Mechanism Linking T-Wave Alternans to the Genesis of Cardiac Fibrillation

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Background—Although T-wave alternans has been closely associated with vulnerability to ventricular arrhythmias, the cellular processes underlying T-wave alternans and their role, if any, in the mechanism of reentry remain unclear.

Methods and Results—T-wave alternans on the surface ECG was elicited in 8 Langendorff-perfused guinea pig hearts during fixed-rate pacing while action potentials were recorded simultaneously from 128 epicardial sites with voltage-sensitive dyes. Alternans of the repolarization phase of the action potential was observed above a critical threshold heart rate (HR) (209±46 bpm) that was significantly lower (by 57±36 bpm) than the HR threshold for alternation of action potential depolarization. The magnitude (range, 2.7 to 47.0 mV) and HR threshold (range, 171 to 272 bpm) of repolarization alternans varied substantially between cells across the epicardial surface. T-wave alternans on the surface ECG was explained primarily by beat-to-beat alternation in the time course of cellular repolarization. Above a critical HR, membrane repolarization alternated with the opposite phase between neighboring cells (ie, discordant alternans), creating large spatial gradients of repolarization. In the presence of discordant alternans, a small acceleration of pacing cycle length produced a characteristic sequence of events: (1) unidirectional block of an impulse propagating against steep gradients of repolarization, (2) reentrant propagation, and (3) the initiation of ventricular fibrillation.

Conclusions—Repolarization alternans at the level of the single cell accounts for T-wave alternans on the surface ECG. Discordant alternans produces spatial gradients of repolarization of sufficient magnitude to cause unidirectional block and reentrant ventricular fibrillation. These data establish a mechanism linking T-wave alternans of the ECG to the pathogenesis of sudden cardiac death. (Circulation. 1999;99:1385-1394.)

Key Words: mapping ■ repolarization ■ fibrillation ■ electrical alternans ■ reentry ■ electrocardiogram

Electrical alternans is defined as a beat-to-beat change in the amplitude of the ECG that repeats once every other beat. Shortly after the ECG was introduced to clinical medicine, electrical alternans of the T wave was recognized as a precursor to ventricular arrhythmias.1 T-wave alternans was subsequently observed in a surprisingly wide variety of clinical2–6 and experimental7–12 conditions associated with ventricular arrhythmias. We have used sensitive ECG processing techniques to establish a close quantitative relationship between microwatt-level, visually inapparent T-wave alternans and vulnerability to ventricular arrhythmias in humans.13 Despite convincing evidence that T-wave alternans is closely associated with the development of reentrant ventricular arrhythmias and sudden cardiac death, it is not known how, or whether, T-wave alternans is causally linked to the underlying mechanism of ventricular arrhythmias.

There are 2 prevailing hypotheses on the mechanisms of T-wave alternans. One states that a spatial dispersion of refractoriness gives rise to alternations in propagation and repolarization. According to this hypothesis, repolarization alternans is secondary to propagation alternans, which occurs when the time between successive activations is shorter than the refractory period. This hypothesis was supported by an experimental study in which ECG alternans during regional ischemia was generated by alternating conduction block into the ischemic zone.14 However, such alternating conduction block has not been observed in the absence of regional ischemia. The second hypothesis states that T-wave alternans is caused primarily by alternations in repolarization of the action potential, which may give rise to secondary propagation alternans.11,12,15–18 However, the regional membrane changes that underlie the development of T-wave alternans in the intact heart and the possible role such changes play in the mechanism of ventricular arrhythmias are poorly understood because (1) conventional recording techniques cannot be used to monitor cellular membrane potential with sufficient spatial resolution.
resolution during the development of ECG T-wave alternans and (2) experimental studies have focused on transient alternans during an abrupt change in cycle length (CL) or alternans during myocardial ischemia, whereas the majority of patients at risk for sudden cardiac death exhibit T-wave alternans at a relatively constant heart rate (HR) and in the absence of acute ischemia. Therefore, we used the technique of high-resolution optical action potential mapping in an intact heart model of pacing-induced T-wave alternans to determine if T-wave alternans is causally linked to the mechanism of reentry.

Methods

Experimental Preparation

Male retired breeder guinea pigs (n = 8) were anesthetized (pentobarbital sodium 30 mg/kg IP), and their hearts were rapidly excised and perfused as Langendorff preparations with oxygenated (95% O2, 5% CO2) Tyrode’s solution containing (mmol/L) NaCl 130, NaHCO3 25.0, MgSO4 1.2, KCl 4.75, dextrose 5.0, and CaCl2 1.25 (pH 7.40, 27°C). Previously, it was shown that reduced temperature is an effective means of eliciting T-wave alternans in a controlled fashion. In a subset of experiments (n = 5), the experimental protocol was repeated at 37°C to confirm that our experimental results could be reproduced at physiological temperatures. During all experiments, the endocardial surface was eliminated by use of a cryoadipalysis procedure described previously. This procedure produces a thin (100-μm-thick) viable rim of epicardium having normal electrophysiological properties and served to restrict propagation to the surface from which action potentials were recorded. Hearts were stained with 100 mL of the voltage-sensitive dye di-4-ANEPPS (15 μmol/L) by direct coronary perfusion for 10 minutes.

Beating and perfused hearts were placed in a custom-built imaging chamber. To avoid surface cooling and the formation of intracardiac temperature gradients, the heart was immersed in the coronary effluent, which was maintained at a constant temperature (equal to the perfusion temperature) with a heat exchanger. Gentle pressure was applied to the posterior surface of the heart with a movable piston to stabilize the anterior surface of the ventricle against an imaging window so as to eliminate motion artifacts without altering action potential properties. Cardiac rhythm was monitored with 3 silver disk electrodes fixed to the chamber in positions corresponding to ECG limb leads I, II, and III. ECG signals were filtered (0.05 to 300 Hz), amplified (×10), and displayed on a digital oscilloscope. Preparations were stable for at least 3 hours of perfusion.

Optical Mapping System

Previously, we developed an optical action potential mapping system that is capable of resolving membrane potential changes as small as 0.5 mV from 128 simultaneous sites across the anterior epicardial surface of the intact guinea pig ventricle (see References 22 through 24 for details). In the present study, an optical magnification of 1:1.8 was used, which corresponded to a total mapping field of 10×10 mm, 0.83-mm interpixel spatial resolution, with 1-ms temporal resolution, permitting detailed and quantitative analysis of action potential shape and duration. To ensure consistency and objectivity, depolarization and repolarization times were determined from each action potential by use of previously described algorithms.

Stimulation Protocol

The ventricular epicardial surface was stimulated at 5 times diastolic threshold current with a Teflon-coated silver bipolar electrode (1.0-mm interelectrode spacing). Recordings were made during steady-state (>1 minute) pacing starting at a CL of 400 ms and then at faster CLs (by 50-ms decrements) until T-wave alternans was visually observed in at least 1 ECG lead, at which point CL was shortened by 10-ms intervals. The CL was decreased until 1-to-1 capture was lost or until ventricular fibrillation (VF) ensued. Action potential alternans was measured simultaneously from 128 epicardial sites for 64 consecutive beats at each CL tested.

Voltage Calibration of Optically Recorded Action Potentials

The fluorescent signal measured with voltage-sensitive dyes conveys relative but not absolute transmembrane potential. To estimate the magnitude of action potential alternans (in millivolts) from optically recorded action potentials, we developed and validated a method for calibrating beat-to-beat changes in membrane potential (ΔV) from action potential amplitude (APA) measured at a baseline CL of 400 ms by microelectrode (APAme) and optical (APAO) techniques, and from measured beat-to-beat changes in fluorescence (ΔF) as follows.

\[
\Delta V (mV) = \left( \frac{APA_{me}}{APA_{AO}} \right) \times \Delta F.
\]

In a series of preliminary studies, we found that APAm was measured with microelectrodes during constant CL pacing of 400 ms did not vary significantly (P = 0.24) between cells across the epicardial mapping surface spanning from the left ventricular base (n = 7, 108.5 ± 2.8 mV) to apex (n = 8, 111.6 ± 6.2 mV). Therefore, because APAm was essentially constant (∼110 mV) at each recording site, ΔV was estimated from APAO and ΔF measured optically at each recording site.

The aforementioned calibration technique was validated with floating microelectrode recordings from ventricular myocytes in 2 intact hearts. Action potentials were recorded at a sampling rate of 20 000 Hz from 10 cells corresponding to ventricular sites throughout the optical mapping array. APAs measured with microelectrodes were compared with those measured and calibrated by use of voltage-sensitive dye (ie, calculated in mV).

Measurement of Alternans From Optically Recorded Action Potentials

Beat-to-beat fluctuations of APA were measured from each ventricular site by a previously validated spectral technique, which was modified for action potential analysis in this investigation. Briefly, action potentials from 64 consecutive beats were aligned by the stimulus artifact. For every point along the action potential, a power spectrum was calculated from a time series representing amplitude fluctuations of that point over 64 consecutive beats. The resulting spectra were then averaged over 2 intervals: depolarization (defined from an 8-ms window centered around the maximum derivative of the action potential upstroke) and repolarization (defined from the end of the depolarization interval to the end of phase 3 of the action potential). Action potential alternans was determined from the noise-corrected magnitude of the averaged power spectrum registered at a frequency of 0.5 cycles per beat. This technique is capable of distinguishing alternans-type action potential fluctuations from action potential fluctuation occurring at other frequencies and from random, “white” action potential fluctuations.

Results

Estimating Transmembrane Voltage Changes With Voltage-Sensitive Dye

We validated a technique for estimating transmembrane voltage from the fluorescent signal recorded with voltage-sensitive dye. Figure 1 illustrates the close relationship between APA calculated from optical action potentials (APA0) and APAm recorded from similar sites with a floating microelectrode. As expected, APA decreased progressively as stimulation rate increased. The rate of rise of the optically recorded action potential upstroke (Figure 1, inset) was slower than the upstroke recorded with the microelectrode because the optical signal is derived from a small aggregate of
There was a close linear correlation between APA and APA, falling near the line of identity, indicating that the technique used to calibrate APA from the fluorescent signal provides a reasonable approximation of the actual change in transmembrane potential. Therefore, it was valid to compare the magnitude of alternans (in mV) between cells across the surface of the heart.

Changes in Membrane Potential Responsible for ECG T-Wave Alternans

The changes in membrane potential that underlie the development of ECG T-wave alternans are shown in Figure 2. In this representative example, the magnitude of alternans measured for each point of the ECG and the ventricular action potential are compared. At HRs of 270 and 285 bpm, there was a small peak of alternans during the upstroke of the action potential that coincided with alternans of the ECG QRS complex and a larger peak of alternans during phase 3 of the action potential that coincided with alternans of the ECG T wave. Alternation in the T wave was caused by alternation in the slope of the plateau and onset of phase 3 of the action potential. Note that the amplitude of repolarization alternans of the action potential (eg, 9 mV at 270 bpm) was more than an order of magnitude larger than the amplitude of T-wave alternans in the ECG (0.3 mV at 270 bpm).

The tracings recorded at a pacing CL of 315 ms (Figure 2D and 2E) illustrate an important point regarding the relationship between action potential alternans and alternans of the QRS complex versus the T wave. Note that despite relatively small beat-to-beat alternation in the timing of the action potential upstroke (5 ms in Figure 2D) and large alternation in cellular repolarization time (50 ms), the magnitude of QRS complex alternans is much larger than the magnitude of T-wave alternans. This paradox is further illustrated in Figure 2E, in which action potentials are shown from 2 ventricular sites on opposite sides of the mapping array during ECG alternans. Note that the repolarization gradient between these sites encompasses the ECG T wave and changes markedly in amplitude and direction from beat to beat. However, the gradient produces relatively small T-wave alternans. In con-

Figure 1. Comparison of APA recorded with a floating microelectrode (abscissa) with APA calculated from fluorescence change of voltage-sensitive dye (ordinate) with a fluorescence calibration technique (see Methods). APA was varied by changing rate of stimulation (stimulation rates in bpm shown beside each data point). Note that APA as calculated from optical action potentials closely correlates with APA measured with standard microelectrode techniques. Inset illustrates action potential upstrokes recorded by optical (OP) and microelectrode (ME) techniques for 3 HRs.

Figure 2. Changes in transmembrane potential underlying T-wave alternans on surface ECG. A through D, ECG lead (top) and representative action potential (bottom) recorded simultaneously from 1 of 128 mapping sites. Tracings recorded from 2 consecutive beats are superimposed to illustrate electrical alternans. Magnitude of electrical alternans during each time point of cardiac cycle is represented by difference between amplitudes of signals recorded on consecutive beats. As stimulus rate was increased to 270 bpm, T-wave alternans in range of 200 to 400 mV was distributed symmetrically around T wave and was explained by beat-to-beat alternation in phases 2 and 3 of action potential. E, T-wave alternans of ECG can be accounted for by gradients of cellular repolarization measured within mapping array. An ECG lead and 2 action potentials are shown at a HR of 315 bpm (measured at same time as data in D). Note that range of repolarization times for a given beat (arrows) spans majority of T wave. AP1 and AP2 are action potentials recorded from 2 ventricular sites.
trast, very subtle propagation alternans (not visibly apparent on the tracing) between action potentials causes marked QRS complex alternans.

A similar distribution of alternans within the action potential was measured with a floating microelectrode, confirming that the optical recordings did indeed accurately reflect transmembrane potential changes at the level of the single cell. In addition, these results were reproduced after contraction was eliminated with 10 mmol/L diacetyl monoxime, verifying that the changes were not caused by mechanical alternans.

Heart Rate Dependence of Cellular Alternans

Figure 3 illustrates the HR dependence of action potential alternans as a function of HR from a representative experiment. At a HR of 200 bpm, low levels of repolarization alternans were present at each recording site in the absence of any depolarization alternans, demonstrating again that $T_D$ is less than $T_R$. Although repolarization alternans was present throughout the mapping field, there was no visible alternation in the T wave of the ECG. At a HR of 240 bpm, the magnitude of repolarization alternans increased substantially and was heterogeneous between cells across the epicardial surface. At a somewhat faster rate (285 bpm), the pattern of repolarization alternans changed importantly. Although the HR was faster, the magnitude of repolarization alternans was markedly reduced across a linear band (blue contour in Figure 4, right panel) spanning the epicardial surface. This band was surrounded on either side by cells exhibiting a relatively large magnitude of repolarization alternans extending toward the base and apex of the ventricle.

Spatial Heterogeneity of Cellular Alternans

The characteristics of action potential alternans varied considerably between cells across the epicardial surface of the heart. The median and range of $T_D$, $T_R$, and maximum alternans of cellular depolarization and repolarization measured from all 128 recording sites are shown in the Table. In all experiments, $T_R$ and $T_D$ varied between cells across the epicardial surface. The maximum magnitude of action potential alternans also varied substantially (by as much as 60%) between epicardial cells (Table).

Figure 4 illustrates the spatial distribution of action potential alternans as a function of HR from a representative experiment. At a HR of 200 bpm, low levels of repolarization alternans were present at each recording site in the absence of any depolarization alternans, demonstrating again that $T_D$ is less than $T_R$. Although repolarization alternans was present throughout the mapping field, there was no visible alternation in the T wave of the ECG. At a HR of 240 bpm, the magnitude of repolarization alternans increased substantially and was heterogeneous between cells across the epicardial surface. At a somewhat faster rate (285 bpm), the pattern of repolarization alternans changed importantly. Although the HR was faster, the magnitude of repolarization alternans was markedly reduced across a linear band (blue contour in Figure 4, right panel) spanning the epicardial surface. This band was surrounded on either side by cells exhibiting a relatively large magnitude of repolarization alternans extending toward the base and apex of the ventricle.

Characteristics of Cellular Alternans Across the Epicardial Surface

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Alternans HR Threshold, bpm</th>
<th>Maximum Alternans, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_D$</td>
<td>$T_R$</td>
</tr>
<tr>
<td>1</td>
<td>285 (272–300)</td>
<td>272 (240–272)</td>
</tr>
<tr>
<td>2</td>
<td>300 (240–300)</td>
<td>240 (171–300)</td>
</tr>
<tr>
<td>3</td>
<td>285 (222–300)</td>
<td>171 (171–222)</td>
</tr>
<tr>
<td>4</td>
<td>240 (200–261)</td>
<td>171 (150–240)</td>
</tr>
</tbody>
</table>

Comparison of HR threshold required to elicit action potential alternans and the maximum amplitude of action potential alternans in 4 experiments. All data are shown as median (range) of values measured from all mapping sites. $T_D$ and $T_R$ correspond to the HR threshold for the depolarization and repolarization components of the action potential, respectively. $\Delta$ indicates the difference between median values, and the $P$ value indicates the significance level of the difference determined by the Wilcoxon matched-pairs test. The maximum amplitude of action potential alternans measured over the entire range of steady-state HRs was used.
The mechanisms responsible for producing the band of low alternans were investigated by analyzing action potentials recorded from sites in close proximity to the band. Action potentials shown in Figure 5 were recorded from cells located within 3 mm of the low-alternans band, either toward the base (top potentials) or apex (bottom potentials) of the ventricle, and from cells within the band (potentials in center). At the slower HR (285 bpm, Figure 5, left), APDs during each beat either prolonged or shortened at all sites (ie, concordant alternans). In contrast, at a critical HR (315 bpm, Figure 5, right), the low-alternans band was present, as evidenced by the black line on the isoalternans plot (Figure 5, right), which marks the sites at which the difference in local repolarization time between 2 consecutive beats was zero. Action potentials recorded from the most basal site (site A, Figure 5) alternated in a long-short pattern, whereas the most apical site (site E, Figure 5) alternated in a short-long pattern. In other words, adjacent regions of myocardial cells were alternating with opposite phase, ie, discordant alternans. Therefore, the band of low alternans could be explained by the discordant alternation in the action potentials on either side of the band leading to cancellation of repolarization alternans along the band. Discordant alternans between cells was consistently oriented in a base-to-apex direction, which closely follows regional heterogeneities of membrane kinetics known to exist across the epicardial surface of guinea pig ventricle.24 Moreover, this pattern of heterogeneity was largely independent of pacing site, suggesting that it was caused by heterogeneities of repolarization properties intrinsic to each cell rather than heterogeneous propagation delays.

Effects of Discordant Alternans on the Sequence, Pattern, and Dispersion of Repolarization
The spatial patterns of depolarization and repolarization during concordant and discordant alternans are compared in Figure 6. During concordant alternans (Figure 6, left), the pattern and sequence of ventricular depolarization and repolarization were similar from beat to beat. In contrast, during discordant alternans, the patterns of repolarization varied substantially (Figure 6, right), because the direction of repolarization reversed nearly 180° on consecutive beats. Furthermore, repolarization was twice as slow during discordant alternans because of steep gradients of repolarization (ie, crowding of isochrones) that were not present during concordant alternans (Figure 6). Discordant alternans–induced gradients of repolarization were sufficiently large to cause secondary propagation delays, as conduction velocity near the base of the heart (Figure 6, beat 2) slowed by 44% relative to concordant alternans.

The importance of discordant alternans in producing spatial heterogeneity of repolarization is demonstrated quantitatively in Figure 7. Spatial dispersion of repolarization was calculated from the variance of the repolarization times recorded from all 128 recording sites within the mapping array. Dispersion of repolarization measured on consecutive beats is plotted as a function of HR. Dispersion of repolarization was not substantially increased over baseline values during concordant alternans. In contrast, dispersion of repolarization increased >13-fold during discordant alternans.

Role of Discordant Alternans in the Mechanism of Initiation of VF
Discordant alternans produced a state of marked electrical instability, because VF was always preceded by discordant alternans and never by concordant alternans. The mechanism of initiation of VF during discordant alternans was investigated by mapping propagation and repolarization in detail as steady-state CL was shortened by 10-ms decrements. As shown in Figure 8, during steady-state discordant alternans, the patterns of depolarization were similar from beat to beat, whereas the patterns of repolarization changed markedly on alternating beats (Figure 8, bottom).
Although the patterns of repolarization were complex, they were nearly identical on alternating beats (Figure 8, compare beats 3 and 5). Notice that during beat 5 (ie, when CL was shortened by 10 ms), the area of most delayed repolarization and steepest repolarization gradient was in the upper right corner of the mapping array. This region corresponded to the area in which the depolarizing wave front blocked on the next beat (beat 6) and propagated around either side of the line of block (off of the mapping field). However, 90 ms later, the zone of block regained excitability and the impulse reentered it from the opposite direction, forming the first spontaneous beat of VF.

During discordant alternans (Figure 8, beats 1 through 5), propagation proceeded consistently from the site that is proximal (site 1) to the site that is distal (site 2) to the stimulus electrode. Note that there is a considerable difference in the magnitude and phase of action potential alternans between these sites. Although the action potentials recorded from site 2 have relatively small amplitudes on beats 2 and 4, inspection of action potentials at sites immediately distal to site 2 (eg, site 3) confirmed that these were indeed propagated and not electrotonic responses (ie, no decrement in APA). Moreover, if these were nonpropagating (ie, 2:1) responses, the effective pacing CL for these cells would be 360 ms. At this CL, APD at site 2 was 218 ms, whereas in Figure 8 (beats 1, 3, and 5), APD is 148 ms, 32% shorter than would be expected had 2:1 conduction been present. After the stimulus CL was shortened by 10 ms (beat 5), the impulse propagated successfully into site 2, because this beat followed a long diastolic interval providing sufficient time for the cells of site

Figure 5. Alternans in local repolarization time between consecutive beats shown on iso-alternans contour plots. Red (positive) and blue (negative) colors indicate prolongation and shortening of repolarization, respectively. Shown below each contour plot are optical action potential tracings recorded from selected ventricular sites (A through E). Action potentials recorded from 2 consecutive beats (thick and thin tracings) are superimposed to demonstrate alternation in transmembrane potential. Measurements were obtained from same preparation during concordant alternans (left) and discordant alternans (right).
2 to regain excitability. However, as a direct consequence of discordant alternans, the next beat (beat 6) followed a short diastolic interval at sites 2 and 3 and a long diastolic interval at site 1, resulting in successful propagation of the impulse through site 1 and failure to propagate (ie, block) through site 2. Therefore, unidirectional block was caused by critical repolarization gradients established during discordant alternans.

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. Therefore, an understanding of the mechanisms responsible for T-wave alternans may provide important insights into the pathophysiology of sudden cardiac death. Previously, it was not clear whether T-wave alternans is causally linked to the pathogenesis of sudden cardiac death or whether it is simply an epiphenomenon related to another process. This is a critical distinction, particularly because T-wave alternans is now being used increasingly to stratify arrhythmia risk in patients. Therefore, we sought to determine a mechanism by which T-wave alternans may be causally linked to the pathogenesis of reentrant excitation in the ventricle.

Experimental Model of Steady-State T-Wave Alternans

To investigate the time course of membrane potential at many sites during T-wave alternans, we used a Langendorff-perfused guinea pig model in which electrical alternans could be reproducibly elicited by constant-rate pacing. Although these results must be extrapolated cautiously to patients, T-wave alternans in our experimental model shared many characteristics of ECG T-wave alternans in patients because it (1) could occur at the microvolt level, (2) was not evenly distributed over the T wave but instead was largest in amplitude near the T-wave peak, (3) was dependent on HR, and (4) was closely associated with the onset of VF. Because arrhythmia risk associated with T-wave alternans is in most instances unrelated to ischemia, we purposely avoided an experimental model that required ischemia to induce alternans.

Our model has several potential limitations. For example, measurements were made in a nonworking heart, whereas mechanical loading can potentially influence electrical alternans via mechanoelectrical feedback mechanisms. Also, some evidence suggests that T-wave alternans is dependent on sympathetic stimulation, which is absent in isolated heart preparations. However, sympathetic stimulation does not appear to influence the amplitude of T-wave alternans.

Figure 6. Patterns of ventricular depolarization and repolarization during concordant (left, CL 220 ms) and discordant (right, CL 180 ms) alternans. Shown are 10-ms isochrone plots representing depolarization (depol) and repolarization (repol) within mapping array for 2 consecutive beats.

Figure 7. Representative experiment demonstrating effect of concordant and discordant alternans on spatial dispersion of repolarization. Note that discordant alternans, but not concordant alternans, causes marked dispersion of repolarization.
alternans in patients with structural heart disease and ventricular arrhythmias. Moreover, when autonomically mediated HR changes are carefully controlled for, sympathetic stimulation can actually reduce alternans. Although the present study does not resolve this controversy, our data indicate that sympathetic stimulation is not necessary for the development of T-wave alternans. Finally, our preparation included a thin rim of normal epicardial tissue, which eliminated possible contributions of the His-Purkinje system, the endocardium, and the midmyocardial layers of the ventricle to the generation of T-wave alternans. This was an expected trade-off that ensured that the observed ECG patterns were indeed arising from the epicardial regions that were accessible for mapping. However, the principles set forth by these epicardial mapping studies should apply to any myocardial surface on which spatial heterogeneities of membrane function exist (eg, transmural wall).

**Action Potential Alternans in the Intact Heart**

Limited information is available on regional cellular changes that underlie T-wave alternans in the intact heart. Our data indicate that beat-to-beat alternation of membrane repolarization is a rate-dependent property of cardiac myocytes (Figures 3, 4, and 5), as postulated earlier by Hoffman and Suckling. Alternans most commonly involved the slope of the action potential plateau and the onset of final repolarization (Figure 2). The HR-alternans relation was highly non-linear, such that transmembrane alternation was provoked only above a critical threshold HR, and above this threshold the magnitude of alternation increased markedly (Figure 3). Evidence supporting a HR threshold for action potential alternans was demonstrated previously in isolated cat ventricular myocytes. However, in isolated cells, repolarization alternans increased monotonically with HR, whereas in the intact heart, cellular repolarization alternans typically failed to increase or even decreased at rapid rates (Figure 3). The explanation for this difference is the development of discordant alternans between cells, which caused electrotonic attenuation of cellular alternans, especially along bands of tissue that border neighboring regions of cells whose repolarization times alternate with the opposite phase (Figures 5 and 6).

Although we did not measure ionic currents, our results are consistent with the hypothesis that T-wave alternans is caused by beat-to-beat changes of the membrane ionic and intracellular processes that determine the time course of repolarization. Alternation of membrane potential was provoked above a threshold HR, which most likely corresponds to a time interval that is shorter than the recovery kinetics of one or more time-dependent currents. For example, APD shortening during alternans (as in Figure 2E, second beat at site AP2) followed a short diastolic interval and is potentially attributable to incomplete deactivation of outward potassium current or lesser activation of inward calcium plateau currents secondary to reduced action potential upstroke amplitude. It is expected, therefore, that pathological conditions that impair ion channel function may also reduce the HR threshold required to elicit T-wave alternans. This may explain why T-wave alternans was accentuated by hypothermia in this and other experimental studies and why T-wave alternans is observed at relatively slow HRs in patients at risk for sudden cardiac death but is provoked only by very rapid HRs in normal hearts. Further studies are required to determine...
how disease states lower the HR threshold for alternans and thereby increase vulnerability to arrhythmias.

Our data also suggest that alternation of action potential propagation is secondary to repolarization alternans, because propagation alternans never occurred in the absence of repolarization alternans and because conduction slowing on alternating beats typically followed prolongation of repolarization from the previous beat. Had propagation alternans caused repolarization alternans, the opposite relation between the phases of propagation and repolarization alternans would be expected. The effects of repolarization alternans on conduction were particularly important during discordant alternans (see Mechanism Linking T-Wave Alternans to the Initiation of Reentrant VF).

**Cellular Basis for ECG T-Wave Alternans**

The results of this study indicate that T-wave alternans arises from repolarization alternans at the level of the single cell. Although this is the first study to directly map patterns of cellular repolarization during T-wave alternans, earlier recordings from single ventricular sites support our finding that T-wave alternans is produced by alternations of membrane repolarization. Interestingly, a primary role of repolarization in the mechanisms of T-wave alternans was supported by a systematic investigation of T-wave alternans in humans in which T-wave alternans but not QRS alternans was common in high-risk arrhythmia patients.

It is important to emphasize that beat-to-beat alternation of propagation and repolarization are not equally reflected on the surface ECG. In general, propagation alternans causes accentuated alternation of the QRS complex, whereas repolarization alternans causes relatively subtle T-wave alternans. This apparent discrepancy can be explained by biophysical principles that state that the ECG is generated by spatial gradients in transmembrane potential, which are larger during the upstroke of the action potential compared with cellular repolarization, which is a slower process. Therefore, even subtle alternation of action potential propagation generates relatively large alternans of the ECG QRS complex. Conversely, relatively subtle T-wave alternans can be associated with marked alternation in the timing and amplitude of cellular repolarization. These data are relevant to recent findings that microvolt-level T-wave alternans is closely associated with susceptibility to sudden cardiac death in humans. Microvolt-level T-wave alternans was a feature of our experimental model (see Figure 4, 240 bpm and Figure 2B and 2C) and was associated with cellular alternans across large regions of the ventricle (Figure 4, 200 bpm). The ECG manifestation of cellular alternans is probably attenuated to a greater extent in patients in whom impedance barriers between the heart and body surface are much larger than those imposed by the perfusate-filled bath used in this study. Therefore, even subtle microvolt-level T-wave alternans in humans are most likely associated with considerable alternation of repolarization within the heart.

**Mechanism Linking T-Wave Alternans to the Initiation of Reentrant VF**

To the best of our knowledge, this is the first study to establish a mechanism directly linking T-wave alternans to the initiation of reentry. We found that repolarization alternans at the level of the single cell triggered a predictable cascade of events leading to VF. Discordant alternans, which was characterized by simultaneous prolongation and shortening of repolarization in different myocardial regions, was central to this mechanism. Recent data suggest that the phase of APD alternation in a cell (ie, prolongation versus shortening) is determined by the ionic properties of the cell and the timing of the stimulus used to induce alternans. We observed extensive inhomogeneities in the phase and amplitude of alternans between neighboring regions of cells, suggesting that the ionic currents that determine repolarization differ substantially between these regions so as to overcome electrotonic forces that ordinarily act to synchronize repolarization. Interestingly, the spatial pattern of discordant alternans was not random. Instead, cells typically alternated with the opposite phase on the base and apex of the heart (Figures 4 and 6). We recently found that APD restitution, which is an index of membrane ionic kinetics, also varies systematically from base to apex, further supporting a role of ion channel heterogeneity in the development of discordant alternans. One would predict, therefore, that pathological conditions that increase spatial heterogeneity of membrane ionic properties or impair coupling between cells may facilitate the development of discordant alternans.

Consequently, it is not surprising that Konta et al. reported a close association between discordant alternans of extracellular electrograms recorded over ischemic border zones and the development of VF. However, the mechanisms by which spatially discordant alternans facilitated the initiation of VF were not established. Our data indicate that discordant alternans is responsible for the development of steep spatial gradients (ie, dispersion) of repolarization of sufficient magnitude to cause unidirectional block and reentry. In contrast, concordant alternation between cells never produced substantial gradients of repolarization (Figures 7 and 8), unidirectional block, or VF. It is well established that a critical dispersion of repolarization is an important condition for the development of reentrant arrhythmias. The present study demonstrates that repolarization alternans is a property that is intrinsic to a cell and therefore does not require spatial dispersion of repolarization to develop. However, in the presence of relatively minor heterogeneities of repolarization properties between cells, discordant alternans can be transformed into discordant alternans, causing marked dispersions that form the substrate for VF.

Because discordant alternans and VF were initiated by pacing, it is important to consider the possibility that elevation of HR alone, rather than discordant alternans, contributed to the mechanism of initiation of VF. Our data do not support a major contributory role of HR, however, because VF occurred only in the presence of discordant and not concordant cellular alternans, irrespective of HR. Finally, in these studies, we did not simply identify an association between discordant alternans and VF but rather were able to directly map the interaction between repolarization gradients and propagating wave fronts that cause conduction block and reentry during discordant alternans (Figure 8).
Mechanism of T-Wave Alternans

The mechanisms responsible for initiating discordant alternans are unknown. One can speculate that physiological perturbations that are known to affect repolarization alternans, such as transient ischemia, PVCs, or sympathetic stimulation, may trigger discordant alternans in patients. Further studies aimed at delineating these mechanisms are expected to improve our ability to understand and potentially prevent the complex sequence of events that precipitate sudden cardiac death episodes in patients.

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References
