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Cardiovascular Research 57 (2003) 599–614

Cardiovascular Research

www.elsevier.com/locate/cardiores

Review

Repolarization alternans: implications for the mechanism and prevention of sudden cardiac death

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Received 6 August 2002; accepted 21 October 2002

Abstract

For nearly 100 years, beat to beat alternation of T wave amplitude, termed T wave alternans (TWA), has been closely linked to electrical instability in the heart. TWA is now established among the strongest markers of susceptibility to sudden cardiac death. Since computer technology allows for detection of very subtle yet clinically significant TWA during standard exercise testing, TWA has been used increasingly as a noninvasive clinical tool for identifying and treating patients at risk for sudden cardiac death. The observation of TWA hastening ventricular tachyarrhythmias in an extraordinary variety of clinical and experimental conditions suggest potential universality of TWA in the pathophysiological mechanism of sudden death. High resolution optical mapping studies have shown that TWA arises from alternans of repolarization at the level of the ventricular myocyte. Cellular alternans is likely due to the actions of one or more ionic currents and is closely related to, if not directly dependent on, the kinetics of intracellular calcium cycling. Impairment in calcium cycling at the cellular and sub-cellular levels has been implicated in the mechanism of cellulcar alternans. Importantly, spatially discordant alternans between cells is most likely a consequence of heterogeneities of electrophysiological properties between cells which span the ventricular wall, serving to amplify spatial heterogeneities of repolarization, and forming a substrate for reentrant excitation. Through this mechanism, TWA is linked directly and mechanistically to the pathogenesis of arrhythmias. Although available data would suggest that TWA is certainly closely related to a mechanism of arrhythmogenesis, and is a strong marker of clinical risk, the precise sequence of events which triggers sudden cardiac death, and the potential role of TWA in this process remains elusive.

Keywords: Arrhythmia (mechanisms); ECG; Sudden death; Ventricular arrhythmias

1. Introduction

Alternans of the electrocardiogram (ECG) is defined as a change in the amplitude and/or morphology of a component of the ECG that occurs on an every-other-beat basis. It was first reported in 1908 by Hering [1] in an experimental animal after parenteral administration of glycolytic acid. Two years later, Sir Thomas Lewis [2] observed alternans in a man during paroxysmal atrial tachycardia, leading him to later hypothesize that ''alternation occurs either when the heart muscle is normal and the heart rate is very fast, or when there is serious cardiac disease and the rate is normal''. It was not until nearly 100 years later that we could appreciate how prophetic these insights were. Based on his astute observations in 1913 of pulsus alternans in patients with advanced cardiomyopathy, Windle [3] concluded that alternans ''always adds to the gravity of the prognosis''. These insights pointed early on to the prognostic implications of alternans, as well as the potential role of contractile proteins in its mechanism.

Alternans has since been reported under a wide variety of clinical situations such as Prinzmetal's angina [4], acute myocardial infarction [5], coronary vasospasm [6], antiar-

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0008-6363/03/$ – see front matter
doi:10.1016/S0008-6363(02)00737-X

rhythmic drug therapy [7,8], HIV cardiomyopathy [9], and long QT syndrome [10]. It has also been observed in such disparate situations as arsenic poisoning [11], chlorpromazine therapy [12], alcoholism [13], and electrolyte imbalance [14]. Importantly, the appearance of alternans under such varied circumstances suggests that arrhythmias due to a variety of cardiac diseases may be related via common mechanisms that are expressed as various forms of ECG alternans.

2. T-wave alternans is a marker of cardiac electrical instability in humans

T-wave alternans (TWA) is a specific form of ECG alternans that is related to repolarization abnormalities. TWA is of particular interest because there is a close, quantitative relationship between the appearance of TWA and electrical instability in the heart. The classic experiments of Schwartz et al. [10] served to focus attention on TWA as a precursor or possible mechanism for ventricular arrhythmias. However, further development of this hypothesis was impaired by lack of methodologies for detecting and quantifying TWA from the surface ECG. In the early 1980s, Adam and co-workers discovered that TWA occurs at levels undetectable by visual observation alone (i.e. on the order of microvolts) [15,16]. They developed the spectral analysis method for detecting microvolt-level TWA in experimental animals, which was later refined for use in humans [17]. This method was used to reaffirm that TWA recorded from experimental animals is closely associated with ventricular fibrillation (VF) [18], which was a necessary and critical step in later establishing TWA as a clinically-useful marker of risk for sudden cardiac death (SCD) in patients.

Early clinical studies revealed that TWA induced by atrial pacing was comparable to electrophysiologic testing with respect to prediction of arrhythmia-free survival (see Fig. 1) [17,19]. Later it was found that elevating heart rate by moderate exercise was sufficient to induce TWA that correlated closely with arrhythmic risk [20–23]. The ability to measure TWA noninvasively is an obvious advantage over electrophysiologic testing, which requires implantation of pacing electrodes and induction of arrhythmias. When compared directly to other noninvasive markers, such as heart rate variability, signal averaged ECG, or reduced ejection fraction, TWA appears to predict arrhythmic events with greater accuracy [24,25], and has been successfully applied to risk-stratified patients following myocardial infarction [26], during electrophysiological testing [17], or during routine follow-up in heart failure clinics [27]. It appears that TWA is a particularly sensitive marker for SCD such that, when used for the purpose of primary prevention of SCD, a negative TWA test is associated with event rates $<5\%$ [26,27]. Positive and negative predictive values of TWA testing is about 50–80% and 75–98%, respectively, depending on the nature of the population being tested [17,28,29]. These encouraging

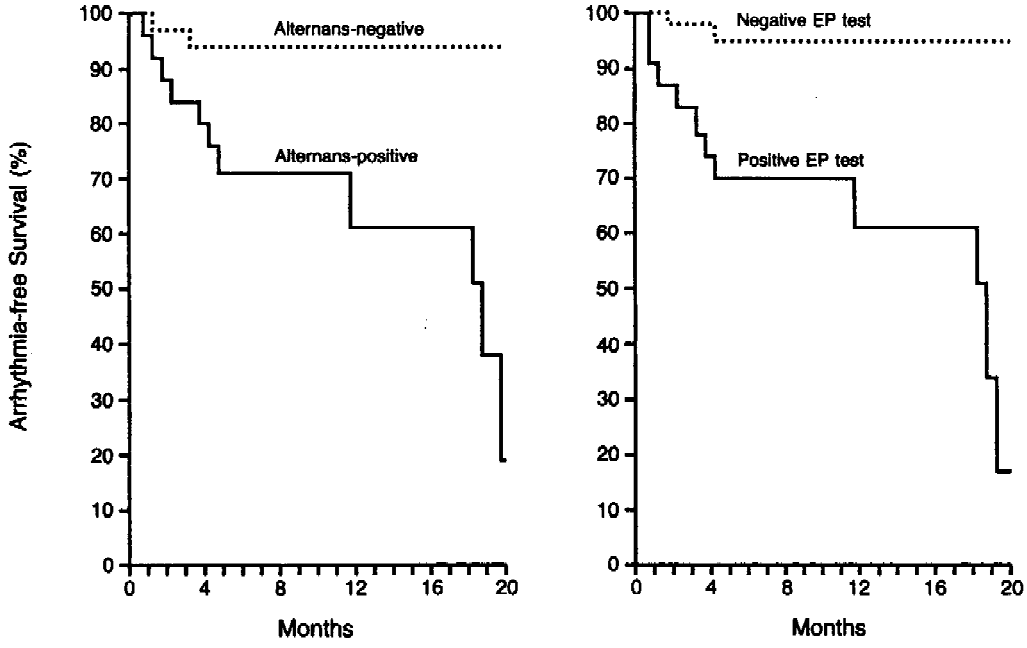

Fig. 1. T-wave alternans and electrophysiological testing as predictors of arrhythmia-free survival. Left panel: arrhythmia-free survival according to Kaplan–Meier life-table analysis is compared in patients ($n=66$) with T-wave alternans (alternans ratio>3.0) or without (alternans ratio≤ 3.0) (note: alternans ratio is the magnitude of alternans divided by noise, normalized to the uncertainty of the measurements). Right panel: arrhythmia-free survival among same patients with positive (ventricular arrhythmias were induced) or negative (ventricular arrhythmias were not induced) electrophysiologic testing results. The predictive value of EP testing and T-wave alternans is essentially the same in these two plots. (Reproduced from Rosenbaum et al. [17]).

results have motivated the recent implementation of the alternans before cardioverter defibrillator (ABCD) trial, which is designed to establish an innovative clinical strategy for primary prevention of sudden death using TWA screening.

3. Electrophysiological basis for electrocardiographic T-wave alternans

Alternans can occur in various components of the electrocardiogram, such as the QRS complex, the ST segment, or the T-wave. QRS alternans is generally associated with gross mechanical abnormalities such as those seen in association with large pericardial effusions, and may also be associated with conduction abnormalities such as alternating bundle branch block. ST segment alternans is associated with acute myocardial ischemia. There can also be alternans of heart rate (RR alternans), such as atrial or ventricular bigeminy and in some forms of supraventricular tachycardia [30]. At present, there is no clear clinical or mechanistic relationship between RR, QRS or ST alternans and ventricular tachyarrhythmias. In contrast, TWA is the most commonly observed form of ECG alternans, and has been implicated in the pathophysiological mechanism and clinical prognosis of SCD.

The underlying electrophysiological basis for TWA was originally thought to be alternating conduction pathways, arising from regional areas of refractoriness that alternated spatially from beat to beat [31]. However, experimentally, this was only demonstrable during acute myocardial ischemia [32], where the mechanism of conduction block is unrelated to repolarization. Additionally, the fact that individual cardiac cells could alternate with respect to action potential duration (APD) strongly suggested that TWA arose from the level of the single cell [33–36]. In 1999, Pastore et al. [37] recorded optical action potentials from 128 sites on the epicardial surface of guinea pig hearts during ventricular pacing. As heart rate was elevated, alternation of repolarization phases of the action potential coincided with alternation of the T-wave of the ECG (Fig. 2). Interestingly, repolarization alternans at the cellular level was several orders of magnitude greater than the corresponding magnitude of TWA, which may explain why even microvolt-level TWA detected from the body surface ECG can be physiologically and clinically important. Recently, alternans of cellular repolarization was also observed in patients with LQTS [38]. In summary, the weight of experimental and clinical evidence suggests that TWA arises from alternation of repolarization at the level of the myocyte. Consequently, the underlying mechanisms of TWA most certainly arise from sarcolemmal ion channels or intracellular calcium handling processes that are responsible for cellular repolarization.

4. Dynamics of T-wave alternans

An important aspect of TWA is its relationship to heart rate. One hypothesis suggests that alternans occurs when heart rate exceeds the capability of certain ionic channels involved in repolarization to fully recover from activation or inactivation based on their time-dependent gating

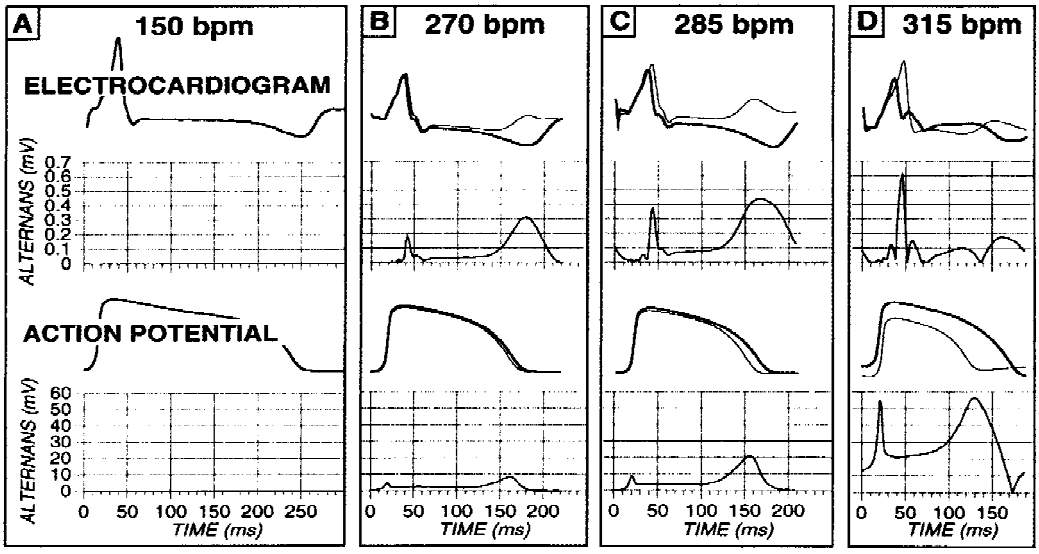

Fig. 2. Changes in transmembrane potential underlying T-wave alternans on surface ECG. ECG lead (top) and representative action potentials (bottom) recorded simultaneously from 1 of 128 mapping sites. Tracings recorded from two consecutive beats are superimposed to illustrate electrical alternans. Magnitude of electrical alternans during each time point of the cardiac cycle is represented by the difference in amplitude of signals recorded on consecutive beats. As stimulus rate was increased to 270 bpm, T-wave alternans in the range of 200–400 μV was distributed symmetrically around the T wave and was explained by beat-to-beat alternation in phases 2 and 3 of the action potential. (Reproduced from Pastore et al. [37]).

kinetics. For example, the first beat of a rate change may occur before key channels have recovered from inactivation on the previous beat, and therefore are not able to contribute fully to the next beat, causing it to have a shortened APD. This results in a longer diastolic interval (DI), which allows channels to fully recover by the next beat, giving rise to a longer APD. The long APD gives rise to a shorter DI, and the cycle of alternation begins again.

Various ionic currents have been implicated in repolarization alternans. Fox et al. [39] have shown in a computer model that reduction in the time constant of the inactivation gating variable for the L-type calcium current (I_{Ca}), an increase in the magnitude of the inward sodium current, I_{Na}, and any modification of the Na^+–Ca^{2+} exchanger current can reduce alternans amplitude. Increasing the amplitudes of the inward rectifier current, I_{K1}, and the two components of the delayed rectifier current, I_{Kr} and I_{Ks}, suppresses alternans by reducing APD and prolonging DI. The relative roles of I_{Kr} and I_{Ks} in determining APD and its rate-dependence have been highlighted in computer studies by Viswanathan et al. [40] Nevertheless, it is still not clear exactly which specific ion channels are involved in alternans, or whether such channels represent a primary or secondary mechanism for alternans.

Despite not having a clear understanding of ionic mechanisms, the relationship between alternans and heart rate is well-established. Alternans can be induced both experimentally and clinically by pacing or elevation of heart rate with exercise [20,41] and, furthermore, the heart rate at which significant alternans appears in patients is the prime determinant of the degree of risk [17,42]. Whereas normal patients will often exhibit TWA at heart rates exceeding 120 bpm, patients at risk for SCD exhibit TWA at heart rates under 110 bpm [42]. This shift in the alternans–heart rate relationship towards lower heart rate thresholds no doubt represents an important clue to the mechanism of alternans-related arrhythmogenesis.

4.1. Restitution

The alternans–heart rate relationship may be closely related to APD restitution, which describes the duration of an action potential relative to its degree of prematurity (S1–S2 interval) or the DI which preceded it. Restitution has been attributed to the action of time-dependent repolarization currents that, with progressive shortening of DI, fail to fully reactivate (inward current) or deactivate (outward current), generating progressively shorter action potential durations. Restitution can be thought of in terms of a direct relationship between the APD of a given beat, and the DI of the beat that preceded it. In other words, when DI is short, the APD that follows is also short. This is similar to the pattern of alternans: during alternans, short DIs lead to short APDs, which in turn result in longer DIs, and longer APDs on the next beat, and so on.

The kinetics of restitution are described by the 'restitution curve', a plot of APD vs. DI. It has been suggested that when the slope of the restitution curve is greater than or equal to one, stable alternans can occur [39,43,44]. This is known as the 'restitution hypothesis' for alternans. In simple terms, when the slope is steep, the change in DI from beat to beat is matched by a change in APD of similar magnitude, which allows stable alternation between two APDs while maintaining a constant BCL (Fig. 3A). However, when the slope is shallow, large changes in DI result in only small changes in APD. Such fluctuations are not stable and 'dampen down' to a nonalternating state (Fig. 3B). Experimentally, the slope of the restitution curve can be flattened by changing the kinetics of one or more membrane currents. This has been shown to prevent the development of cellular alternans [45], spiral wave breakup [46], and VF initiation [46–48].

Studies exploring the relationship between restitution and alternans must be interpreted cautiously. Although flattening of the restitution curve could be a valuable strategy for drug development [47], this focus lacks an appreciation of the fundamental cellular and subcellular processes governing restitution, which may differ from species to species [49,50] and amongst cell types [51,52]. In other words, restitution is a phenomenological process representing a number of cellular mechanisms and numerous physiological processes, and its relationship to alternans may be difficult to interpret in the absence of such specific information. Moreover, such studies also cannot explain why alternans is readily observed during ischemia, when the slope of the restitution curve is flat. Furthermore, there are varied approaches to measuring restitution [53] and it remains unclear how restitution relates to transient versus steady-state alternans. Some investigators have suggested that steady-state alternans may be better described by the kinetics of APD adaptation, which are dependent upon both the restitution curve and the steady-state relationship between BCL and APD [54]. This is important because clinical studies clearly demonstrate that, in patients at risk for SCD, arrhythmogenic alternans is not a transient occurrence, but is persistent at constant heart rates above threshold.

4.2. Alternans memory

Another aspect of the relationship between alternans and heart rate is the 'alternans memory' effect. Narayan and Smith [55] reported that microvolt-level TWA, once induced by atrial pacing in patients, persisted even when pacing rate was subsequently decreased. This effect had been previously suggested by computer modeling studies of repolarization [56]. The implications of alternans memory are important, not just for further elucidating the mechanism of repolarization alternans itself, but also with respect to the clinical aspects of TWA-related risk assessment.

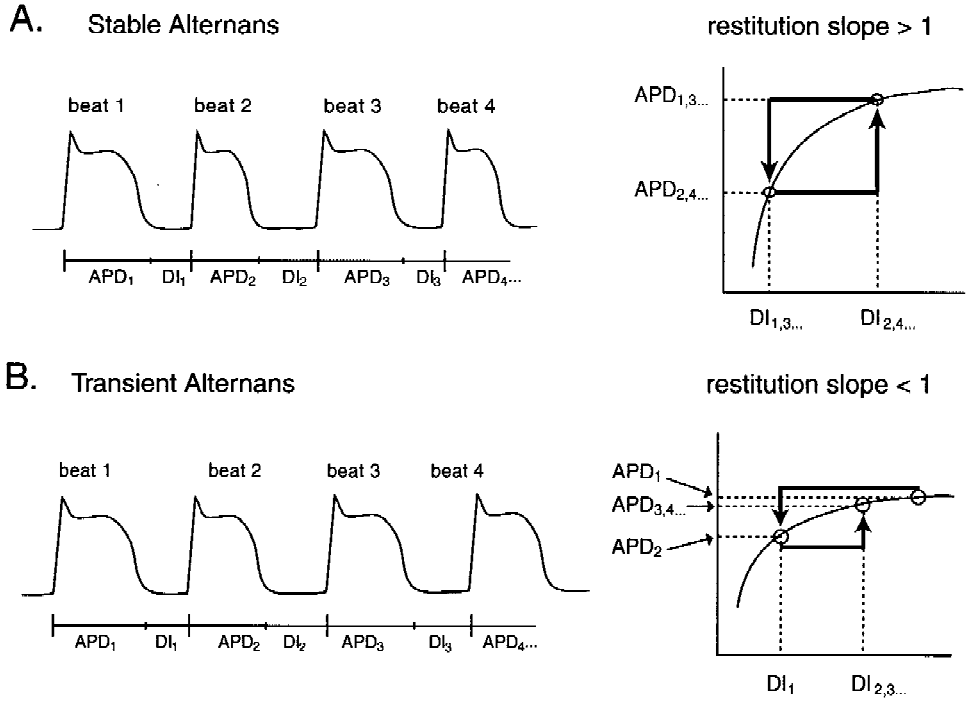

Fig. 3. Restitution hypothesis for mechanism of T-wave alternans. Restitution hypothesis states that stable alternans can develop when slope of the restitution curve is >1. (A) Stable alternans occurs when there is a balance between changes in DI and changes in APD. APD and DI fluctuate between two constant points on the curve, maintaining stable alternation where the APD of odd beats are all the same, and APD of even beats are all the same. (B) When slope of the restitution curve is <1, changes in DI are not matched by equivalent changes in APD and stable alternans is not maintained. Instead, alternans is transient and dampens down to a nonalternating state. DI is short on beat 1, causing APD to be shorter on beat 2, and DI becomes longer. However, the APD that results is not much different from the previous one, due to the flatness of the restitution curve. Subsequent changes in DI and APD are negligible, so both APD and DI stabilize to one point on the curve (i.e. no alternans).

Recently, we reported that 'alternans memory' applies to APD alternans in whole hearts and at the level of the single cell [57]. Action potentials were mapped from the epicardial surface of Langendorff-perfused guinea pig hearts when pacing rate was accelerated and decelerated in stepwise fashion. Following development of alternans at increased pacing rates, alternans persisted even when rate was subsequently decreased below the threshold at which it was induced. As a result, the alternans threshold was shifted to lower heart rates during deceleration relative to acceleration. We also observed alternans memory in isolated guinea pig myocytes, demonstrating that the mechanism has a cellular origin. Presumably, the mechanisms causing alternans to persist are related to those mechanisms which cause cells to alternate in the first place, so the observation of alternans memory in single cells lends support to a cellular mechanism for repolarization alternans. Alternans memory studies illustrate that the dynamics of heart rate, and not just absolute rate, are important factors in the mechanism of alternans. Furthermore, these results suggest that, under clinical conditions, TWA could persist following a period of tachycardia, providing a possible mechanism for SCD occurring at relatively normal heart rates.

5. Cellular and subcellular mechanisms

From the above discussions it is clear that TWA arises from alternans at the level of the single cell. Alternans of membrane repolarization may arise from primary alternations in the activity of sarcolemmal ion channels, or from secondary alternations of ion channels in response to alternations in intracellular calcium cycling. Clearly, these mechanisms are not mutually exclusive. Additional evidence supporting the notion that TWA arises from the level of the single cell includes: (1) alternation of membrane potential may be provoked by delivery of a critically coupled premature beat [58], (2) alternation of membrane potential is provoked experimentally with a regular pacing rate above a threshold value [37], and (3) pharmacological probes that affect L-type calcium currents are able to simultaneously affect APD and mechanical alternans [51,52,59]. It is important to determine the underlying cellular mechanisms for alternans because, presumably, an abnormality in one of these processes can contribute to the pathophysiology of SCD.

There is strong evidence that intracellular calcium (Ca) cycling plays a key role in the mechanism of TWA. It has been known for several decades that alternans of APD is

associated with alternans of tension (i.e. mechanical alternans), and alternans is inhibited or suppressed by verapamil [59,60], caffeine [51,59], BayK8644 [52], nisoldipine [52], and ryanodine [51], all of which point to a mechanism related to cellular Ca cycling. Rubenstein and Lipsius [58] showed that reversal of the phase of electrical alternans (i.e. from long–short–long to short–long–short) was dependent on recovery of mechanical activity, not electrical activity. Changes in contraction during alternans are paralleled by changes in the underlying Ca transient [61,62], and the technology now exists to perform simultaneous recording of Ca transients and action potentials. It has since been demonstrated in isolated rabbit [63] and cat ventricular myocytes [64] that Ca transient alternans can occur during rapid pacing when membrane potential alternans is prevented by voltage-clamping, strongly suggesting that Ca transient alternans is the underlying trigger for voltage alternans, and not vice versa (see Fig. 4). Recently, we found that when voltage-clamped cells are given pulses of alternating duration, Ca alternans is not induced except at rates equal to or above the threshold for Ca alternans in unclamped cells (unpublished data), further suggesting that the rate-limiting factor for alternans is rate-induced alternation of intracellular Ca handling. The correlation between spatial heterogeneity of Ca transients and APD dispersion during T-wave alternans also supports this theory [65].

Before discussing the specific role of intracellular Ca handling in the mechanism of alternans, a review of the key processes involved in the uptake, buffering, and release of intracellular Ca may be helpful. The Ca cycle is illustrated in Fig. 5. Briefly, activation of I_{Ca} by a depolarizing wavefront causes free Ca to cross the sarcolemmal membrane in close proximity to the T-tubules. This cytosolic Ca binds to the ryanodine receptors (Ryr) of the sarcoplasmic reticulum (SR) which, in turn, release abundant SR Ca into the cytosol via the Ca-induced Ca-release mechanism [66]. When $I_{Ca}$is blocked, or during prolonged depolarization of membrane voltage, SR Ca release can also be stimulated by inward Ca current from the Na–Ca exchanger (NCX) operating in 'reverse mode'. After each contraction, the majority of cytosolic Ca is sequestered back into the SR by SR-Ca-ATPase (SERCA) and, to a lesser extent, by NCX operating in 'forward mode' [67]. The Ca cycle is completed when SR Ca is translocated to the junctional SR prior to its release by Ryr on the subsequent beat.

In order to maintain homeostasis, the amount of Ca released from Ryr during each heart beat must be fully reclaimed from the cytoplasm. Therefore, impairment in the kinetics of any component of cellular Ca cycling (Ryr, NCX, SERCA, translocation) can create a situation where cytoplasmic Ca cannot be fully reclaimed during each beat (as during normal homeostasis) but only on alternating beats (i.e. steady state alternans). For example, an elegant study by Huser et al. [64] suggests that alternation of Ca-induced Ca release (i.e. Ryr kinetics) may be responsible for alternans. They found that amplitude of I_{Ca} did not alternate during mechanical alternans (although they did note a slight alternation in inactivation time), nor did they find any beat-to-beat differences in SR Ca load, but they were able to induce alternans by metabolic inhibition, which deprives the cell of ATP and affects glycolytic enzymes closely associated with the Ryr channel. Other experiments suggest that a delay between the reuptake of calcium into the SR and its release may explain mechanical alternans [61,62].

Despite strong evidence pointing to a role for Ca cycling in the mechanism of alternans, the potential interdependence of membrane voltage and intracellular Ca cycling presents a difficult chicken and egg problem that remains unresolved. At least two hypotheses can be considered: (1) alternans of intracellular Ca cycling causes alternans of membrane voltage via some Ca-dependent electrogenic sarcolemmal current which, in turn, results in TWA; or (2) alternation of membrane voltage is a primary cause of TWA and causes alternation of intracellular Ca cycling as a secondary effect. There is also the possibility that alternans of voltage and Ca cycling are independent with no causal relationship or, conversely, that both result from some other cellular mechanism.

If Ca transient alternans represents the primary mechanism for cellular alternans (hypothesis 1, above), what is the electrogenic mechanism that translates alternation of Ca handling into alternation of membrane repolarization? Mechanical activity is capable of affecting membrane voltage via feedback mechanisms inherent to the Ca cycling process, and there are at least two important electrogenic feedback mechanisms that may be involved in alternans: (1) SR Ca release tends to inactivate I_{Ca}, hence lowering membrane voltage and shortening APD (Fig. 5: $\ominus$ symbol), and (2) SR Ca release enhances Ca extrusion by NCX, resulting in an increase in membrane voltage and APD lengthening (Fig. 5: $\oplus$ symbol). The nature of the association between electrical and mechanical alternans (electromechanical alternans, EMA) may yield some insight as to which of these mechanisms predominates. Long APDs associated with weak developed tension (i.e. discordant EMA) have been reported in rabbit papillary muscle [68], ferret ventricular muscles [62], and canine myocardium [69]. Huser et al [64] also observed discordant EMA in isolated cat myocytes at room temperature where action potentials and Ca transients were measured simultaneously. They reported that during large amplitude Ca transients, inactivation of I_{Ca} was slightly faster, causing APD to shorten. Hirayama et al. [59] noted that while caffeine suppressed both repolarization and mechanical alternans, verapamil (which blocks I_{Ca}) suppressed only repolarization alternans. Discordant EMA therefore points to I_{Ca} as an electrogenic mechanism during alternans.

On the other hand, concordant EMA has also been

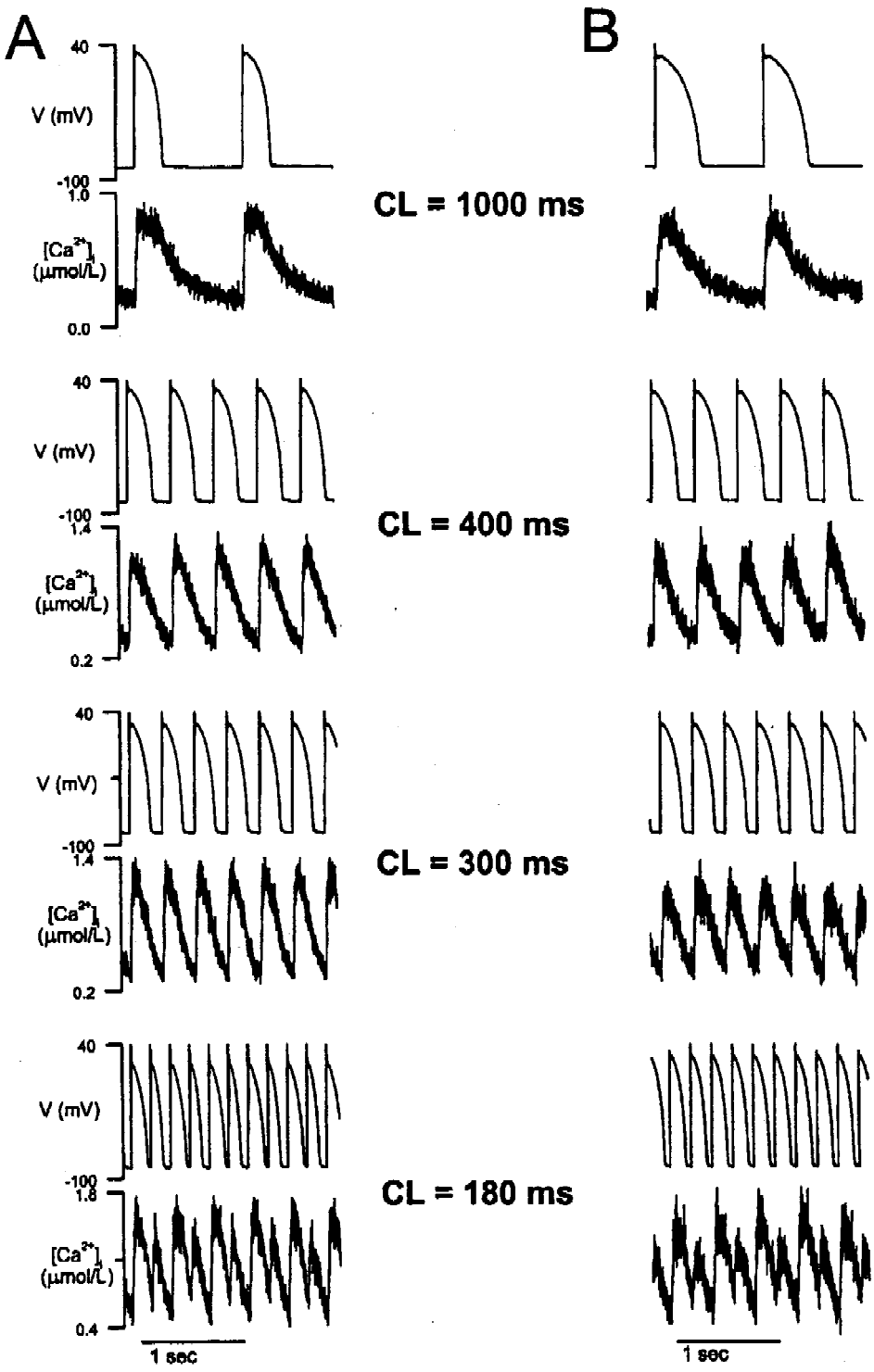

Fig. 4. Changes in the action potential and intracellular calcium transient during rapid pacing in isolated rabbit ventricular myocytes. (A) Membrane potential (V) and intracellular calcium transient during rapid pacing at cycle lengths (CL) indicated. (B) Same as (A) except the myocyte is paced with an action potential clamp. Note that alternation in the calcium transient still occurs at CL = 180 ms, despite a fixed APD, consistent with a primary role for Ca in the mechanism of alternans. (Reproduced from Chudin et al. [63]).

observed experimentally. In isolated dog myocytes long APs are associated with stronger developed tension and short APs with weaker tension [52]. Rubenstein and Lipsius [58] also observed electromechanical concordance in cat ventricular myocytes at 35 °C. Concordant EMA suggests that NCX might serve as an important electrogenic mechanism, as described above. The question of whether EMA is concordant or discordant, and what this

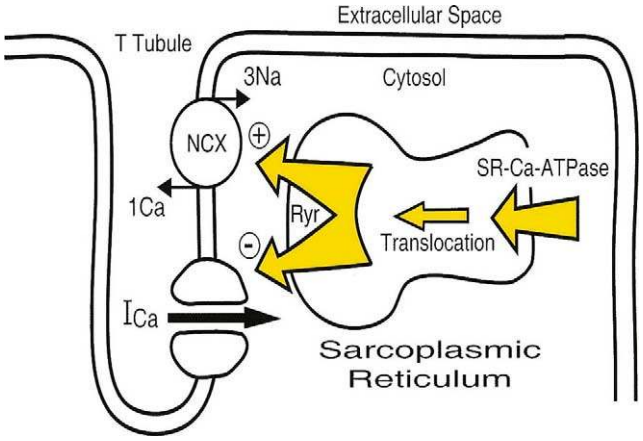

Fig. 5. Schematic representation of intracellular calcium cycling. With each heartbeat, calcium flows into the cell via L-type calcium channels (I_{Ca}), triggering release of calcium from the sarcoplasmic reticulum (SR) via ryanodine-sensitive release channels (Ryr). Release of calcium via Ryr promotes inactivation of I_{Ca}. Before the next heartbeat, the cell must eliminate this extra cytosolic calcium by either extrusion from the cell via the sarcolemmal sodium–calcium exchanger (NCX), which is stimulated by the release of calcium via Ryr, or by reuptake into the SR via the SR-calcium-ATPase channel (SERCA) in the SR membrane. Calcium is then translocated through the SR to be available for release upon the next beat. Cellular alternans can occur when heart rate exceeds the capability of the cell to cycle calcium.

implies regarding underlying electrogenic mechanisms is complicated by the fact that in the experiments above, alternans is produced under a variety of conditions such as rapid pacing, hypothermia, metabolic inhibition, or ischemia, which may have different underlying mechanisms.

While there are compelling data suggesting that alternation of Ca controls alternation of membrane voltage, the possibility that voltage causes Ca transient alternans remains a consideration. Computer simulation studies suggest that specific sodium and potassium currents appear to control the initial and terminal parts of the APD restitution curve, respectively, which is consistent with experimental findings in cardiac tissue [45,70,71]. However, there is also experimental evidence for a role of Ca handling in action potential restitution kinetics. L-type Ca channel blockers flatten APD restitution curves and prevent APD alternans, VF induction, and convert VF to a periodic rhythm (see above). I_{Ca} appears to control the intermediate, steep portion of the restitution curve [39], since any reduction in I_{Ca} increases the alternans heart rate threshold or abolishes APD alternans, while at the same time flattening the restitution curve and shortening APD for any given DI.

To summarize the above, it has become increasingly apparent that alternans of intracellular Ca cycling is an important, if not primary, mechanism for cellular alternans. Various processes involved in maintaining intracellular Ca homeostasis may be subject to time-dependent alternation in their activation or inactivation kinetics, and there exist plausible electrogenic mechanisms for translating such fluctuations into alternans of membrane voltage. At this point, evidence favors a primary role for Ca cycling (i.e. that Ca cycling alternans causes voltage alternans) as the principle mechanism underlying TWA. The role of Ca handling in TWA is particularly interesting from a clinical perspective, since many cardiac diseases are associated with abnormalities of intracellular Ca handling. This may provide a basis for the apparently wide applicability of TWA testing as a measure of risk for SCD.

6. Discordant alternans: a novel mechanism linking TWA to cardiac arrhythmogenesis

APD alternans does not occur uniformly throughout the heart. Just as APD and restitution are distributed as gradients across the epicardium, from apex to base and epicardium to endocardium, so are the magnitude and phase of alternans at a given heart rate [37,59,72,73]. Action potentials at the ventricular base may alternate in a long–short–long fashion, while action potentials at the apex alternate simultaneously in a short–long–short pattern. This is referred to as spatially discordant alternans between cells, and should not be confused with discordant electromechanical alternans, which refers to the phase relation between the alternating calcium transient and membrane repolarization in the same cell.

Until recently, the technology available for mapping action potentials from multiple sites on the heart was limited to arrays of electrograms and monophasic action potential electrodes. The introduction of optical mapping allowed for simultaneous recordings of action potentials from hundreds of sites with enough spatial, temporal, and voltage resolution to measure depolarization, repolarization, magnitude and phase of alternans, and to follow wavefronts as they fractionate and initiate arrhythmias [74,75]. We used optical mapping to study discordant alternans in a guinea pig model of pacing-induced TWA [37,76] In this model, TWA can be reproducibly elicited by constant-rate pacing. The TWA thus produced shares many characteristics of TWA in patients because: (1) it occurs at the microvolt level, (2) it is not evenly distributed over the T-wave but instead is largest in amplitude near the T-wave peak, (3) it does not substantially involve either the QRS complex or ST segment, (4) it is dependent on heart rate, (5) it is closely associated with the onset of VF and (6) this model does not require ischemia to induce alternans (arrhythmia risk associated with TWA is, in most instances, unrelated to ischemia).

Using this model, we demonstrated that discordant alternans occurs by increasing stimulation rates and is distributed spatially across the surface of the epicardium in a predictable and reproducible fashion [37]. Cells at the apex alternate with opposite phase to those at the base. Regions of opposite phase are separated by one or more zones of ‘zero alternans’, also referred to as ‘nodes’, which

tend to be oriented in a base-to-apex direction, following underlying gradients of APD (Fig. 6). As one moves away from the node, toward either the base or apex, alternans magnitude increases.

Discordant alternans is a critical step in the process of alternans-induced arrhythmogenesis. Using the guinea pig model of TWA, we recently demonstrated that discordant alternans is key to the mechanistic link between TWA and arrhythmogenesis [37]. Under normal conditions, gradients of repolarization across the epicardial surface of the guinea pig heart average 3–4 ms/mm [77]. However, during discordant alternans, gradients of repolarization can exceed 10 ms/mm [76] as action potentials are preferentially prolonged at the apex relative to the base. Furthermore, these gradients reverse direction on opposite beats, such that when APD is short at the apex it is prolonged at the base and then vice versa on the next beat (Fig. 7A). This creates significant spatial and temporal dispersion of repolarization (Fig. 7B) that is sufficient to create conduction block and reentry leading to VF (Fig. 8). The presence of discordant cellular alternans was an absolute requirement for the development of reentrant ventricular arrhythmias in this experimental model, suggesting potentially why cellular alternans may be a common precursor of arrhythmias in many clinical situations. Therefore, discordant alternans serves to transform relatively minor

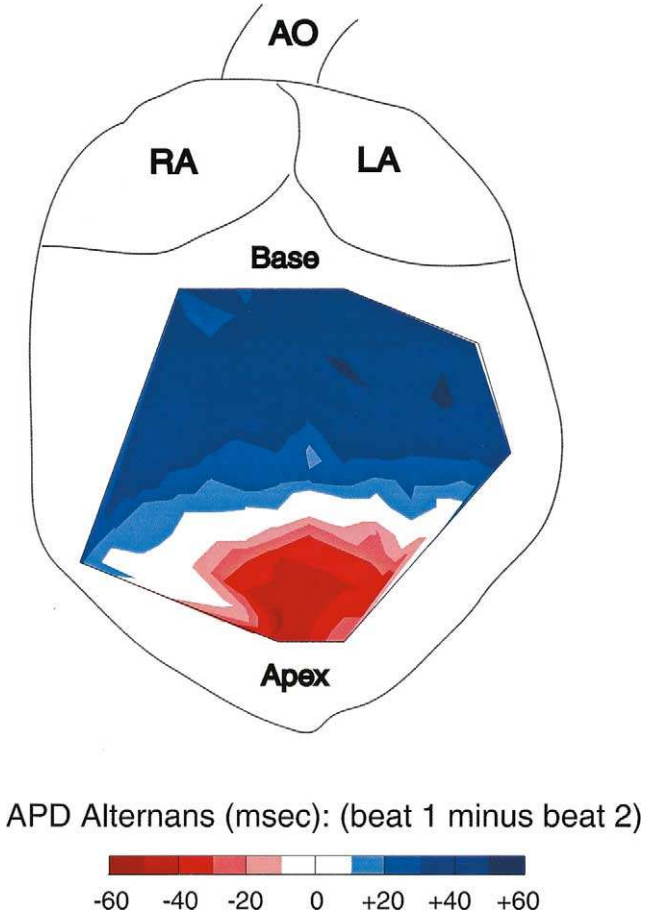

Fig. 6. Contour map of discordant electrical alternans. Map shows recording area superimposed on a schematic diagram of the epicardial surface of a Langendorff-perfused guinea pig heart, paced at 300 bpm. AO, aorta; RA, right atrium; LA, left atrium. Ventricular apex and base are also labeled. Optical action potentials were recorded from 256 sites within the recording area. Alternans was calculated as APD of beat 1 minus APD of beat 2 for two consecutive beats. Long–short sequences are shown in blue (positive values) and short–long sequences are shown in red (negative values). Areas where there is no significant alternans (alternans > 10 ms) are shown in white ('node' of zero alternans). Note that the base and apex regions are alternating simultaneously, yet out of phase with one another (i.e. discordant alternans).

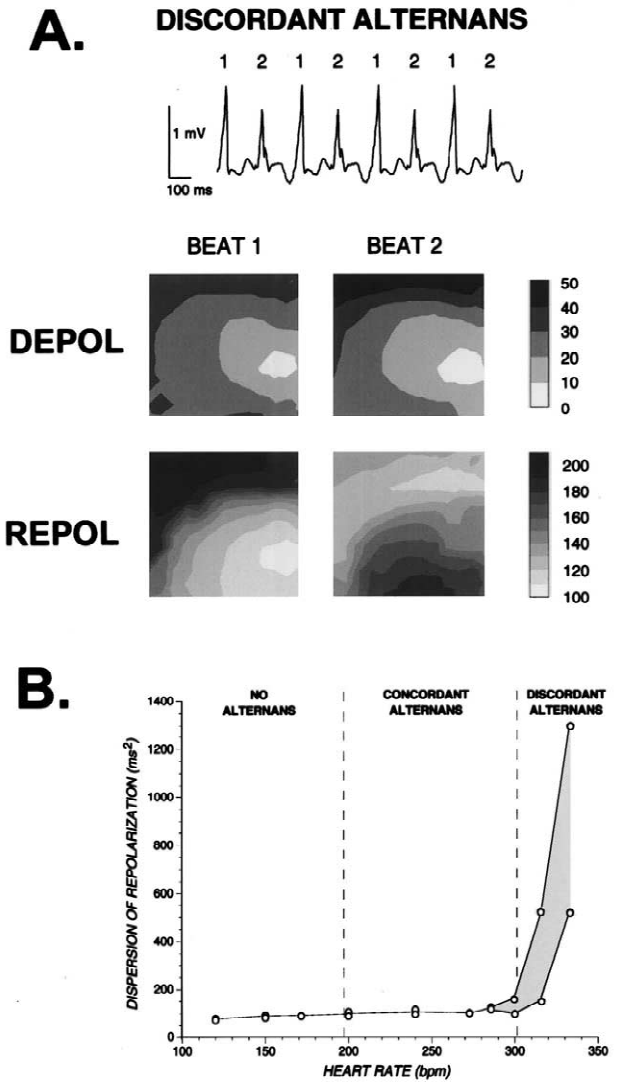

Fig. 7. Patterns of ventricular repolarization and depolarization during discordant alternans. Optical action potentials were recorded from 128 sites on the epicardial surface of a Langendorff-perfused guinea pig heart. (A). Shown are 10 ms isochrone plots representing depolarization (Depol) and repolarization (Repol) within the mapping array for two consecutive beats during discordant alternans at a pacing cycle length of 180 ms. The corresponding ECG recording is shown above indicating visible alternation of the T-wave. (B) Representative experiment demonstrating marked effect of discordant alternans on spatial dispersion of repolarization. (Reproduced from Pastore et al. [37]).

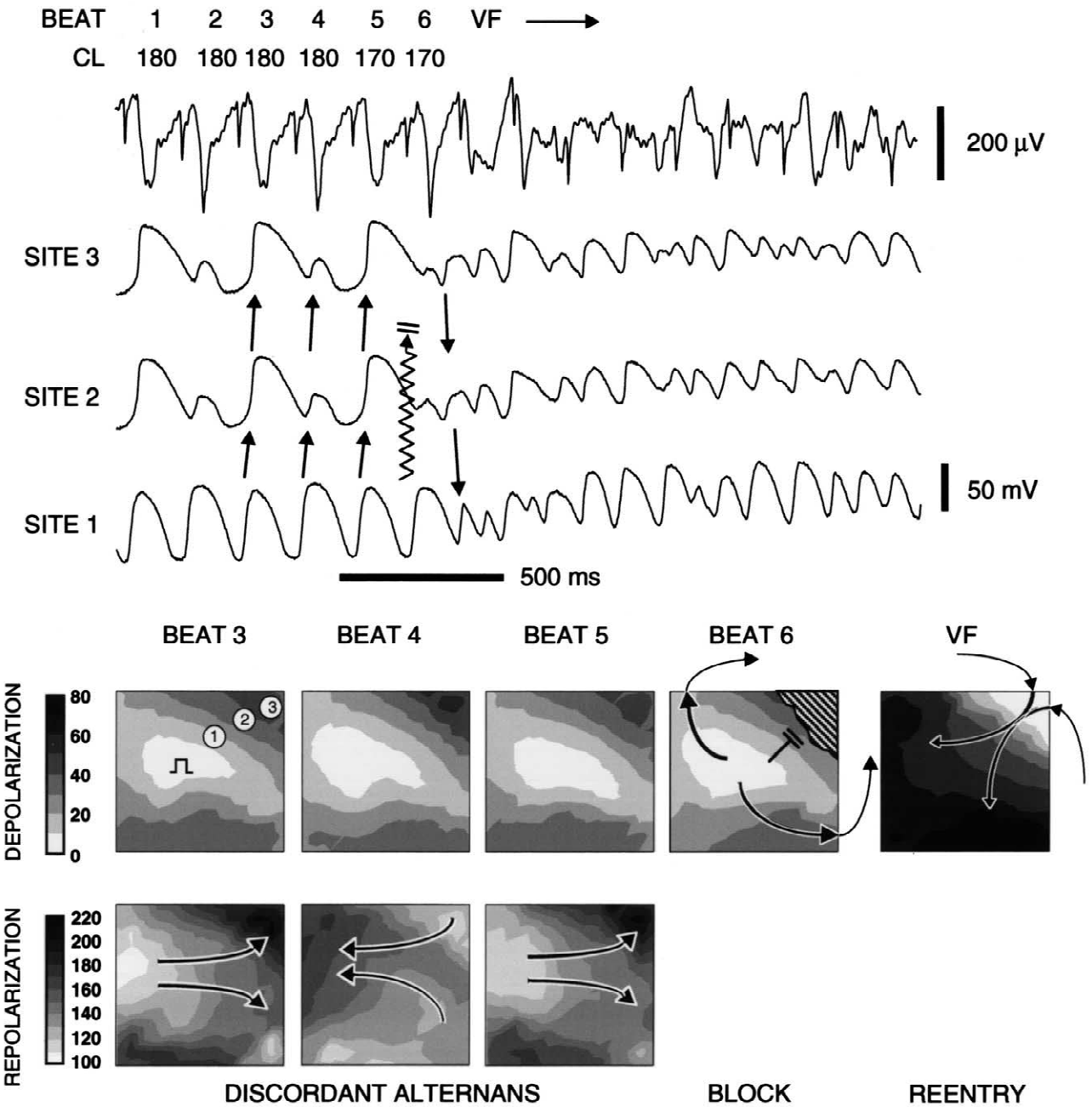

Fig. 8. Mechanism of initiation of VF during discordant alternans. Shown are 10-ms isochrone plots of depolarization and repolarization for beats that immediately preceded VF. (Top) ECG and action potentials recorded from 3 ventricular sites marked on isochrone map below (beat 3). Depolarization and repolarization times are referenced to stimulus artifact during pacing and to earliest activation time during the first beat of VF. On beats 1–5, the depolarizing wavefront propagated uniformly from site of stimulation. However, patterns of repolarization differed substantially but reproducibly on alternating beats (compare beats 3 and 5). Pacing cycle length was decreased by 10 ms during beat 5. During beat 6, block occurred, as represented by hatched area in depolarization map. Block is shown in top panel by failure of propagation from site 1 to site 3. After block occurred, pattern of depolarization reversed from (site 1→ site 2→ site 3) to (site 3→site 2→site 1), indicating first reentrant beat that led to VF. First beat of VF occurred 120 ms after pacing artifact of beat 6. (Reproduced from Pastore et al. [37]).

physiological heterogeneities of repolarization between cells into pathophysiological heterogeneities which dynamically form a substrate for unidirectional block and reentry. Discordant alternans is a mechanism for amplifying underlying heterogeneities of repolarization which, in the absence of discordance, are not arrhythmogenic [37].

These studies also served to illustrate how discordant alternans can potentially lead to a variety of experimental ventricular arrhythmias which have been observed in association with TWA in patients. For example, when discordant alternans is produced in the presence of structural discontinuities in the ventricle produced by artificial

barriers, a substrate for unidirectional block develops but, in this case, monomorphic reentrant VT develops rather than VF, as the discontinuity forms an anchor around which a stable reentrant rotor can form [76]. It is, therefore, intriguing to speculate that discordant alternans is a common mechanism for generating substrates for conduction block, but that the specific arrhythmia that ensues is dependent on the presence or absence of structural abnormalities within myocardium. These experimental studies parallel the clinical observation that TWA occurring in patients with structurally normal hearts (e.g. LQTS) versus structurally abnormal hearts (e.g. scar associated with healed MI) most often leads to polymorphic (e.g. Torsades de Points) and monomorphic VT, respectively.

From the aforementioned discussion, it is apparent that it is critically essential to develop a clear understanding of the mechanisms responsible for generating discordant alternans between cells. It is well established that repolarization properties, such as the restitution relationship or the kinetics and densities of various potassium channels, vary between cell types both across the surface of the epicardium [78] and transmural wall [79]. Experimental data suggest that discordant alternans does not occur randomly across the heart, but instead arises predictably from underlying heterogeneities of repolarization between cells. Myocytes that exhibit different repolarization properties, tend to alternate with opposite phase [37]. Evidence for a primary role of repolarization heterogeneities in the mechanism of discordant alternans is as follows: (1) patterns of discordant alternans appear to orient themselves along the same gradients as APD and restitution [37], (2) patterns of discordance are independent of pacing site in intact hearts [37], and (3) when a precise structural barrier is created parallel to gradients of repolarization and restitution in intact guinea pig hearts, the threshold heart rate for discordant alternans is significantly decreased [76].

However, some computer modeling studies have suggested that heterogeneity of repolarization may not be a requirement for discordant alternans. Logically, any intervention which creates a significant gradient of DI across a sheet or cable has the potential to generate discordant alternans, based on the principles of restitution. This is because, with a sufficient gradient of DI, action potentials following short DIs will be significantly shorter than action potentials following long DIs, and the situation will reverse on the next beat. Thus, for example, conduction velocity (CV) restitution (i.e. slowing of CV at progressively shorter DI) can create sufficient gradients of DI for discordant alternans to develop, even in otherwise electrically homogeneous tissue [45,80,80]. The importance of CV restitution and CV alternans in the mechanism of discordant alternans has been examined under various conditions. In the guinea pig model of TWA, no appreciable CV alternans was measured at heart rates where APD alternans occurred [37]. Furthermore, propagation alternans was not present in the absence of repolarization alternans: i.e., the latter always preceded the former. On the other hand, studies in a canine model show CV alternating at heart rates where VF can be induced, suggesting that both CV and APD restitution may be important in alternans-mediated arrhythmogenesis [81]. It is possible that under conditions of slowed conduction, such as ischemia, CV restitution may play a primary role.

Finally, another important mechanism for discordant alternans is intercellular uncoupling, as is seen a variety of forms of heart disease. Uncoupling can result from disruption of the extracellular matrix by collagen deposition, in addition to reduced expression and remodeling of cardiac gap junction proteins [82]. Intercellular uncoupling increases the propensity for discordant alternans because neighboring regions of cells can more easily express their underlying differences in ion channel composition when they are not under each others' electrotonic influence. This principal was established in the guinea pig model of pacing-induced TWA by using a structural barrier to insulate two neighboring regions of ventricle which exhibit different intrinsic repolarization properties [76]. Without changing the properties of the myocytes, but by severing electrical communication between them, we found substantial reduction in the heart rate thresholds required to induce discordant alternans. Interestingly, when the insulating barrier was introduced in a perpendicular orientation (i.e. along, but not separating, regions having different repolarization properties) the threshold heart rate for discordant alternans was not reduced. Taken together, these data suggest an important principal that may underlie the formation of complex electrophysiological substrates seen in heart disease: i.e. structural barriers are particularly arrhythmogenic when they form between myocardial zones possessing intrinsically different electrophysiological properties (e.g. apex to base, epicardium to M cell).

7. Role of the autonomic nervous system in alternans

Although alternans can be readily induced in isolated heart experiments where the sympathetic nervous system is absent, it may play an important regulatory role in the intact heart. In intact dogs, mechanical alternans induced by rapid pacing was abolished by epinephrine, and this effect was reversed by propranolol [83]. A reflex increase in sympathetic tone caused by decreasing carotid sinus pressure in intact dogs prevent (keep) mechanical alternans induced by rapid pacing [84]. In another dog experiment, stimulation of left stellate ganglia caused a decrease in the heart rate threshold for electrical alternans (measured on an ECG) in a rate-dependent manner which was inhibited by timolol [85]. The mechanism whereby β-stimulation protects against alternans in the above experiments may have a cellular origin. In isolated feline cardiomyocytes, isoproterenol (a β-adrenergic agonist) caused reversible inhibition of both APD and mechanical alternans [64].

β-Adrenergic stimulation enhances cardiac inotropy and affects both cellular calcium regulation and excitation–contraction coupling through a number of different mechanisms resulting in an increase in calcium current and enhanced SR calcium uptake [86]. It is therefore possible that β-adrenergic stimulation, by enhancing the activity of calcium cycling proteins, allows calcium homeostasis to be maintained under conditions that normally trigger alternans.

In contrast with the above findings, there are numerous studies showing that sympathetic activity can, in fact, promote alternans. Schwartz and Malliani [10] noted that a fright response caused visible TWA to appear in a child with hereditary long-QT syndrome, and subsequently evoked TWA in anaesthetized cats using left stellate ganglion stimulation. Bilateral ablation of left stellate ganglia in anesthetized dogs abolished coronary artery occlusion-induced TWA, and this effect was reversed by left stellate ganglion stimulation [18].

Clinical studies also yield conflicting results. The fact that the standard TWA test involves exercise-induced elevation of heart rate suggests that sympathetic stimulation may enhance alternans. While Hohnloser et al. [41] found that both exercise and rapid atrial pacing produced TWA that correlated with arrhythmic risk, the magnitude of TWA was greater during exercise than during pacing. Rashba et al. [87] found that the β-blocker esmolol reduced TWA in patients with coronary artery disease, left ventricular dysfunction and inducible sustained VT. To settle the issue of heart rate versus sympathetic activation, something not addressed in many of the above studies, Kaufman et al. [88] specifically looked at the effects of heart rate versus β-adrenergic stimulation in patients referred for electrophysiological testing. They found that heart rate, and not sympathetic activation, was responsible for TWA measured during exercise testing.

It may be possible to resolve these conflicting results if one examines the nature of the patient populations studied. Subjects in the Kaufman and Hohnloser studies had congestive heart failure and left ventricular dysfunction, suggesting high levels of sympathetic tone at baseline. Also, Rashba et al. [87] looked specifically at TWA in patients having documented ischemic cardiomyopathy and ejection fractions $\leq$40%. All of these patients are expected to have heightened baseline sympathetic tone. Therefore, the ability to further enhance sympathetic tone pharmacologically may be limited, resulting in negligible effects of exogenously administered β-stimulants on TWA. In contrast, administration of β-blockers may greatly diminish β-adrenergic tone and hence make it possible to inhibit or attenuate TWA. In summary, available data would suggest that β-adrenergic stimulation tends to inhibit repolarization alternans in isolated myocytes but, paradoxically, can potentially enhance TWA in vivo. It has been suggested that β-adrenergic activation and increasing heart rate might worsen ischemia by increasing the demand on energy consumption and increasing the likelihood of alternans [89]. In this case, β-blockade might have a protective effect. Such an effect might be mediated by β-blockade induced reduction in intracellular cAMP production, which would be expected to decrease influx of calcium through I_{Ca} [90]. This would allow the cell to maintain calcium homeostasis under conditions that would normally tax the kinetics of calcium cycling, because the cell has less calcium to deal with on each beat.

β-Blockers are known to reduce mortality and prevent SCD in patients with various cardiac diseases such as ischemic and nonischemic cardiomyopathy. This is consistent with an inhibitory role for β-blockade in the regulation of TWA, particularly as evidence mounts for a mechanistic link between TWA and risk of SCD in a variety of patient populations. Further research is warranted before this matter can be resolved completely, but it is certainly a subject that might have important clinical and therapeutic implications.

8. T-wave alternans and ischemia

A review of the literature regarding TWA shows that there is a tendency to combine conclusions from studies where alternans was induced by different means, with the assumption that they all relate to a common mechanism. However, there is significant evidence that alternans induced in the presence and absence of ischemia have substantially different mechanisms.

During ischemia, the appearance of alternans is transient and is often associated with decreased contractility [91,92]. Alternans can occur in the AP upstroke, repolarization, action potential amplitude, and/or magnitude of hyperpolarization [93], whereas during pacing-induced alternans the primary alternation is in APD. Moreover, ischemia is associated more often with alternation of the ST segment, and to a lesser extent, the T-wave, whereas pacing-induced alternans is associated almost exclusively with the T-wave.

During ischemia, conduction alternans can develop as impulses fail to propagate on alternating beats into ischemic zones exhibiting prolonged refractoriness. Here, conduction block and arrhythmias are unrelated to alternans or repolarization, but rather due to postrepolarization refractoriness. Thus, during ischemia the underlying relationship between alternans and arrhythmogenesis may not be mechanistically related, as is clearly the case with pacing-induced alternans. Importantly, there is no evidence that microvolt-level TWA, which is closely associated with enhanced susceptibility to arrhythmias in humans, is in any way dependent on the development of myocardial ischemia.

9. Summary

In the past century much has been learned about TWA and its potential role as a mechanism for ar-

rhythmogenesis. TWA is a marker for sudden cardiac death and arises due to alternation of repolarization at the cellular level. In the heart, spatially discordant alternans provides a substrate for unidirectional block and reentry. Cellular alternans is likely due to the actions of one or more ionic currents and is closely related to, if not directly dependent on, the kinetics of intracellular calcium cycling. These facts, coupled with the close relationship between alternans, heterogeneities of repolarization, and intercellular coupling, provide the basis for a general hypothesis regarding the role of TWA-mediated arrhythmogenesis in a variety of pathophysiological conditions.

This hypothesis is summarized in Fig. 9. Under normal physiological conditions, underlying heterogeneities of repolarization exist. When heart rate exceeds the kinetics of key ionic processes (such as ion channels or exchangers), perhaps those involved in intracellular calcium handling, cellular alternans develops. The heart rate threshold for alternans can be shifted to lower rates by disease-induced changes in intercellular coupling, expression or function of repolarization currents, or alterations in intracellular calcium cycling. Such changes increase the propensity for arrhythmogenic discordant alternans, which creates pathophysiological dispersions of repolarization (i.e. amplifies underlying heterogeneities) and provides a substrate for reentrant arrhythmias. When alternans-induced conduction block develops in the presence of structural barriers (e.g. healed myocardial infarction), monomorphic VT can develop as these barriers serve as anchors around which stable reentrant circuits can form [76]. In structurally normal myocardium (e.g. long-QT syndrome), discordant alternans leads to polymorphic VT or VF. These principles may explain why TWA is a common precursor to monomorphic VT, polymorphic VT, and VF in surprisingly diverse clinical and experimental circumstances.

There remains a great deal left to learn. On the one hand, our understanding regarding the cellular, subcellular, and molecular mechanisms responsible for generating repolarization alternans is far from complete. Which specific calcium cycling proteins and ion channels can be directly implicated? Can spatially heterogeneous expression of these proteins explain discordant alternans between cells across the ventricular wall? Can different mechanisms be implicated in different forms of cardiac disease? On the other hand, the potential role of TWA in the pathophysiological mechanisms of life threatening ventricular arrhythmias and SCD must be further established. Although available data would suggest that repolarization alternans is certainly a mechanism of arrhythmogenesis and a strong marker of clinical risk, the precise sequence of events which triggers SCD, and the potential role of TWA remains elusive.

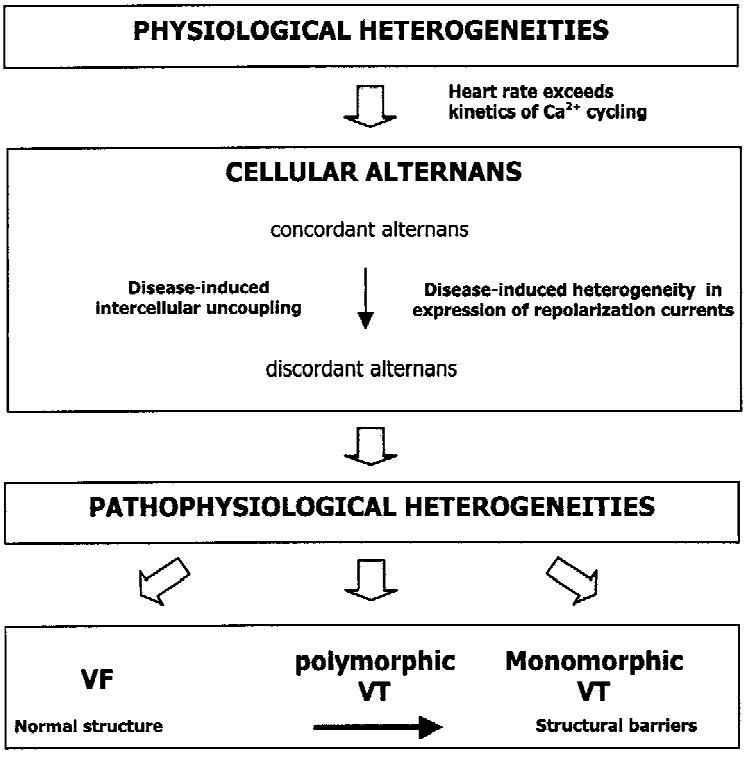

Fig. 9. General hypothesis for the role of repolarization alternans in the mechanism of ventricular arrhythmias. Cellular alternans occurs when heart rate exceeds the ability of the cell to effectively cycle intracellular calcium. Disease-induced changes in intracellular coupling or expression of currents involved in repolarization lower the alternans threshold and promote discordant alternans. Discordant alternans creates pathophysiological dispersions of repolarization that can lead to a wide variety of ventricular arrhythmias. The presence of structural barriers allows reentrant circuits to anchor, promoting ventricular tachycardia. In the absence of such anatomical anchors, VF dominates.

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