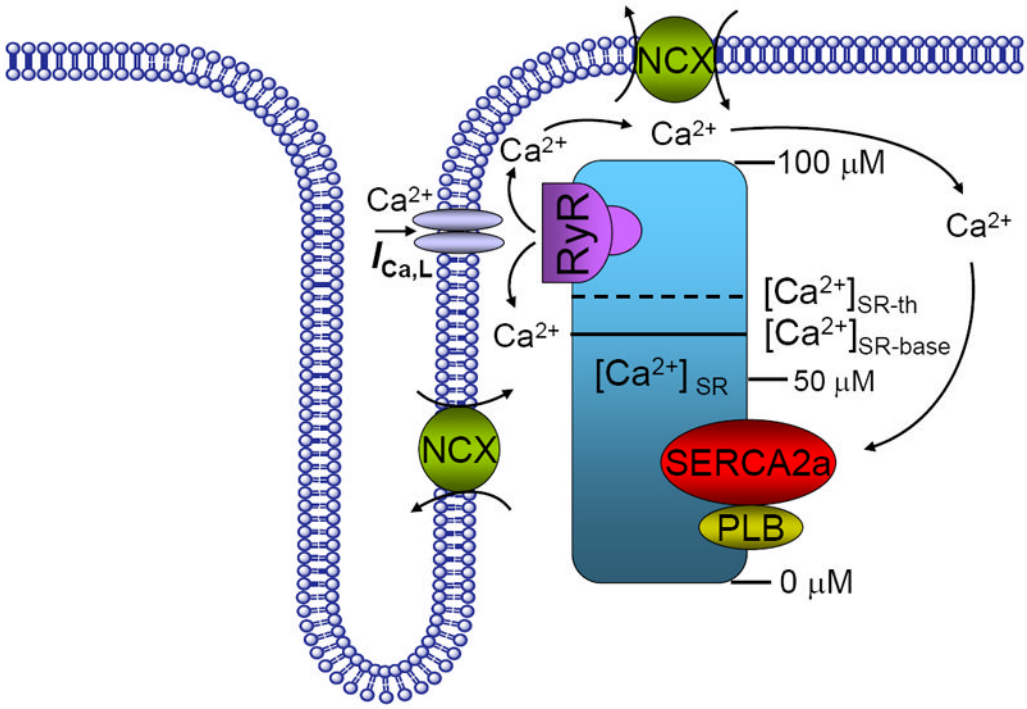

Figure 2. Cellular calcium content and alternans threshold

Schematic diagram of the effect of the SR Ca^{2+} content on a proposed model for cellular alternans. Ca^{2+} cycling though calcium-induced-calcium-release (CICR) includes the L-type Ca^{2+} channel, the SR Ca^{2+} ATPase pump (SERCA2a), phospholamban (PLB), the ryanodine receptor (RyR) channel and the Na^{+}/Ca^{2+} exchanger (NCX). The solid line shows the SR Ca^{2+} baseline ($[Ca^{2+}]_{SR\text{-}base}$) and the dashed line shows the threshold SR Ca^{2+} ($[Ca^{2+}]_{SR\text{-}th}$) content at which Ca^{2+} release occurs. In the normal heart, CICR is manifested by an operational baseline of $[Ca^{2+}]_{SR}$ that is lower than the threshold to trigger spontaneous Ca^{2+} release. However, high stimulation frequency or β-adrenergic stimulation results in SR Ca^{2+} overload that raises the SR Ca^{2+} baseline level above the threshold such that spontaneous sub-threshold Ca^{2+} release may occur.

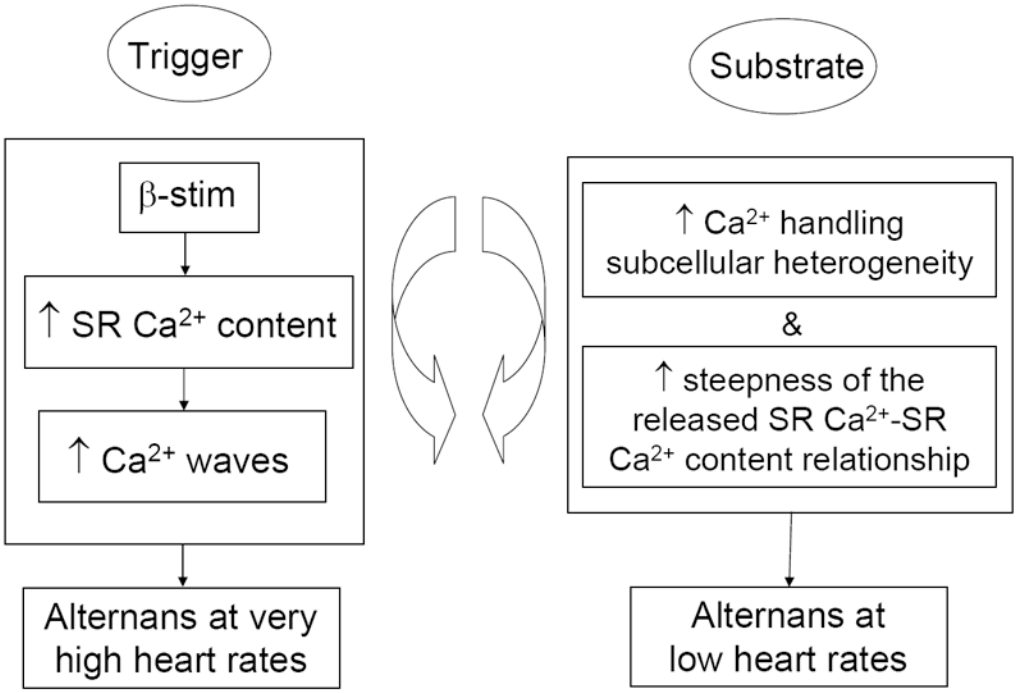

Figure 3. Theoretical paradigm for differential mechanisms of alternans in normal and diseased hearts

In the diseased heart, cellular alternans requires a trigger event and the appropriate substrate to develop. A trigger event alone is sufficient to induce alternans even in the normal heart, however, it requires supra-physiologic heart rates. In the diseased heart, however, the presence of an appropriate sub-cellular substrate makes more favorable the conditions for alternans development, and thus the lower heart rate alternans onset.

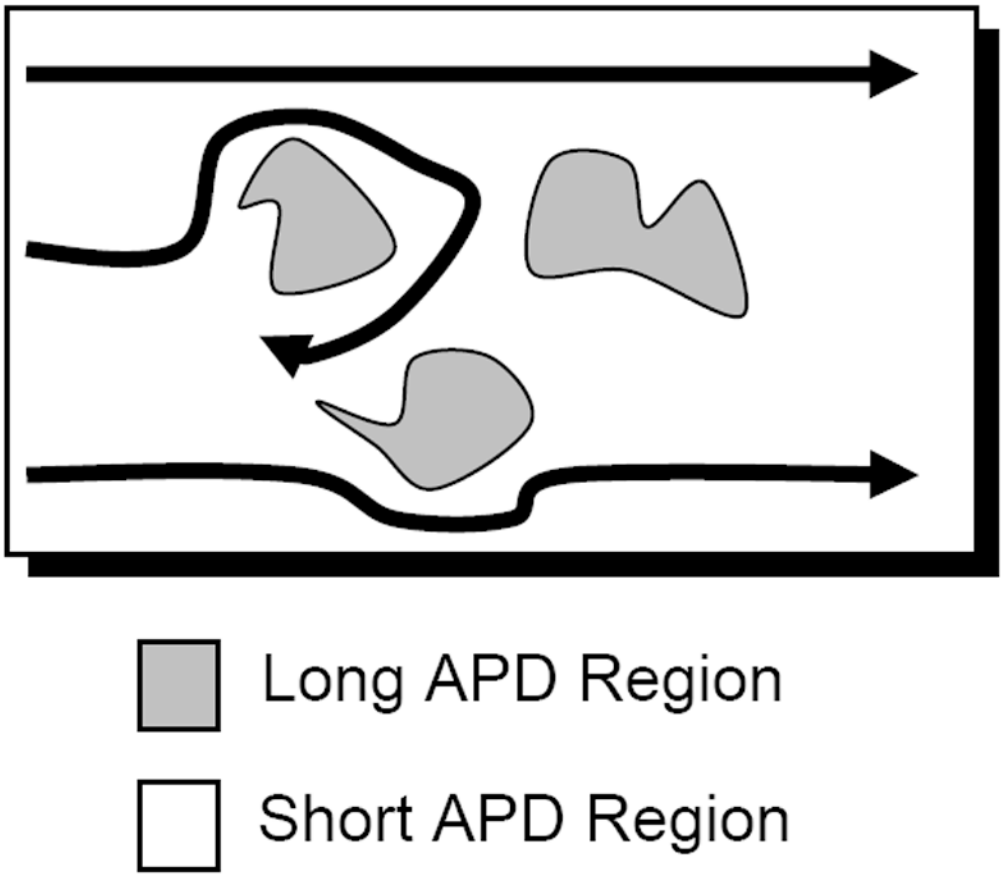

Figure 4. Functional relationship of alternans and re-entry

Localized action potential alternans is manifested as repolarization alternans on the electrocardiogram. Localized regions of tissue exhibiting action potential alternans are associated with delayed recovery on an every other beat basis. These tissue areas of delayed recovery may lead to wavebreak and the development of reentry.

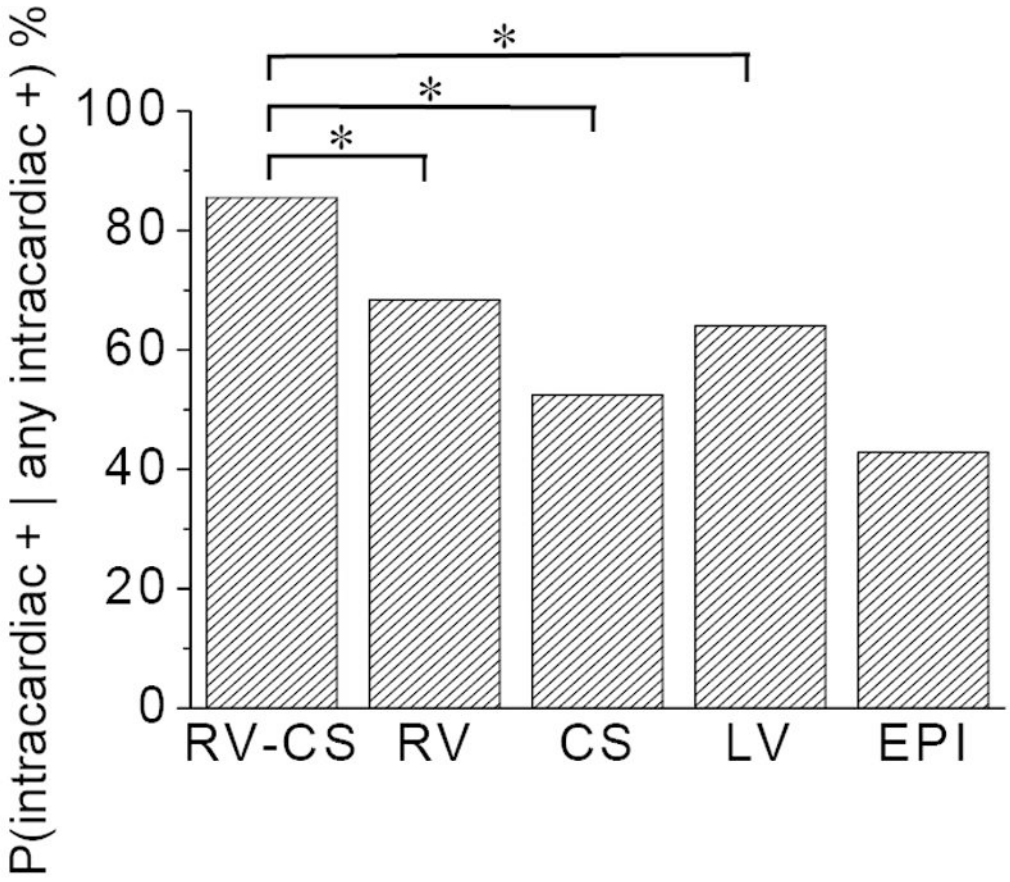

Figure 5. Intracardiac RA detection

Probability that a far-field bipolar intracardiac lead is positive for RA, given that at least one intracardiac far-field lead is positive for RA, for each of the RV, CS, LV, EPI, and triangular RV-CS far-field intracardiac lead configurations [91]. The RV-CS positive percentage was significantly (*) larger than for the RV configuration (p=0.040), the CS configuration (p=0.004), and the LV configuration (p=0.035), but not for the EPI configuration (p=0.270).

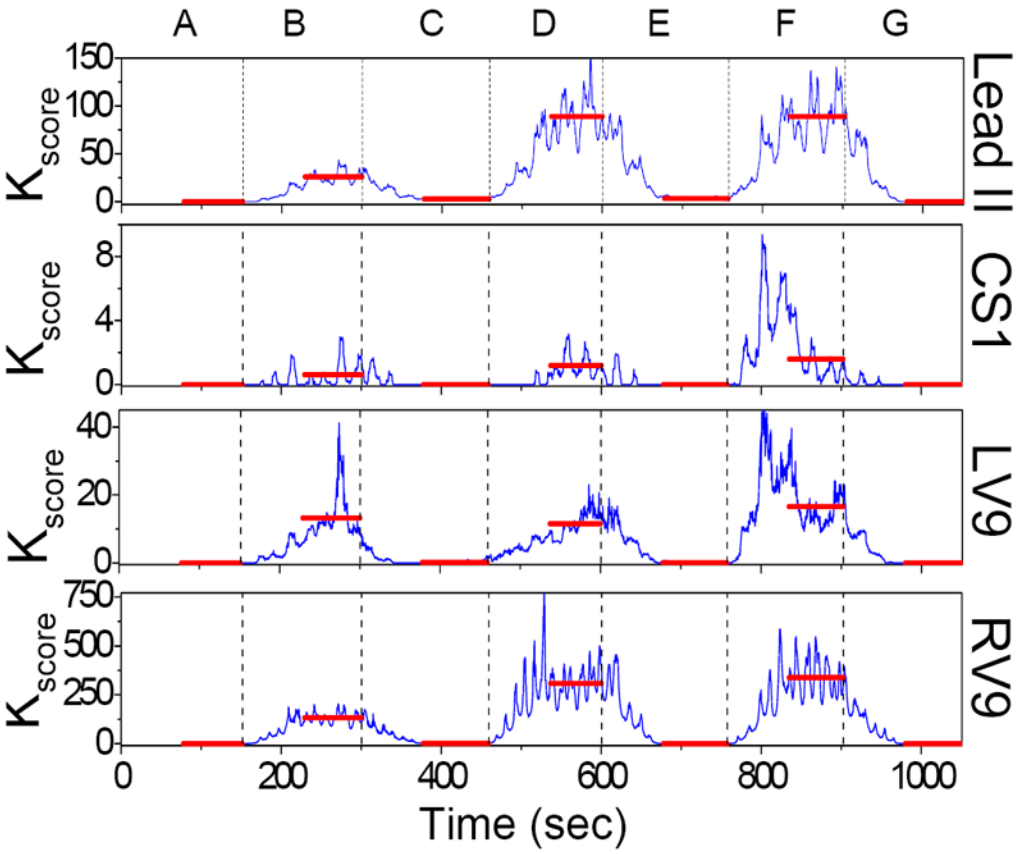

Figure 6. Intracardiac RA control

Repolarization alternans (RA) control during which RA is induced via RV12 triggered pacing (-7 mA, 30 ms width, 30 ms coupling) and suppressed via RV56 triggered pacing (-7 mA, 30 ms width, 30 ms coupling). A: baseline, B: RV12 even beats, C: RV12 even & RV56 odd, D: RV12 even & RV56 odd with polarity flip, E: RV12 even & RV56 even with polarity flip, F: RV12 even & RV56 even, G: RV12 every beat. Interventions B, D, and F induce RA, while C and E suppress RA. Transitions occur at times marked by dashed vertical black lines, while the red horizontal lines during each intervention indicate the mean K_{score} (see reference [117] for a description of the method used to estimate K_{score}).

Table

Definitions of cardiac alternans

- Sub-cellular spatially concordant $[Ca^{2+}]_i$ alternans - oscillation of calcium concentration within the myocyte such that $[Ca^{2+}]$ within all sub-cellular areas oscillates *in-phase* (i.e. all sub-cellular regions demonstrate high or low $[Ca^{2+}]$).
- Sub-cellular spatially discordant $[Ca^{2+}]_i$ alternans - oscillation of calcium concentration within the myocyte such that $[Ca^{2+}]$ within adjacent sub-cellular areas oscillates *out-of-phase* (i.e. some sub-cellular regions demonstrate high $[Ca^{2+}]$ and adjacent sub-cellular regions demonstrate low $[Ca^{2+}]$).
- Cellular concordant $[Ca^{2+}]_i$ and AP alternans - oscillation of intracellular calcium concentration and AP voltage such that these signals are *in-phase* (i.e. a large $[Ca^{2+}]_i$ corresponds to a long APD and vice versa).
- Cellular discordant $[Ca^{2+}]_i$ and AP alternans - oscillation of intracellular calcium concentration and AP voltage such that these signals are *out-of-phase* (i.e. a large $[Ca^{2+}]_i$ corresponds to a short APD and vice versa).
- Tissue/whole heart spatially concordant APD alternans – oscillation of action potential duration such that adjacent areas of the heart are *in-phase* (i.e. adjacent regions demonstrate either long or short APDs).
- Tissue/whole heart spatially discordant APD alternans - oscillation of action potential duration such that adjacent areas of the heart are *out-of-phase* (i.e. one area of the heart demonstrates long APDs while an adjacent area demonstrates short APDs).