Role of Structural Barriers in the Mechanism of Alternans-Induced Reentry

Joseph M. Pastore, David S. Rosenbaum

Abstract—Previously, using an animal model of T-wave alternans in structurally normal myocardium, we demonstrated that repolarization can alternate with opposite phase between neighboring myocytes (ie, discordant alternans), causing spatial dispersions of repolarization that form the substrate for functional block and reentrant ventricular fibrillation (VF). However, the mechanisms responsible for cellular discordant alternans and its electrocardiographic manifestation (ie, T-wave alternans) in patients with structural heart disease are unknown. We hypothesize that electrotonic uncoupling between neighboring regions of cells by a structural barrier (SB) is a mechanism for discordant alternans. Using voltage-sensitive dyes, ventricular action potentials were recorded from 26 Langendorff-perfused guinea pig hearts in the absence (ie, control) and presence of an insulating SB produced by an epicardial laser lesion. Quantitative analysis of magnitude and phase of cellular alternans revealed that in controls, action potential duration alternated at all ventricular sites above a critical heart rate (269 ± 17 bpm), ie, concordant alternans. Also, above a faster critical heart rate threshold (335 ± 24 bpm), action potential duration alternated with opposite phase between sites, ie, discordant alternans. In contrast, only discordant but not concordant alternans was observed in 80% of hearts with the SB, and discordant alternans always occurred at a significantly slower heart rate (by 68 ± 28 bpm) compared with controls. Therefore, the SB had a major effect on the alternans–heart rate relation, which served to facilitate the development of discordant alternans. Whether a SB was present or not, discordant alternans produced considerable increases (by ~170%) in the maximum spatial gradient of repolarization, which in turn formed the substrate for unidirectional block and reentry. However, by providing a structural anchor for stable reentry, discordant alternans in the presence of a SB led most often to sustained monomorphic ventricular tachycardia rather than to VF, whereas in the absence of a SB discordant alternans caused VF. SBs facilitate development of discordant alternans between cells with different ionic properties by electrotonically uncoupling neighboring regions of myocardium. This may explain why arrhythmia-prone patients with structural heart disease exhibit T-wave alternans at lower heart rates. These data also suggest a singular mechanism by which T-wave alternans forms a substrate for initiation of both VF and sustained monomorphic ventricular tachycardia. (Circ Res. 2000;87:1157-1163.)

Key Words: repolarization ■ reentry ■ alternans

T-wave alternans is a beat-to-beat fluctuation in the amplitude of the electrocardiographic T wave that repeats once every other beat and has been closely associated with ventricular arrhythmias and sudden cardiac death. Visually apparent T-wave alternans is known to hasten cardiac electric instability in a surprisingly wide variety of experimental1-4 and clinical5-7 conditions. More recently, using sensitive signal-processing techniques, we found that the detection of microvolt-level, visually apparent T-wave alternans is also a strong predictor of vulnerability to ventricular arrhythmias in patients.8 Interestingly, T-wave alternans has been closely associated with polymorphic ventricular tachycardia (VT),9 ventricular fibrillation (VF),10 torsade de pointes,7,11 and sustained monomorphic VT (SMVT)8 both in the absence of structural heart disease and in patients with advanced cardiomyopathy. Therefore, a greater understanding of the mechanisms underlying T-wave alternans may provide important new insights into the pathophysiology of sudden cardiac death in a variety of clinical and experimental circumstances.

Recently, we used an experimental model to demonstrate that T-wave alternans is caused by primary alternations in the repolarization phase of the action potential.10 Importantly, above a critical heart rate threshold, repolarization of action potentials from adjacent regions of the ventricle alternated with opposite phase, ie, discordant alternans, causing steep spatial gradients of repolarization that formed an electrophysiological substrate for functional block, reentry, and VF.10 It is noteworthy that discordant alternans occurred under conditions of normal intercellular coupling, indicating that re-
regional differences in cellular ionic properties can overcome electrotonic effects that normally act to synchronize repolarization.12,13 However, the mechanisms responsible for triggering discordant alternans are unknown.

A major unresolved question is why patients with structural heart disease and T-wave alternans are at particularly high risk for ventricular arrhythmias. Clearly, patients with contractile dysfunction constitute the vast majority of cases of sudden cardiac death. Among patients with structural heart disease, those with T-wave alternans are more susceptible to inducible SMVT and spontaneous arrhythmic events.8,14 However, the role of structural heart disease in the mechanisms of alternans-induced reentry is unknown. Most forms of myocardial disease are associated with fibrotic barriers,15 alterations of fiber bundle architecture,16 and maldistribution of cardiac gap junctions,17 all of which are expected to produce electric uncoupling between fibers. Because cell-to-cell coupling plays an important role in synchronizing repolarization between cells, we hypothesize that electrotonic uncoupling by structural barriers (SBs) facilitates the development of discordant alternans. This may explain a mechanism by which SBs promote the development of critical dispersions of repolarization that cause functional block and reentry.

To test this hypothesis, we used high-resolution optical mapping in an experimental model of T-wave alternans. This model offered several advantages, as follows. (1) Action potentials could be recorded simultaneously while T-wave alternans was elicited on the ECG. (2) SB could be created with precise control using an argon ion laser in a preparation with previously characterized heterogeneities of repolarization. (3) Propagation, functional block, and dispersions of repolarization could be mapped directly with high spatial resolution. (4) In this model, both monomorphic and polymorphic arrhythmias could be induced. We found that electrotonic uncoupling produced by a SB greatly facilitates the development of discordant alternans, creating critical heterogeneities of repolarization that can form the substrate for a variety of reentrant arrhythmias. These data provide additional evidence linking T-wave alternans to a novel mechanism of arrhythmogenesis.

**Materials and Methods**

Experiments were performed in the guinea pig model of pacing-induced T-wave alternans10 in which a thin (≈800 μm) viable rim of left and right ventricular epicardium having normal electrophysiological properties was created by endocardial cryoablation in Langendorff-perfused hearts.10,16 After staining with voltage-sensitive dye and action potentials were mapped from a 1.5×1.5-cm mapping area (square grid), Left ventricle was stimulated (square pulse symbol) over a broad range of CLs in absence (ie, control) and presence of a SB. ECGs were recorded from electrodes immersed in the coronary effluent. RA indicates right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle; and LAD, left anterior descending coronary artery.

Repolarization time (RT), activation times, and action potential duration (APD) were determined from previously described algorithms.18,20 Alternans of cellular RT was determined by measuring differences in local RT on consecutive beats. Because the error associated with measurement of RT was 3±4 ms, only RT alternans >10 ms was considered significant. A previously validated spectral analysis technique10 was used to measure ECG T-wave alternans. The method of Bayly et al22 was modified for optically recorded action potential maps to accurately quantify the magnitude and direction of conduction velocity and repolarization gradients at each recording site. In all hearts (n=26), action potentials were recorded (Figure 1) over a broad range of steady-state pacing cycle lengths (CLs) until one-to-one capture failed or an arrhythmia was initiated. In each experiment, the left ventricular free wall was stimulated near the atrioventricular groove (Figure 1) to ensure that the sequence of epicardial activation was nearly identical before and after the introduction of the SB. This stimulation protocol was repeated in each experiment to confirm reproducibility of the findings. To ensure that laser energy did not alter properties of cells outside of the precisely demarcated SB, validation studies were performed in 15 hearts. Each heart served as its own control, as the identical stimulation protocol (described above) was performed before (ie, control) and after a SB was introduced. APD, repolarization gradients, conduction velocity, and ECG morphology were compared at the baseline stimulation CL of 400 ms in the absence and presence of a SB. The effects of SB on cellular alternans was determined in a subset of 5 of these experiments in which quantitative analysis of the magnitude and phase of cellular alternans was compared at each steady-state CL both before and after the introduction of a SB.

An expanded Materials and Methods section can be found in an online data supplement available at http://www.circresaha.org.

**Results**

**Effect of SB on Baseline Electrophysiology**

Although the electrophysiological properties of guinea pig epicardium were described in detail previously,19,20 it was important to confirm whether introduction of a SB changed the baseline characteristics of the model. Conduction velocity and APD were compared at a baseline heart rate of 150 bpm during control and in the presence of the SB. Conduction-velocity transverse to fibers measured during control (24±5 cm/second) and in the presence of the SB (25±7 cm/second) did not differ significantly (P=0.79, Wilcoxon matched pairs test).
The presence of SB significantly decreases the heart rate at which alternans heart rate threshold from 5 experiments. Notice that potentials in panel C. B, Effect of the SB on the discordant repolarization alternans is also demonstrated by superposition of even and odd (ie, sequential) action potentials in panel C. B. Effect of the SB on the discordant alternans heart rate threshold from 5 experiments. Notice that presence of SB significantly decreases the heart rate at which discordant alternans is elicited.

Figure 2. Effect of SB on repolarization alternans. A, Relationship between local repolarization alternans and heart rate in absence (ie, control, open circles) and presence (filled circles) of SB. Optically recorded action potentials shown were recorded from the same site (filled pixel, Figure 1) as heart rate varied. Notice in both cases that repolarization alternans occurs above a threshold heart rate that is lower with SB (240 bpm) compared with control (300 bpm). Repolarization alternans is also demonstrated by superposition of even and odd (ie, sequential) action potentials in panel C. B, Effect of the SB on the discordant alternans heart rate threshold from 5 experiments. Notice that presence of SB significantly decreases the heart rate at which discordant alternans is elicited.

Effect of SB on Cellular Alternans

The effect of regional uncoupling produced by a SB on the magnitude and heart rate threshold of action potential alternans is illustrated in Figure 2. Panel A shows a representative example of the change in cellular alternans—heart rate relation at one ventricular site caused by a SB. Such changes were indicative of cellular alternans at all recording sites. At relatively slow heart rates (<200 bpm), no cellular alternans is present either in control or in the presence of SB. However, above a critical threshold heart rate, RT alternans occurs and increases markedly as heart rate further increases. Notice that the heart rate threshold for RT alternans is considerably lower in the presence of a SB (240 bpm) compared with control (300 bpm). Consequently, over a broad range of heart rates, the magnitude of RT alternans is amplified in the presence of a SB. The difference in cellular alternans produced by the SB is further emphasized by the superposition of even and odd action potentials shown in panel C. Note that the heart rate dependence of repolarization alternans in individual cells was profoundly influenced by the insulating SB located some distant from the cell. The SB also decreased the heart rate threshold for T-wave alternans measured from the ECG (not shown), suggesting that SBs can similarly explain changes in heart rate alternans relation manifest on the surface ECG.

Effect of SB on Discordant Alternans Between Cells

In this investigation, the alternans heart rate threshold was defined as the slowest heart rate at which significant alternans was recorded at one or more ventricular sites, and the heart rate threshold for discordant alternans occurred at the heart rate at which 2 sites had significant alternans with opposite phase between them. As shown in Figure 2B, the SB significantly (P<0.05, Wilcoxon matched pairs test) decreased the heart rate threshold for discordant alternans (by 68±28 bpm) compared with controls. Also, in controls, a progression from no alternans to discordant alternans to discordant alternans always occurred as heart rate increased. In contrast, in the presence of a SB, a progression directly from no alternans to discordant alternans was observed in 80% of experiments. Consequently, the heart rate that produced discordant alternans in hearts with SBs (276±6 bpm) was essentially the same as the heart rate that produced discordant alternans in control hearts (269±17 bpm, P=0.35). Therefore, a SB greatly increased the propensity for discordant alternans. Preliminary experiments performed over long time periods (>3 hours) without introduction of a SB (ie, time controls) demonstrated stable electrophysiological properties of this preparation and, importantly, no change in the heart rate dependence or distribution of cellular alternans. Therefore, changes in magnitude or phase of cellular alternans that followed the application of the SB could not be attributed to degradation of the preparation over time.

We hypothesize that electrically insulating 2 regions of cells with heterogeneous intrinsic repolarization properties such as those known to exist across the epicardium will influence the development of discordant alternans. Figure 3 demonstrates that electrically uncoupling 2 regions of functionally distinct myocardium by introducing a SB creates an anatomic substrate across which discordant alternans can form. Shown are RT alternans phase plots delineating regions of cells where local repolarization alternates in a short-long phase (Figure 3, black region) or a long-short phase (Figure 3,
Areas without significant RT alternans are represented in white. All plots correspond to the slowest heart rate at which discordant alternans was recorded. Note that in all experiments, the SB forms at least a portion (and in experiments 1, 3, and 5, a large portion) of the line separating regions of cells alternating with opposite phase. Below each phase plot are representative action potentials from both the long-short (Figure 3, site A) and short-long (Figure 3, site B) regions demonstrating discordant alternans. Importantly, in the absence of a SB, stimulation at the identical heart rate produced concordant RT alternans in the same hearts. Therefore, the SB lowered the heart rate threshold for discordant alternans.

Because of its effect on the phase of RT alternans, the SB substantially affected spatial gradients of repolarization. Shown in Figure 4 are isochrone maps representing patterns of activation, APD, and repolarization for 2 consecutive beats in the absence (ie, control) and presence of SB at an identical heart rate. Activation propagates as expected from site of stimulation (square pulse symbol) in all cases. SB produces large changes in magnitude and orientation of APD gradients (right middle panel) that were not present during control (left middle panel). This creates large spatial gradients of repolarization in presence of SB. These effects are evident on the ECG (top) as visible T-wave alternans is apparent with SB.

Role of SB on Alternans-Induced Arrhythmias
To determine the effect of the SB on alternans-induced reentry, we attempted to induce discordant alternans and arrhythmias in all 26 hearts. In 15 of these hearts, the arrhythmia-induction protocol was performed in the absence and presence of a SB, whereas in 11 hearts the protocol was performed only in the absence of a SB. In all 26 experiments, reentrant VF or SMVT was only initiated after discordant alternans formed; ie, reentrant excitation never occurred in the absence of discordant alternans. In the absence of a SB,
VF was initiated in 93% of hearts, whereas SMVT was initiated in only 7%. In contrast, the presence of a SB resulted in reentrant SMVT in 70% of hearts and VF in 30%. Therefore, the SB was a significant and specific factor for the development of SMVT (χ²=11.1, P<0.001). Figure 5 illustrates representative examples of the induction of VF and SMVT in the same heart before and after the introduction of a SB. Therefore, discordant alternans occurring in the presence and absence of a SB formed the electrophysiological substrate for SMVT or VF, respectively.

To ascertain the role of discordant alternans in forming an arrhythmogenic substrate, the maximum repolarization gradient within the mapping array was measured at baseline (CL=400) and during discordant alternans at the CL immediately preceding the initiation of either reentrant VF or SMVT. We found that discordant alternans created an electrophysiological substrate for unidirectional block by amplifying gradients of repolarization independent of underlying structure. Figure 6 shows the maximum gradient of repolarization measured within the mapping array at a baseline heart rate of 150 bpm as compared with during discordant alternans immediately preceding the initiation of VF (in hearts without a SB) and SMVT (in hearts with a SB). Notice that the maximum repolarization gradient is significantly larger preceding SMVT or VF compared with baseline, whereas the maximum repolarization gradients preceding SMVT and VF are similar. These data suggest that discordant alternans transforms minor gradients of repolarization into critical gradients sufficient for development of functional block that leads to both SMVT and VF. This is illustrated in the representative example shown in Figure 7 demonstrating the initiation of reentrant SMVT after the application of a single premature stimulus (S2) during discordant alternans. Reentry is immediately preceded by discordant alternans as shown by the action potential tracings from 2 selected sites, sites A and B, (Figure 7, bottom panel). Notice the large repolarization gradient between these 2 sites, which are only 5 mm apart. Although the wavefront propagates successfully along both sides of the SB on the S1 beat, the gradient caused by discordant alternans is of sufficient magnitude to cause unidirectional block of the premature (S2) beat. Notice that the wavefront fails to propagate against the repolarization gradient created by discordant alternans on the basal side of the SB but does propagate along the apical side. About 130 ms later, the wavefront reenters the area of functional block in a clockwise direction and propagates around the SB forming the first beat of reentry. Similarly, discordant alternans produced critical gradients of repolarization, which formed the substrate unidirectional block, which led to reentrant VF when no SB was available to stabilize the reentrant circuit. Therefore, in the presence of a SB, discordant alternans underlie the mechanism of SMVT because the SB served as an anchor around which a stable reentrant circuit could form.

Discussion

For more than three-quarters of a century, T-wave alternans has been closely associated with susceptibility to ventricular arrhythmias in remarkably broad patient populations both with and without structural heart disease. Furthermore, T-wave alternans is now emerging as a clinical tool to screen for patients at risk for sudden cardiac death based on the principle that the heart rate threshold for T-wave alternans is significantly reduced in high-risk patients. Recently, we demonstrated that T-wave alternans is linked to the mechanism of reentry by discordant alternans that develops between cells possessing different electrophysiological properties. Importantly, discordant alternans greatly amplifies dispersion of repolarization, producing conditions necessary for functional block and reentry. We hypothesized that electrotonic uncoupling by a SB increases the propensity for discordant alternans, which may explain a mechanism of T-wave alternans and arrhythmias in patients with structural heart disease. In this report, we show conclusively that discordant alternans is a novel mechanism by which the electrophysiological substrate for block and reentry can form and that SBs greatly increase the propensity for discordant alternans to develop.

Although results obtained from the guinea pig pacing-induced model of T-wave alternans must be extrapolated cautiously to patients, the model offers several distinct advantages. As is the case in patients, electrical alternans in this model persists over time (ie, alternans are not transient), does not require ischemic damage, and principally involves the T-wave rather than the QRS complex. Although spontaneous initiation of VT or VF in patients is often not preceded by visible T-wave alternans as seen in these experimental studies, it is possible that microvolt-level T-wave alternans is present but unrecognized during clinical episodes of sudden cardiac death. Finally, cellular alternans elicited by steady-state pacing produces an electrophysiological substrate for reentrant VT and VF, which typically lasts indefinitely unless actively terminated by pacing or defibrillation, respectively.
The effect of the SB on the development of T-wave alternans and arrhythmogenesis was ascertained using optical action potential mapping, which served to monitor the development of cellular alternans both in the absence (ie, control) and presence of a SB. This barrier was produced with a laser to create a well-defined anatomic obstacle that electrically insulated 2 regions of myocardium without altering the electrophysiological properties of cells surrounding the barrier. Therefore, our observation of profound changes in cellular repolarization and alternans caused by SBs emphasizes the importance of investigating cell physiology in the intact heart.

Previously, we have shown that the orientation of discordant alternans closely follows heterogeneities of restitution properties between cells that are known to exist across guinea pig epicardium, suggesting that the reason neighboring regions of cells alternate with opposite phase is that the cells possess different ionic properties. One would predict that in the presence of reduced intercellular coupling, electrotonic forces that normally act to maintain synchronization of repolarization between cells are reduced, thereby enhancing dispersion of repolarization. Conversely, several studies have shown that increased coupling attenuates repolarization gradients. The present investigation demonstrates an independent and important effect of cell-to-cell uncoupling on repolarization. In the presence of minor heterogeneities of repolarization properties between cells, electrotonic uncoupling promotes the development of discordant alternans, which in turn causes marked gradients of repolarization. Importantly, arrhythmias only occurred in the presence of steep repolarization gradients caused by discordant alternans. Discordant alternans has also been reported during ischemia, in which cell-to-cell coupling is impaired, further supporting a role of uncoupling in the mechanism of discordant alternans.

There are several lines of evidence indicating that a SB facilitates the development of discordant alternans. First, in structurally normal myocardium, discordant alternans always preceded discordant alternans, whereas in the presence of a SB, a direct transition from no alternans to discordant alternans occurred. Second, myocardial cells alternating with opposite phase were typically located in proximity to the SB. Third, the heart rate threshold required to elicit discordant alternans was reduced significantly by the SB (Figure 3), which was also reflected in the ECG by a decrease in the T-wave alternans heart rate threshold. This corresponds well with recent clinical observations that suggest that patients in whom SMVT can be initiated during electrophysiological study have a significantly lower heart rate threshold for T-wave alternans compared with patients with negative electrophysiological tests. The findings in this study provide a possible mechanism explaining these clinical observations.

Our data suggest that discordant alternans can potentially form in the following 2 situations: (1) where large heterogeneities in cellular repolarization properties exist in structurally normal myocardium and (2) where minor heterogeneities of repolarization exist in the presence of uncoupling caused by SBs. For example, significant repolarization heterogeneities may exist in structurally normal myocardium in the congenital long-QT syndrome in which discordant alternans has been documented and may explain the association of long-QT syndrome with torsade de pointes and VF. In contrast, hearts of patients with structural heart disease have reduced cell-to-cell coupling in part because of interdigitation of fibrous tissue between myocyte bundles. Even though these patients may have only minor heterogeneities of repolarization between cells, electrical uncoupling may promote the formation of discordant alternans. Moreover, the precise relation between the location of a SB and repolarization gradients may also explain why a scar can predispose to reentry in some cases but not others. Our data do not suggest that SBs of any type or having any orientation constitute a requisite condition for monomorphic and polymorphic reentry. For instance, if a barrier is made along rather than across the gradients of repolarization, reentry is not easily induced in this model. Biological variability in location of repolarization gradients relative to the SB and pacing site may explain why discordant patterns varied between experiments (Figure 3) and why, in turn, the site of block also varied (Figure 7).

The initiation of SMVT, rather than VF, in the presence of a SB was explained by the barrier serving as an anchor to stabilize a reentrant circuit (Figures 5 and 7). The concept is supported by studies of both atrial and ventricular reentry. During atrial flutter, the reentrant circuit can stabilize on structural discontinuities in atria such as the crista terminalis and eustachian ridge or on the tricuspid annulus. Similarly, in ventricle, a meandering core of a spiral wave anchors to structures such as small arteries, which stabilize the wave. Following this reasoning, it is not surprising that discordant cellular alternans provided a common mechanism for VF and SMVT in our experiments. Such findings suggest a singular mechanism by which T-wave alternans forms a substrate for initiation of VF and polymorphic and monomorphic VT.

The structural alterations produced by heart disease are obviously much more complex than those of our experimental model. Although a requirement for unidirectional block in these studies, discordant alternans is clearly only one component of a more complex substrate for reentry that is undoubtedly present in patients and other models of reentry. For example, in these experiments a single SB served to promote both discordant alternans and an anchor for monomorphic reentry, whereas in patients it is more likely that multiple SBs produce these effects independently. In addition, our model does not account for potential disease-induced changes in the expression of ionic currents. However, the physical principles set forth by our experiments should apply to more complex disease states.

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