Cardiac Alternans: Mechanisms and Clinical Utility in Arrhythmia Prevention

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Sudden cardiac death (SCD) is frequently the initial manifestation of a cardiac arrhythmia, resulting in about 350,000 deaths annually in the United States. Devices such as the implantable cardioverter-defibrillator (ICD) seek to restore normal rhythm and may abort SCD. However, given the complex spatiotemporal dynamics of cardiac electrophysiology, predicting the onset of an arrhythmia and preventing the transition from a stable to an unstable rhythm is highly challenging. Deciphering the mechanisms that lead to an unstable heart rhythm and developing therapies to prevent unstable rhythms is an urgent clinical need.

In 1908, Heinrich Hering first described ECG alternans, a pattern of beat-to-beat oscillation in the ECG waveform. Subsequently, repolarization alternans (RA), or alternans that manifests during ventricular repolarization, has been associated with an increased risk for ventricular tachyarrhythmic events (VTEs) and SCD under a wide range of pathophysiological substrates including ischemic and nonischemic cardiomyopathy and recent acute coronary syndromes. RA may also be seen in structurally normal hearts under conditions of significant metabolic stress and chronotropic stimulation. Early pioneering work has shown that different regions of the heart may alternate out of phase to form spatially discordant RA, and that phenomenon alone was a key factor promoting arrhythmogenesis by predisposing the heart to reentrant wave propagation. Furthermore, in in silico studies, it was demonstrated that spatially discordant alternans led to markedly increased dispersion in repolarization (DR) that formed an ideal substrate for an ectopic trigger beat to instigate spiral-wave breakups leading to the onset of lethal arrhythmias, such as ventricular tachycardia (VT) and ventricular fibrillation (VF).

This review provides a contemporary perspective of the subcellular and cellular mechanisms that give rise to cardiac alternans and potential therapeutic approaches based on this mechanistic understanding.

Mechanisms of Cardiac Alternans

Two prevailing hypotheses have been put forth to explain the pathogenesis of cardiac alternans. The first posits that alternans is a membrane voltage or action potential (AP)–driven phenomenon. Under this hypothesis, alternation in cellular sarcolemmal currents, AP duration (APD) and AP amplitude drive alternation in intracellular Ca$^{2+}$ concentration on an every-other-beat basis. In silico and in vitro studies support this hypothesis by demonstrating that the stability of Ca$^{2+}$ homeostatic processes and the transition to stable alternans is driven by modulation of sarcolemmal Ca$^{2+}$-12 and K$^{+}$-13,14 currents, driven primarily by fluctuation in AP morphology.

The second hypothesis postulates that intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]) alternans is the primary driver, which then results in membrane voltage (AP morphology) alternation. Under the second hypothesis, stress-induced disruptions in Ca$^{2+}$ transport processes can initiate [Ca$^{2+}$] alternans, which then results in AP alternans. Perturbations in intracellular Ca$^{2+}$ transport can impact Ca$^{2+}$ entry into the cytoplasm, sarcoplasmic reticulum (SR) Ca$^{2+}$ uptake, intra-SR Ca$^{2+}$ redistribution, SR Ca$^{2+}$ release, recovery of inactivated ryanodine receptors (RyRs) and coupling between intracellular Ca$^{2+}$ cycling and surface membrane voltage.

Figure 1 exhibits Ca$^{2+}$ cycling via calcium-induced calcium release, the impact of SR Ca$^{2+}$ content, and the role of mitochondria on a proposed model for the subcellular and cellular pathogenesis of alternans. The solid line indicates the SR Ca$^{2+}$ baseline and the dashed line represents the threshold of SR Ca$^{2+}$ content at which Ca$^{2+}$ release occurs. In addition...
to providing ATP for excitation/contraction, mitochondria are centrally involved in Ca\textsuperscript{2+} signaling by serving as a Ca\textsuperscript{2+} buffer by taking up Ca\textsuperscript{2+} via the mitochondrial Ca\textsuperscript{2+} uniporter.\textsuperscript{27} Because of the spatial proximity of mitochondria to the RyR, mitochondria have been directly implicated in excitation-contraction coupling. Whether mitochondrial Ca\textsuperscript{2+} uptake occurs on a beat-to-beat basis\textsuperscript{28,29} or occurs in a more slowly integrated fashion\textsuperscript{30,31} remains unclear.

**Metabolic Mechanisms of Alternans in Isolated Cardiac Myocytes**

A preponderance of data have emerged that support the second hypothesis and invokes perturbations in Ca\textsuperscript{2+} handling as the primary driver of subcellular and cellular alternans. The effect of mitochondrial dysfunction on sarcoplasmic Ca\textsuperscript{2+} content during alternans has been studied by our group\textsuperscript{32} and others.\textsuperscript{33} These studies have provided insight on the changes that occur in Ca\textsuperscript{2+} handling in myocytes in diseased hearts and may open the door to novel therapeutic interventions. Our study used a customized photometry system in which Ca\textsuperscript{2+} dyes were excited at 2 discrete wavelengths to simultaneously excite 2 different dyes. Figure 2A presents examples of simultaneously measured cytosolic Ca\textsuperscript{2+} (Fluo-4, AM; Thermo Fisher Scientific) and mitochondrial Ca\textsuperscript{2+} (x-Rhod-1, AM; Thermo Fisher Scientific) alternans. Figure 2B presents simultaneously measured cytosolic Ca\textsuperscript{2+} (Rhod-2, AM; Thermo Fisher Scientific) and sarcoplasmic Ca\textsuperscript{2+} (Fluo-5N, AM; Thermo Fisher Scientific) alternans.

In the same study, we demonstrated that blocking cytochrome c oxidase, F\textsubscript{0}F\textsubscript{1}-synthase, complex I and II, and
α-ketoglutarate dehydrogenase of the electron transport chain increased alternans in both control and SERCA2a upregulated mice. The increase in alternans in SERCA2a upregulated mice was significantly less than in control mice under 7 of 9 conditions tested ($P<0.04$). However, N-Acetyl-L-cysteine reduced alternans in myocytes previously exposed to an oxidizing agent and CGP (an antagonist of mitochondrial sodium calcium exchanger). Blocking the mitochondrial permeability transition pore with cyclosporin A reduced CGP-induced alternans.

In summary, our work demonstrates that mitochondrial Ca$^{2+}$ handling impairments and energy production deficiencies increase alternans. This effect is lessened in SERCA2a upregulated mice, suggesting that these mice are better able to maintain electrical stability under conditions of stress. The data support a functional relationship between mitochondrial dysfunction, sarcoplasmic Ca$^{2+}$ content, and the genesis of alternans and may help explain perturbations in Ca$^{2+}$ signaling in myocytes from patients with heart failure.

While mitochondrial Ca$^{2+}$ buffering can lead to alternans, studies have also shown that increased reactive oxygen species, especially following myocardial infarction (MI), may reduce SERCA2a function and lead to enhanced alternans. Furthermore, redox modulation of RyRs has been shown to promote Ca$^{2+}$ alternans and create a proarrhythmic substrate following MI.

**Interplay of [Ca$^{2+}$]$_i$ and AP Alternans**

To further delineate the relationship between ionic currents and the SR Ca$^{2+}$ uptake and release fluxes with the sarcolemmal membrane potential, we used a novel reverse engineering approach of a simultaneous AP and [Ca$^{2+}$]clamp of experimentally obtained data to a previously described left

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Representative examples of simultaneously measured (A) cytosolic Ca$^{2+}$ (Fluo-4, AM) and mitochondrial Ca$^{2+}$ (x-Rhod-1, AM) alternans, and (B) cytosolic Ca$^{2+}$ (Rhod-2, AM) and sarcoplasmic Ca$^{2+}$ (Fluo-5N) alternans.
ventricular (LV) canine myocyte model. This hybrid (experimental and computational) approach was used to investigate whether model-derived APs correlate with the APs obtained experimentally and to elucidate the subcellular (Figure 3C) and cellular mechanisms underlying cardiac alternans.

Our work has demonstrated that APD prolongation is associated with a large [Ca\(^{2+}\)] and coincides with a secondary, much smaller, SR Ca\(^{2+}\) release (Figure 3B) manifested in the RyR state-1 open probability \(P_{101}\) on an every-other-beat basis (where primary/secondary SR Ca\(^{2+}\) releases are indicated by an “↑” and an “↓”, respectively). This, in turn, triggers a larger inward depolarizing current attributed to both the L-type Ca\(^{2+}\) channel (LTCC) and the sodium calcium exchanger (NCX), as shown in Figure 3D. This depolarizing current results from a large [Ca\(^{2+}\)], coincident with a smaller secondary SR Ca\(^{2+}\) release and a smaller RyR state-1 open probability, as shown in Figure 3C and 3D. Also, careful inspection of Figure 3A reveals a small deflection on the AP, a subthreshold early afterdepolarization, which is associated with this depolarizing current. Importantly, this every-other-beat secondary Ca\(^{2+}\) release (Figure 3B) does not occur in recordings that do not exhibit alternans. The shape of the AP waveform is dependent on balance between the NCX and the LTCC. The NCX contributes either a depolarizing or repolarizing current during the AP, while the LTCC contributes a depolarizing current. Both the NCX and LTCC are directly mediated by [Ca\(^{2+}\)]. A large calcium transient causes the NCX to reverse earlier, thus contributing a smaller repolarizing current and leading to AP prolongation. It also causes acceleration of the LTCC Ca\(^{2+}\)-mediated inactivation, leading to AP shortening. A small calcium transient would expectedly cause opposite effects. Thus, the net balance of NCX and LTCC defines the effect of [Ca\(^{2+}\)], on the AP. Therefore, Ca\(^{2+}\) induced inactivation of the LTCC and Ca\(^{2+}\) transport across the sarcolemma through the NCX, which are both dependent on [Ca\(^{2+}\)], mediate the relationship between [Ca\(^{2+}\)], and APD during alternans. Furthermore, under varying pathological conditions, this intricate balance between the NCX and LTCC could be altered leading to concordant or discordant alternans.

This observation further suggests that AP alternans is closely associated with the incidence of a spontaneous secondary SR Ca\(^{2+}\) release on alternate beats that occurs early during the AP plateau and provokes a subthreshold early afterdepolarization, which ultimately results in RA at the whole heart level. In prior studies it has been shown that elevation in SR lumenal Ca\(^{2+}\) is more likely to cause RyRs to be triggered by cytosolic Ca\(^{2+}\) . In addition, spontaneous SR Ca\(^{2+}\) release and delayed afterdepolarization amplitude can ascend closer to AP trigger threshold with an increase in SR Ca\(^{2+}\) content. Delayed afterdepolarization have, in turn, been shown to trigger abnormal electrical activity in response to catecholamines or high stimulation rates in normal ventricular myocytes, heart failure preparations, and cardiomyopathic human hearts. These studies support the hypothesis that SR Ca\(^{2+}\) stabilization can abolish alternans as evidenced by studies in which ryanodine and thapsigargin markedly reduced [Ca\(^{2+}\)], and eliminated AP alternans. Ryanodine has also been shown to eliminate both AP and tension alternans in papillary muscles. Xie and Weiss provided further evidence for the relationship between SR Ca\(^{2+}\) content and alternans by demonstrating that rapid stimulation rates make myocytes more susceptible to Ca\(^{2+}\) overload and interactions between spontaneously occurring Ca\(^{2+}\) waves and AP-triggered [Ca\(^{2+}\)], result in subcellular spatially discordant alternans. Therefore, the combination of increased likelihood of cytosolic Ca\(^{2+}\) to activate neighboring RyR clusters and increased sensitization of RyR luminal Ca\(^{2+}\) to cytosolic Ca\(^{2+}\) may reset local [Ca\(^{2+}\)] and trigger subcellular alternans. These results also align well with the unified theory of Ca\(^{2+}\)-mediated alternans recently proposed by Qu et al., wherein alternans was shown to arise as a result of an instability in 3 properties of the Ca\(^{2+}\) release units, namely, randomness of Ca\(^{2+}\) sparks, recruitment of a Ca\(^{2+}\) spark by neighboring Ca\(^{2+}\) sparks, and refractoriness of the Ca\(^{2+}\) release units. In addition, they have successfully demonstrated that SR Ca\(^{2+}\), RyR sensitivity, and SR Ca\(^{2+}\) uptake rate all play an important role in Ca\(^{2+}\)-mediated alternans.

To test the hypothesis that secondary RyR openings are involved in AP alternans, we used isolated ventricular myocytes from mice hearts that were whole-cell (in current-clamp) patch-clamped (37°C) at progressively faster rates until AP alternans was elicited (Figure 4A). Following the onset of alternans, pulses of −3.78 pA/pF and 5 ms duration were delivered 10 ms after the AP upstroke, on every other beat, which resulted in elimination of APD alternans (Figure 4B). Upon termination of stimulation (Figure 4C), APD alternans reappeared.

Mechanisms of Alternans in the Whole Heart

Early work demonstrated that electrical restitution, an intrinsic property of cardiac myocytes, can cause APD alternans at high heart rates. It has been shown in both in silico and in vitro studies that steep APD restitution slope (the relationship between the APD and the previous diastolic interval) and abnormal [Ca\(^{2+}\)], handling are the reasons for [Ca\(^{2+}\)], and APD alternans. In addition, tissue level studies demonstrate that ectopic beats and conduction velocity restitution promote spatially discordant alternans. However, despite evidence suggesting that sustained APD alternans occurs when the slope of the APD restitution at a given pacing cycle length is >1, the restitution hypothesis has not been validated in experimental studies. In both isolated ventricular myocytes and intact tissue, the onset of APD

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Figure 3. A, Examples of concordant calcium transients ([Ca\textsuperscript{2+}]) and action potential (AP) alternans recorded in a left ventricular canine myocyte (at 0.8 seconds). Arrows indicate location of subthreshold early afterdepolarizations. L and S indicate Large/Long and Small/Short [Ca\textsuperscript{2+}] AP, respectively. B, Superposition of intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) and APs from 2 consecutive beats during alternans. C, Left ordinate indicates the AP during alternans, while the right ordinate (in red) indicates open probability of state 1, P\textsubscript{01}, of the ryanodine receptor (RyR). The green area under the P\textsubscript{01}V curve indicates the limits and the magnitude of the secondary RyR, while the same figure also indicates the timing of the peak of that secondary release with respect to the AP upstroke. D, Left ordinate presents the sum (black line) of the $I_{\text{Ca,L}}$ (blue line) and $I_{\text{NCX}}$ (red line), and the right ordinate the RyR state 1 open probability $P_{01}$ (magenta line). The primary and secondary RyR releases are indicated by "*/" and an "**", respectively.
alternans occurs when APD restitution slope is considerably \(<1\) and interventions that suppress \([\text{Ca}^{2+}]_{\text{SR}}\) cycling eliminate AP alternans irrespective of the APD restitution.\(^{18,54}\) While it was proposed that in certain cases, the complex interaction between the transient outward current \(I_{\text{to}}\) and the \(I_{\text{Ca,L}}\) can lead to alternans at slower heart rates,\(^{55}\) there is compelling evidence that in the intact heart, the onset of APD alternans is primarily attributable to an instability in \([\text{Ca}^{2+}]_{i}\) handling rather than steep APD restitution.\(^{11,56}\) Furthermore, in simultaneous voltage-calcium optical mapping studies in isolated whole rabbit hearts, it has been demonstrated that the local onset of \(\text{Ca}^{2+}\) amplitude alternans precedes and triggers APD alternans.\(^{57}\) In aggregate, studies in isolated myocytes, intact tissue, and isolated hearts provide evidence that perturbations in \(\text{Ca}^{2+}\) cycling processes are the principal factors underlying APD alternans in the whole heart.

Localized alternans may lead to increased DR and susceptibility to VT/VF,\(^{22}\) and increased DR has been linked to concordant or discordant alternans (in which DR is found to be greater at sites of discordant compared with concordant alternans) and to VT/VF,\(^{58,59}\) while many other studies have shown that APD alternans may become the substrate for reentry.\(^{22,23,60–64}\) Furthermore, it has been demonstrated that under conditions of reduced repolarization reserve such as long QT syndrome and bradycardia, RA can cause increased DR, which can trigger premature ventricular complexes and lead to reentrant ventricular arrhythmias.\(^{65}\)

It should be noted, however, that the mechanisms that give rise to APD alternans may differ under different pathophysiological conditions and it remains unclear whether the presence of alternans always reflects a proarrhythmic substrate. It has been suggested that chronotropically
induced alternans is nonspecific and does not necessarily reflect a proarrhythmic substrate. In contrast, discordant alternans resulting from acute ischemia or heart failure appears to be caused by subcellular Ca\(^{2+}\) handling perturbations, which are reflective of a proarrhythmic substrate.\(^{66-68}\) It seems likely that the more advanced the underlying heart disease, the higher the probability of inducing alternans with progressively smaller trigger events resulting in greater arrhythmia susceptibility.\(^{69}\)

In addition to the voltage- and calcium-mediated alternans hypotheses, over the past few years, some alternative theories pertinent to the formation of cardiac alternans have emerged. The presence of dynamic instabilities in the substrate that cause EADs were shown to lead to APD restitution discontinuities causing APD alternans.\(^{69,70,71}\) Sato et al\(^{70}\) demonstrated using in silico experiments that, while in smaller tissue sizes, EADs were able to synchronize globally, in larger tissues the spatial heterogeneity of EADs could lead to complex rhythms like APD alternans. In addition, EADs created a substrate conducive to the formation of premature ventricular complexes that then degraded into lethal arrhythmias such as VT or VF.

Furthermore, it has been shown that fibroblasts, which can electrotonically couple to myocytes via gap junctions, can affect APD and \(\text{Ca}^{2+}\) cycling dynamics. Xie et al\(^{72}\) demonstrated that modulation of fibroblast-myocyte coupling can alter repolarization and \(\text{Ca}^{2+}\) cycling alternans at both the cellular and tissue levels, hence playing an important role in arrhythmogenesis, especially in diseased hearts with fibrosis. Both the conduction velocity and pacing frequency at which the alternans onset occurred have been shown to increase with increased gap junction coupling between cells.\(^{73}\) While lower gap junctional coupling enhanced \(\text{Ca}^{2+}\) alternans,\(^{73,74}\) intermediate coupling enabled maximum spatial spread of alternans.\(^{73}\) Additionally, it has been proposed that tissue heterogeneity and the presence of structural barriers accentuate the presence and magnitude of alternans.\(^{75}\) The increased DR caused by the presence of tissue heterogeneities is conducive to the onset of spatially discordant alternans and potentiates the transition to VT/VF. And the DR affecting the presence of spatially discordant alternans is in effect not only caused by spatial dispersion of APD restitution, but likely also caused by dispersion of conduction velocity restitution.\(^{65,76}\)

**RA and Short-Term Arrhythmia Susceptibility**

It is believed that RA is a marker of long-term cardiac electrical instability.\(^{4,5}\) In addition to risk stratifying patients for ICD therapy, recent clinical observations have also suggested that heightened RA may be an important predictor of short-term arrhythmia susceptibility. Previous studies have established a plausible link between RA and susceptibility to VTEs and suggest that suppression of RA may prevent VTEs and SCD.\(^{10,77,78}\) This idea is supported by observations that increases in RA magnitude occur within minutes before spontaneous VTEs in patients with a history of cardiac arrest.\(^{79}\) In this study, compared with baseline, a 25% higher T-wave alternans (TWA) magnitude was seen 10 minutes before the onset of a VTE. Similarly, increased TWA has also been demonstrated using ECG analysis (leads V1, V5, and aVF) in patients hospitalized for acute heart failure\(^{80}\) where an upsurge in TWA was observed 15 to 30 minutes before the onset of VTEs.

These data provide proof of concept that measuring changes in TWA from body surface ECGs may be capable of predicting acute arrhythmia susceptibility. Compared with body surface ECGs, intracardiac electrograms (EGMs) have significantly larger RA magnitude and may provide even more robust assessment of the link between surges in RA and short-term arrhythmia susceptibility. Studies from our group\(^{81}\) and others\(^{82}\) have shown close correlation between simultaneous measurements of RA from body surface ECGs and intracardiac EGMs, suggesting that these methods are measuring the same phenomena.

The magnitude of RA measured from intracardiac EGMs from ICDs has been shown to rise sharply before spontaneous VTEs.\(^{83,84}\) However, similar surges have not been noted before inappropriate ICD shocks or induced VTEs.\(^{83}\) In a prospective multicenter study,\(^{85}\) it was noted that the amplitude of alternans and nonalternans T-wave variability (TWA/V) is significantly greater before spontaneous VTEs compared with during baseline rhythm, time-matched ambulatory EGMs (from the same time of day at which spontaneous VTEs occurred), rapid pacing (at 105 bpm), and EGMs before the onset of supraventricular tachycardia. Each \(\mu\text{V}\) increase in TWA/V was associated with a >2-fold increase in the odds ratio for experiencing a VTE.

These data suggest a close temporal relationship between surges in RA and spontaneous ventricular arrhythmias. To further these observations, in a tachypacing–induced heart failure model,\(^{86}\) we have noted a transition profile from concordant to discordant alternans in unipolar (as well as near-field bipolar and far-field bipolar [not shown here]) EGMs recorded from multiple sites across the left ventricle (base to apex) (Figure 5). The representation demonstrates that discordant alternans observed in both QRS and T waves originates in the region of the heart spanned by leads LV5 to LV10 with spatiotemporal propagation to neighboring regions. The presence of spatially discordant alternans furthers the DR, which, in turn, enables the triggering of VTEs.

In summary, a robust body of evidence supports the hypothesis that there is a close relationship between surges in RA and short-term susceptibility to VTEs. Elevated RA
occurs either in conjunction with the development of a VTE or the heart passes through a state of elevated RA en route to a VTE. In either case, these observations suggest that detection of elevated levels of RA may be an important short-term predictor of an impending VTE and raise the possibility that upstream therapies may be able to suppress RA and prevent the onset of VTEs.

**Use of RA in Guiding Antiarrhythmic Therapy**

RA is known to exhibit spatiotemporal heterogeneity. Therefore, attempts to deliver upstream RA suppressive therapy depend on the ability to detect RA regardless of where it originates in the heart. We have developed a novel intracardiac electrode configuration to detect RA in a highly reproducible manner despite spatiotemporal heterogeneity (using pairs of electrodes from catheters in the right ventricle and the coronary sinus [CS]). In an acute ischemia animal model, our data demonstrate that if significant RA is present, the right ventricular (RV) CS lead configuration will detect it >85% of the time.

Using the same animal model, in Figure 6A we demonstrate the use of body surface as well as intracardiac leads comprised of catheters in the right ventricle, the left ventricle, and the CS to monitor RA immediately before the onset of a VTE. Figure 6B shows results (N=17) of alternans voltage and Kscore from body surface and LV CS leads before the onset of VT/VF, where Kscore reflects the statistical significance of the alternans voltage, relative to the background noise. Significant RA (ST segment and T wave) are present before VT/VF, at least in 1 lead in which the alternans voltage and Kscore are significant (alternans voltage >0.55 and 2.2 μV for body surface and LV CS leads, respectively; Kscore >3.0), indicating that RA can be accurately measured from intracardiac electrograms immediately before the onset of a VTE, and therefore may be used as an index to initiate upstream antiarrhythmic therapy.

Overall, despite the substantial spatiotemporal heterogeneity of RA, the RV CS lead configuration system can detect RA with a high degree of sensitivity. It also has clinical applicability because many implantable devices already utilize RV and CS leads (ie, in cardiac resynchronization therapy). The ability to detect heightened levels of RA from implantable devices opens the door to delivering upstream therapy from the device with the aim of suppressing RA and potentially preventing the development of a proarrhythmic substrate. Such upstream therapy may prevent the need for ICD shocks and mitigate the adverse impact of ICD shocks on quality of life. Upstream therapy may take the form of adaptive pacing protocols, which could be incorporated into an implantable device such that upon detection of an unstable substrate, as evidenced by detection of a surge in RA, the adaptive pacing protocol would be triggered to restabilize the unstable substrate and could be terminated when the RA magnitude

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**Figure 5.** Transition from concordant alternans to discordant alternans during 4 consecutive beats for unipolar, near-field bipolar, and far-field bipolar signals from left ventricular (LV) catheter in the failing heart model. Amplitudes are normalized with red corresponding to higher amplitudes and blue pointing to lower amplitudes. The representation demonstrates that the discordant alternans observed in both QRS and T waves are originating in the region of the heart spanned by leads LV5 to LV10 with similar spatiotemporal propagation effect on the neighboring leads.
drops below a predefined threshold. Homogenization of the electrical substrate, via adaptive pacing to suppress RA, would render the substrate less vulnerable to a trigger, such as a premature beat, which might have initiated a VTE under other circumstances.

**Suppression of RA**

Control of cardiac alternans has been the focus of several studies in in silico, in vitro, and in vivo models. These studies have demonstrated control of alternans at the single cell level, as well as in in vitro preparations that have employed an adaptive negative feedback loop algorithm to adjust the pacing cycle length based on the alternans magnitude. This approach has demonstrated control of alternans in a tissue region of \( \approx 2 \) to \( 2.5 \) cm. However, alternans control in a more complex spatiotemporal setting has been difficult to demonstrate. Recently, Kulkarni et al demonstrated prevention of chronotropically induced alternans through real-time control of the diastolic interval in optical mapping studies in healthy, isolated whole rabbit hearts. In an elegant in vivo demonstration of dynamic pacing therapy to control alternans, Christini et al demonstrated the ability to control AV nodal conduction alternans in humans undergoing electrophysiologic evaluation. However, AV nodal conduction alternans is a spatially constrained phenomenon that differs from the complex spatial-temporal nature of ventricular alternans.

We have recently explored the utility of adaptive pacing to suppress RA in vivo in an acute coronary artery occlusion swine model. In Figure 7A and 7B, we plotted the alternans voltage and \( K_{score} \) respectively, of an alternative, clinically relevant triangular intracardiac lead configuration comprising catheters in the CS and left ventricle. Upon detection of significant spontaneous RA at baseline, the phase of RA is estimated in real time and in phase, with positive polarity R-wave triggered pacing delivered from a lead in the RV apex (intervention “pacing”) resulting in a significant decrease of RA (alternans voltage: \( \approx 4 \)-fold decrease compared with baseline in panel a, \( P<0.0001 \); \( K_{score} \): \( \approx 12 \)-fold decrease compared with baseline in panel a, \( P<0.0001 \)). In panel c, RV12 pacing is stopped, resulting in a rise of the alternans voltage and \( K_{score} \) (alternans voltage: \( \approx 3 \)-fold rise compared with pacing in...
panel b, \( P<0.0001; \) \( K_{\text{score}} \): \( \approx \)7-fold rise compared with pacing in panel b, \( P<0.0001 \).

Furthermore, Figure 8A and 8B present summary results across all experiments \( (N=7 \text{ animals}; \ n=11 \text{ records}) \) where R-wave triggered pacing during the absolute refractory period was used to suppress spontaneously occurring RA. The figure demonstrates the alternans voltage (top row) and \( K_{\text{score}} \) (bottom row) measured from RV CS leads depicting the suppression of RA when pacing is on. During balloon occlusion (baseline) in the presence of acute ischemia, markedly elevated levels of RA are observed. When triggered pacing is initiated, using the customized parameters (amplitude, pulse width, coupling interval, and phase), a significant decrease in alternans voltage and \( K_{\text{score}} \) is observed. With cessation of pacing, alternans magnitude again returns to the elevated levels seen during baseline recordings in the presence of acute ischemia.

In summary, these studies provide a proof of concept that RA can be suppressed using an adaptive (RA-triggered and in real time) pacing protocol, and thus potentially preventing the formation of a proarrhythmic substrate. The concept of pacing during the absolute refractory period to suppress RA is supported by prior studies investigating its use in modulating cardiac contractility, \(^{101-103}\) where it has been shown in experimental studies and computer simulations that stimulation during the absolute refractory period may control the APD. This observation is in agreement with studies that have shown that pacing stimuli applied early during the absolute refractory period result in modulation of the transient outward current \( I_{\text{to}} \) (see also Figure 4), which, in turn, may result in activation of the LTCC and \([Ca^{2+}]_i\) modulation, suggesting that this form of stimulation aiming to control APD and RA and the pathogenesis of alternans at the myocyte level may share the same mechanisms.

**Atrial Alternans**

The majority of the mechanisms and understanding pertaining to cardiac alternans today has been derived from experimental and theoretical investigation of ventricular

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**Figure 6.** Continued
myocytes. More recently, however, there has been strong evidence supporting the role of alternans in promoting arrhythmogenic substrates in the atria as well.\textsuperscript{104,105} In fact, it has been proposed that similar to RA in the ventricles, atrial alternans can serve as a precursor to severe atrial arrhythmias such as atrial fibrillation (AF).\textsuperscript{106,107} Not only has the presence of atrial alternans been reported preceding episodes of AF,\textsuperscript{105,107} clinically, P-wave alternans have also been observed before and during atrial flutter preceding the transition to AF.\textsuperscript{108,109} Furthermore, in a recent study, P-wave abnormalities were shown to predict recurrence of AF in patients after electrical cardioversion.\textsuperscript{110}

Despite the differences in atrial and ventricular physiology and AP morphologies, many similarities have been noted in terms of mechanisms of alternans origin. It was recently shown that similar to ventricular alternans, intracellular Ca\textsuperscript{2+} cycling abnormalities played a major role in initiating atrial alternans and blocking [Ca\textsuperscript{2+}]i abolished alternans in isolated rabbit atrial myocytes.\textsuperscript{111} In addition, both in silico and in vitro experiments have demonstrated that recovery of RyR also

\emph{Figure 7}. Example of the use of R-wave triggered pacing during the absolute refractory period to suppress spontaneous repolarization alternans (RA) during acute ischemia. Alternans voltage (A) and K\textsubscript{score} (B) are plotted for intracardiac leads CS2CS7, CS2LV7, and CS2LV10. R-wave triggered pacing (amplitude: +4 mA, pulse width: 10 ms, R-wave coupling: 10 ms) is delivered from the right ventricular apex (RV12). (a) spontaneous visible RA at baseline; (b) pacing delivered on every even beat results in RA suppression; (c) termination of pacing results in rising of the alternans voltage and K\textsubscript{score} to the baseline level. Transitions between interventions are indicated by dashed vertical lines, while red horizontal lines indicate the mean values of the alternans voltage and K\textsubscript{score} during each intervention. C, ECG morphology changes during the above-described interventions. (a), visible RA at baseline, (b) pacing decreases the RA level, (c) pacing is interrupted and RA becomes again visible. Panels show the median odd (red)/even (blue) beats of a 128-beat sequence during each intervention.
plays a key role in initiation and maintenance of atrial Ca$^{2+}$ alternans. However, Kanaporis and Blatter$^{112}$ highlighted some important differences between atrial and ventricular alternans that could affect control and treatment strategies. Using isolated rabbit myocytes, they demonstrated that atrial alternans had a higher pacing frequency threshold for induction of alternans compared with ventricular alternans. Since there is higher SERCA activity in the atria,$^{113}$ end-diastolic [Ca$^{2+}$]$\text{_{SR}}$ did not alternate during Ca$^{2+}$ alternans in atrial myocytes,$^{114}$ whereas imbalances in [Ca$^{2+}$]$\text{_{SR}}$ load have been shown to promote ventricular alternans. In addition, they also showed that atrial myocytes have a higher density of the Ca$^{2+}$-activated Cl$^{-}$/C0 channels, which play a key role in maintaining APD alternans in the atria.$^{115}$ Finally, the structural complexity of the atria and increased spatiotemporal heterogeneity can affect the maintenance and propagation of atrial arrhythmias in a unique way, compared with the ventricles.

**Effect of Autonomic Modulation on Cardiac Alternans**

It is well established that both the parasympathetic and sympathetic branches of the autonomic nervous system innervate the heart and control normal cardiac function.$^{116-118}$ A balance between these branches is essential for regulating cardiac function. Under pathophysiological conditions such as heart failure, hypertension, and MI, an offset in the autonomic regulation characterized by an increased sympathetic drive and decreased parasympathetic activity has been observed.$^{119,120}$ Extensive research in the past decade has focused on neuromodulation techniques to restore this autonomic imbalance by stimulating the parasympathetic nervous system as a potential therapy for the treatment of cardiovascular diseases. Several studies have demonstrated beneficial cardiovascular effects of vagus nerve stimulation (VNS), which is already a Food and Drug

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**Figure 7.** Continued

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**Figure 8.** Demonstration of the utility of pacing during the absolute refractory period to suppress spontaneous repolarization alternans (RA) during acute ischemia (N=7 animals). (A) Alternans voltage and $K_{\text{score}}$ for each animal at baseline during balloon occlusion, during triggered pacing and during baseline again after cessation of pacing. (B) Summary results for alternans voltage and $K_{\text{score}}$ across all animals, during the three interventions. At baseline, during balloon occlusion, markedly elevated levels of RA are observed. When triggered pacing is initiated, a significant decrease in alternans voltage and $K_{\text{score}}$ is observed. With cessation of pacing, the RA magnitude again returns to the elevated levels seen at baseline. CS indicates coronary sinus; LV, left ventricular; RV, right ventricular. * denotes statistical significance of $p<0.05$. DOI: 10.1161/JAHA.119.013750

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Administration–approved therapy for epilepsy and depression.120–125

Initially, it was believed that while vagal fibers densely innervated the atria, sinoatrial node, and atrioventricular junction, there was little or no parasympathetic innervation of the ventricles.116 Hence, although the effect of parasympathetic activation on slowing the heart rate was well documented, a mechanistic understanding of its influence on ventricular function and electrophysiology was missing. Since then, studies have demonstrated the presence of parasympathetic innervation in the ventricles and highlighted its role in regulating ventricular electrophysiological properties.116,126,127 Several preclinical studies using heart failure animal models have reported significant improvements in ventricular hemodynamics with decreased mortality,122 significantly improved LV ejection fraction,128 and attenuated LV remodeling.129 In addition, VNS has been shown to exhibit anti-inflammatory128,130,131 effects and inhibit SCD132 while markedly suppressing arrhythmias.133–135

The antiarrhythmic effects of VNS have been widely studied over the past decade, with promising results reported regarding suppression of both atrial and ventricular arrhythmias. Recently, in a randomized patient study, low-level VNS was shown to suppress postoperative AF.136 Paroxysmal AF was also shown to be suppressed in patients using transcutaneous low-level tragus stimulation along with a decrease in inflammatory cytokines.137 Similarly, VNS was shown to decrease VT inducibility in MI rats by preserving connexin43,138 reduce the occurrence of spontaneous ventricular arrhythmias and VT after coronary artery occlusion in dogs,139 decrease the maximum slope of restitution and electrical alternans, and increase VF threshold in isolated innervated rabbit hearts,140 and reduce the levels of TWA in patients with drug-refractory partial-onset.141

The effect of sympathetic nervous system stimulation on the heart is complex and governed by the state of the myocardium. In the normal ventricle, sympathetic stimulation shortens the APD and reduces the DR, both of which have been associated with a decrease in the arrhythmogenic tendency.142 However, in pathological states, sympathetic stimulation is a potent stimulus for the generation of arrhythmias, perhaps by enhancing the DR, which may be why β-blocker therapy reduces SCD in patients with heart failure.143,144 In that context, interventions that reduce cardiac sympathetic activity have been shown to protect against arrhythmias, whereas those that enhance sympathetic activity provoke them.145–148

An upsurge in the magnitude of RA has been reported during periods of elevated sympathetic activity in humans and in an end-stage heart failure animal model.151 On the other hand, β-blockers152–155 have been reported to reduce the amplitude of RA. In patients with documented or suspected ventricular tachyarrhythmias who underwent RA testing, acute administration of the β-blockers metoprolol and dl-sotalol reduced overall RA amplitude by 35% and 38%, respectively, indicating that RA can be modulated, at least in some patients, by sympathetic activity. The possible effect of β-blockers on the clinical utility of RA is mediated by at least 2 factors: blunting the chronotropic response to exercise, which may prevent some patients from reaching the specific threshold heart rate to develop RA, and reducing the magnitude of alternans.153

In basic science studies, alternans occurred at significantly longer cycle lengths and the peak alternans level was greater with sympathetic stimulation, compared with baseline. In all hearts, alternans level increased at progressively shorter pacing cycle length until VF occurred, albeit the cycle length at which VF occurred was not altered with sympathetic stimulation. VNS has caused a decrease in alternans level (as a result of a small decrease in the cycle length at which alternans occurred) and a small increase in cycle length at which VF occurred.140

The promising preclinical studies led to several clinical trials testing the efficacy of VNS to treat cardiovascular diseases in patients, which unfortunately showed mixed results. While the ANTHEM-HF (Autonomic Neural Regulation Therapy to Enhance Myocardial Function in Heart Failure) study demonstrated significant improvements in LV function and decreased TWA in patients with heart failure, both the INOVATE-HF (Increase of Vagal Tone in Heart Failure) and NECTAR-HF (Neural Cardiac Therapy for Heart Failure) trials failed to demonstrate significant improvements with VNS therapy.157–159 A major factor for the conflicting results was the difference in stimulation parameters used in the different studies. Ardell et al recently demonstrated that the effects of VNS depend on the balance between the efferent and afferent vagal fiber responses, which, in turn, is dependent on the stimulation parameters used. They proposed the existence of an optimal frequency–amplitude–pulse width–based operating point, also called neural fulcrum, when the efferent and afferent stimulation effects are balanced, producing a null heart rate response. They showed that it is possible to achieve cardiac control and beneficial cardiovascular effects of VNS by appropriate selection of stimulation parameters within the neural fulcrum. While many groups continue to decipher the underlying mechanisms behind the cardiovascular effects of VNS, it continues to be an active area of both preclinical and clinical research, offering a potentially promising nonpharmacological treatment for cardiac arrhythmias and cardiovascular diseases such as heart failure, hypertension, and MI.

Conclusions

While many hypotheses have been proposed to explain the genesis of cardiac alternans, the prevailing one proposes that subcellular disruptions of intracellular Ca²⁺ homeostatic
mechanisms occurring dynamically on a beat-to-beat basis give rise to $[\text{Ca}^{2+}]$, alternans, which, in turn, results in APD and ECG alternans. The manifestation of discordant APD alternans at the whole heart level is associated with increased spatial dispersion of refractoriness, waveform fractionation, and the onset of reentrant VTEs. Thus, this conceptual framework regarding the pathophysiology of cardiac alternans suggests that an RA surge, beyond being linked to medium- and long-term risk of VTEs and SCD, is likely to play a more central role in creating the necessary conditions for short-term arrhythmia susceptibility. The temporal relationship between RA and short-term arrhythmogenesis has significant clinical implications for triggering and guiding upstream antiarrhythmic therapy.

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