Electrophysiological Basis of Arrhythmogenicity of QT/T Alternans in the Long-QT Syndrome

Tridimensional Analysis of the Kinetics of Cardiac Repolarization

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Abstract—Tachycardia-dependent QT/T alternans occurs in patients with the congenital or idiopathic form of long-QT syndrome (LQTS) and may presage the onset of polymorphic ventricular tachyarrhythmias. To examine the electrophysiological basis of arrhythmogenicity of QT/T alternans in LQTS, the tridimensional repolarization pattern of QT/T alternans was studied in the anthopleurin-A model of LQTS, a surrogate for LQT3. In 11 anesthetized mongrel puppies, tridimensional repolarization and activation patterns were analyzed from 256 to 384 unipolar electrograms. Cardiac repolarization was evaluated as the activation-recovery interval (ARI) of local electrograms. To induce QT/T alternans, the pacing cycle length (CL) was abruptly shortened in steps of 50 ms from a basic drive of 1000 ms. ARIs were calculated at epicardial (Epi), midmyocardial (Mid), and endocardial (End) sites. ARI restitution at each site was assessed by using a single premature stimulation delivered after the basic drive. ARI alternans occurred at longer CLs at Mid sites compared with End and Epi sites, and the magnitude of alternans at Mid sites was greater. Two factors contributed to the modulation of ARI during QT/T alternans: (1) differences in restitution kinetics at Mid sites, characterized by larger $\Delta$ARI and a slower time constant ($\tau$), and (2) differences in diastolic intervals resulting in different input to restitution at the same constant CL. These 2 factors could explain not only the onset of alternans at Mid sites at longer CLs but also the critical observation that ARI dispersion between Epi and Mid sites during alternans was greater than during the slower basic CL. Marked ARI alternans could be present in local electrograms without manifest alternation of the QT/T segment in the surface ECG. This suggests that the phenomenon may be more prevalent than previously recognized and may represent an important marker of vulnerability to ventricular tachyarrhythmias.

It has long been known that tachycardia-dependent T-wave alternans occurs in patients with the congenital or idiopathic form of the long-QT syndrome (LQTS) and may presage the onset of polymorphic VTs known as torsade de pointes. The canine anthopleurin-A (AP-A) experimental model of LQTS has been well documented as a surrogate for the clinical LQT3. The onset of torsade de pointes in this model is characteristically bradycardia-dependent. The relatively long cardiac cycle is associated with a marked tridimensional dispersion of cardiac repolarization that provides the substrate for reentrant tachyarrhythmias. However, a previous report on the same model from our laboratory indicated that in some experiments an abrupt increase in heart rate was associated with manifest QT/T alternans in the surface ECG and the onset of VT. Those experiments may represent a counterpart to clinical observations. In the present report, a systematic study was performed to investigate tridimensional dispersion of repolarization associated with tachycardia-dependent QT/T alternans in this model and

Key Words: QT/T alternans \(\bullet\) long-QT syndrome \(\bullet\) ventricular tachyarrhythmia
the electrophysiological basis for the arrhythmogenicity during QT/T alternans in general.

Materials and Methods

Surgical Preparation

The present study was approved by the animal studies subcommittee of the local institutional review board and conformed to the guiding principles of the Declaration of Helsinki. Experiments were performed on 11 purpose-bred mongrel puppies, 12 to 14 weeks old, weighing 4.0 to 6.8 kg. Puppies were preanesthetized with sodium thiopental (17.5 mg/kg IV) via the cephalic vein. Puppies were then intubated and anesthetized with 1.0% to 2.0% isoflurane (vaporized in 100% O2) via anesthesia machine (F500, The Forreger Co). Catheters were inserted into the femoral vein for administration of fluids and drugs and into the femoral artery to monitor blood pressure. ECG leads I, aVF, and V1, and blood pressure (Statham transducer, Gould, Inc) were continuously monitored on a physiological recorder (VR12, PPG Industries). The heart was exposed through a midsternotomy. After instrumentation and electrode placement (see the following section), the sternotomy was approximated and covered with a plastic sheet. Intrathoracic and core temperatures were continuously monitored using an electronic thermometer (Yellow Springs Instrument Co) and maintained constant at 37°C by the use of a thermostatically controlled thermal blanket and heat lamp. Warm (37°C) saline was applied intermittently to the heart to moisten the epicardium and prevent surface cooling.

To obtain a slow heart rate, complete atrioventricular (AV) block was accomplished by radiofrequency catheter ablation, and the ventricles were paced through bipolar stainless-steel wire electrodes connected to a digital stimulator (DTU-101, Bloom Inc). In 2 experiments, the AV junctional escape rhythm after the induction of AV block had a cycle length (CL) of <1000 ms. In these experiments, vagal stimulation was applied to slow the escape rhythm and to allow ventricular pacing at a CL of 1000 ms. Vagal stimulation was accomplished by insertion of polyimide-coated silver wire (75-μm diameter), which was exposed 2 to 3 mm at the tip, into the right and left cervical vagosympathetic trunks. A square pulse of 0.3 ms was delivered at 0.1 to 3 V and a frequency of 20 Hz. We have already reported that the behavior of the experimental model is not different whether bradycardia is induced by the induction of AV block or by vagal stimulation.12

Recording Electrodes and Electrode Localization

Thirty-two to 48 plunge needle electrodes were inserted throughout both ventricles (Figure 1). Needle electrodes were fabricated with 50-μm-diameter polyimide-coated tungsten wires contained within a 21-gauge stainless-steel needle. Left ventricular plunge electrodes consisted of 8 unipolar electrodes; each pole was separated by 1 mm. Right ventricular electrodes contained 4 to 6 unipolar electrodes, each separated by 1 mm. Septal electrodes contained 10 unipolar electrodes grouped in pairs; each pole was separated by 1 mm, and each pair was separated by 2 mm. The most proximal electrode was located ~0.5 mm from the epicardial surface. Plunge electrodes were placed throughout the heart, and the distance between plunge electrodes was 4 to 8 mm. Transmural unipolar electrograms were simultaneously recorded from the epicardial (Epi), midmyocardial (Mid), and subendocardial (End) sites by a computerized mapping system. Details of the recording methods and data acquisition system were previously reported.10

Data Acquisition and Isochronal Mapping

Unipolar electrograms were acquired using 3 variable-gain 128-channel multiplexed data acquisition systems (DSC 2000, INET Corp), allowing simultaneous recording of 256 to 384 channels. Each electrogram was amplified and analog-filtered with a fixed high-pass setting of 0.05 Hz and an adjustable low-pass setting of 500 Hz. The analog data were digitized with 12-bit resolution at a sampling rate of 1000 to 2000 samples per second per channel. The digitized signals were then stored on hard disk on an IBM-compatible computer system (486PC, Touche Co). Where indicated, digitized electrograms were further filtered off-line with a digitally implemented Butterworth filter. Activation times were determined using previously published criteria.11,12 Computer-generated isochrones of activation were derived from the activation time data and delineated by closed contours at 20-ms intervals beginning with the earliest detected site of activation. For the whole ventricular activation maps, zones of functional unidirectional conduction block were identified by using previously defined criteria.14 A continuous line, or surface, was drawn through these regions and was defined as a zone of functional conduction block.

Activation-Recovery Intervals

Activation-recovery intervals (ARIs) were defined as the interval between the time of minimum first derivatives of the intrinsic deflection and the maximum first derivative of the T wave of unipolar electrograms.15,16 For recovery time determination, T waves were low-pass-filtered with a digital 8-pole Butterworth filter (frequency, 50 Hz) before computation of temporal derivatives. Previous experimental studies have shown that ARIs derived from unipolar electrograms reasonably approximate the local effective refractory period regardless of the T-wave morphology.15–18 We have previously demonstrated an excellent correlation of ARI and effective refractory period in the AP-A model of LQTS.10 Furthermore, a recent study by Shimizu and Antzelevitch15 in the perfused wedge preparation has shown good correspondence between Vmax of the T wave of unipolar electrograms and V rim of the phase 3 action potential in the presence of different T-wave morphologies.18

Drug Administration

To simulate LQT3, AP-A was used.15 AP-A was dissolved in 0.9% sterile saline and administered as an intravenous bolus of 25 μg/kg.
followed by a maintenance dose of 1.0 μg/kg per minute. Wild-type AP-A produced by a bacterial expression system (provided by Dr. Blumenthal, University of Cincinnati, College of Medicine, Cincinnati, Ohio) was used in this study. In 4 of the 11 puppies, the stimulation protocol was applied in the absence of AP-A, and these experiments served as a control. In 2 of these 4 puppies, the same stimulation protocol was also applied after AP-A administration. In the remaining 7 puppies, data were collected only after AP-A.

**Stimulation Protocol**

Approximately 1 hour was required after induction of AV block for the insertion of needle electrodes and for the electrograms to stabilize before the pacing protocol was conducted.

Bipolar stimulation was used to stimulate the heart. Diastolic threshold was determined during a basic CL (S1S1) of 1000 ms, with the insertion of needle electrodes and for the electrograms to stabilize before the pacing protocol was conducted. The shortest paced CL was 250 ms in control experiments. Approximately 1 hour was required after induction of AV block for the insertion of needle electrodes and for the electrograms to stabilize before the pacing protocol was conducted.

Stimulation Protocol

Bipolar stimulation was used to stimulate the heart. Diastolic threshold was determined during a basic CL (S1S1) of 1000 ms, with increasing current steps of 0.05 mA at a 2.0 ms square pulse width. Once diastolic threshold was determined, the current level was increased to 1.5 times diastolic threshold. Maximum diastolic threshold was always <0.6 mA.

After administration of AP-A, programmed electrical stimulation was performed. To obtain a stable basic state, the heart was driven for 50 beats at a basic CL of 1000 ms before starting each stimulation protocol.

Two pacing protocols were used in the present study. To study the onset of QT/T alternans, the pacing CL was abruptly shortened from a steady-state rate of 1000 ms in steps of 50 ms for 10 to 15 s at each new CL. The shortest paced CL was 250 ms in control experiments and 300 ms after AP-A. In the present study, the basic driven beat was defined as S1, and the rapid pacing beats were defined as P1, P2, etc. In the second protocol, restitution of ARI was determined after AP-A by using single premature ventricular stimulation delivered after every 28th basic beat at a CL of 1000 ms (S1S2 protocol). The S1S2 interval was progressively decreased by 5 to 50 ms from 1000 ms to determine restitution properties and effective refractory periods at each test site.

**Data Analysis**

In the abrupt CL shortening protocol, ARIs were measured at 3 different layers of the left ventricle (End, Mid, and Epi), and the paced CL associated with the onset of local ARIs alternans at each site was determined. Alternans of ARI was defined as a >10-ms difference between 3 consecutive beats. The alternans of local and surface QT/T wave alternans on abrupt shortening of pacing CL. Shown are surface ECG leads I, aVF, and V1 and 5 unipolar recordings from a needle electrode in the left ventricular free wall. The QT intervals on the surface ECG were measured as the time between the QRS onset and the point at which the terminal slope of the T wave crossed the baseline.

During abrupt CL shortening, transmural dispersion of ARI was measured as the maximum difference of ARI between End and Mid sites (marked by stars) with conduction only to the Epi site. There was a reversal of the gradient of ARI dispersion between Epi and Mid/End sites in alternate beats associated with alternation of polarity of intramyocardial QT and a manifest alternation of the configuration of QT in surface leads. The artifacts on the surface ECG recordings were secondary to vagal stimulation.
Origin 5.0 (Microcal Software, Inc) to the following equation:

\[
ARI(t) = ARI_{\text{max}} - DARI \times \exp\left(-\frac{t}{t}\right),
\]

where \( ARI_{\text{max}} \) represents ARI during the plateau of restitution, \( ARI(t) \) is the ARI of the DI preceding S2, and \( DARI \) and \( t \) are the amplitude and time constant, respectively. 20,21 \( ARI_{\text{max}}, DARI, \) and \( t \) were compared in each layer of the left ventricle, and the relationship between the restitution and QT/T wave alternans induced by abrupt CL shortening was examined.

Finally, tridimensional activation maps were constructed during episodes of spontaneous ventricular arrhythmias that developed after QT/T wave alternans. The role of QT/T wave alternans in the initiation of arrhythmias was examined. To facilitate the induction of arrhythmias during QT/T alternans, the basic paced CL of 1000 ms was sometimes changed to a longer or shorter CL, as will be noted in Results.

Statistical Analysis
Statistical analysis was performed by the Student \( t \) test, ANOVA for multivariate and repeated designs, and the Scheffé multiple range post hoc test where appropriate. 22 A Kolmogorov-Smirnov goodness of fit test for normal distribution was used to verify normal distribution of data before performing ANOVA. A value of \( P < 0.05 \) was considered statistically significant. Values are presented as mean ± SD.

Results
Induction of QT/T Alternans on Abrupt CL Shortening After AP-A
The tridimensional changes in ARI distribution during abrupt CL shortening were analyzed using a basic CL of 1000 ms to CLs of 300 to 900 ms, in steps of 50 ms. Figure 2 illustrates representative recordings from a needle electrode in the left ventricular free wall. During the basic drive, the ARIs were more prolonged at Mid and End sites compared with the Epi site, with an Epi-Mid ARI dispersion of 72 ms. The ARIs were constant from beat to beat (±10 ms variation) throughout the left ventricular wall. On slight shortening of the paced CL to 900 ms, there was shortening of ARIs at all sites compared with the basic CL. Mid sites started to show alternans of ARI (±10 ms), whereas ARIs at End and Epi sites remained constant. On further shortening of the paced CL to 800 ms, ARIs at End and Mid sites showed alternans, with the degree of ARI alternans being greater at the Mid sites; ARIs after the second short paced cycle (P2) was greater than during the basic CL of 1000 ms (90 versus 72 ms). With this degree of alternans, the QT-wave configuration in surface ECG leads showed no discernible change, and the QTI showed only a 20-ms alternation. On further shortening of the paced CL to 600 ms, the Epi site also began to show alternans. Conduction block occurred during the first short paced cycle (P1) in End and Mid sites, with conduction only to the Epi site. Because of the difficulty in distinguishing conduction block from slowed conduction in unipolar electrograms, some of the electrograms designated...
as conduction block may actually represent slowed conduction. The magnitude of ARI alternans was much greater at End and Mid sites compared with the Epi site. This resulted in shorter ARIs at End-Mid sites compared with the Epi site during the odd cycles, which were associated with shorter QTIs (P₃, P₅, etc). In other words, the ARI gradient reversed between Epi and Mid sites in alternate cycles. However, the degree of Epi-Mid ARI dispersion during cycles associated with the longer QT was consistently larger than that during the reversal of Epi-Mid gradient of ARI at beats associated with the shorter QT. At a CL of 600 ms, the surface ECG lead showed manifest alternation of QT-wave configuration as well as a marked alternation of the QT. A consistent observation was that a manifest alternation of QT-wave configuration was seen in the surface ECG when there was a reversal of polarity of intramyocardial QT waves associated with reversal of the gradient of ARIs between Epi and Mid/End sites.

**Time Course of QT/T Alternans**

In the AP-A model, ARI alternans gradually decreased during successive short CLs. ARI alternans decreased faster at Epi sites compared with End and Mid sites. Figure 3, obtained from the same experiment shown in Figure 2, illustrates a recording from a different needle electrode site in the left ventricular free wall during abrupt pacing at a short CL of 600 ms. The figure shows that ARI alternans at the Epi site disappeared after P₆, whereas ARI alternans continued at Mid and End sites up to P₈ (not shown in the figure). A significant observation was that even though the magnitude of ARI dispersion between Epi and Mid sites gradually decreased during successive cycles, the dispersion could remain greater at certain sites than during the slower basic rhythm for several beats (compare the 2 sites shown in Figures 2D and 3A). The degree of ARI dispersion at Epi sites was small, and alternans disappeared faster than at the Mid and End sites. The magnitude of ARI alternans between Epi and Mid sites gradually decreased during successive cycles; however, the dispersion remained greater than during S₁S₂ basic rhythm up to P₄. Panel B is a graphic illustration of mean ± SEM of ARI dispersion between Mid and Epi sites and between Mid and End sites during successive short CLs of 600 ms from 12 different sites from the left ventricular free wall from the same experiment shown in Figures 2D and 3A. The magnitude of ARI alternans between Epi and Mid sites remained significantly greater (P<0.02) for even-paced beats than during the slower S₁ basic rhythm up to P₆. The degree of ARI dispersion between Epi and Mid sites after odd-paced beats (P₃, P₅, P₇, etc), associated with reversal of the gradient between the 2 sites, usually dissipated earlier during successive short cycles.

**Characterization of Restitution of ARI and Its Relation to Onset of QT/T Alternans**

The observation that the onset of ARI alternans at End, Mid, and Epi sites that occurred at different CLs could result from differences in the kinetics of restitution of ARI in each zone was examined. This is illustrated in Figures 4 through 6, obtained from a different experiment. Figure 4 is organized similar to Figure 2 and shows recordings from 3 contiguous needle electrodes (within 7 mm from each other) from the left ventricular free wall. The figure illustrates the onset of alternans of ARI at Mid, End, and Epi sites during abrupt shortening of CLs to 800, 700, and 600 ms, respectively. The degree of Epi-Mid ARI dispersion was different among the 3 needle sites. However, the figure emphasizes the 2 key observations shown in Figure 2: (1) the Epi-Mid dispersion of ARI at the short cycle of 600 ms was consistently greater than the dispersion during the basic CL of 1000 ms, and (2) during abrupt shortening of CL to 600 ms, there was a reversal of the Epi-Mid gradient of dispersion of ARI in alternate cycles.

Figure 5 illustrates the S₃S₄ protocol for analysis of ARI restitution of sites labeled End, Mid, and Epi from the 3 needle electrodes shown in Figure 4. ARIs became gradually shorter as the S₃S₄ coupling interval decreased. At a long S₃S₄...
Figure 4. Recordings obtained from a different experiment showing the onset of ARI alternans at different paced CLs at 800, 700, and 600 ms at Mid, End, and Epi sites from 3 different needle electrodes in the left ventricular wall. The duration of ARI during S₁ and the magnitude of ARI alternans were different at the 3 sites. However, at all sites, the degree of ARI alternans at Mid/End sites was greater than at Epi sites. The ARI dispersion between Epi and Mid sites was greater during P₂ and P₄ than during the basic beat S₁ at sites A and B. There was a reversal of the gradient of ARI dispersion between Epi and Mid/End sites in alternate beats at a CL of 600 ms at all sites.
Figure 5. Selected recordings obtained from sites A to C shown in Figure 4 that illustrate the S1S2 protocol for determination of ARI restitution. Note that at an S1S2 of 900 ms, there was a 43- to 64-ms dispersion of ARI between Epi and Mid sites. The dispersion markedly diminished at intermediate S1S2 of 650 ms (−9 to 15 ms) and reappeared on further shortening of S1S2 to 500 ms but with reversal of the gradient of ARI dispersion between the 2 sites (−18 to −52 ms). Vertical lines in each S2 beat at panel A indicate the maximum negative derivative of the QRS complex and the maximum positive derivative of the T wave.
interval, there was a dispersion of ARI of the S₂ beat between Epi and Mid sites. At intermediate S₁S₂ intervals, the dispersion of ARIs across the left ventricular wall decreased. On further shortening of the S₁S₂ interval, the Epi-Mid dispersion of ARI increased again but with reversal of the gradient between the 2 sites. Figure 6 displays ARI restitution curves at the End, Mid, and Epi sites from the 3 needle electrodes A to C shown in Figure 5 and shows that the restitution kinetics were remarkably similar among the 3 Epi sites. The same was true for the Mid and End sites. However, the restitution parameters differed between Mid, Epi, and End sites. The Mid sites had a longer ARImax, larger ΔARI, and longer τ compared with the Epi sites. Restitution parameters of the End sites were intermediate between Mid and Epi sites.

In 5 of the 9 experiments after AP-A administration, an S₁S₂ protocol was applied for analysis of restitution kinetics. In each of the 5 experiments, recordings from 12 needle electrode sites were analyzed. The sites were selected only from the mid zones of the left ventricular free wall, which were shown to exhibit prominent M cell–like behavior in this experimental model. Table 1 shows the exponential parameters of restitution curves at End, Mid, and Epi sites from each of the 5 experiments as well as grouped data from all experiments. Tests for normal distribution found that no parameter was significantly different, as assessed by the Lilliefors (normal and standardized) distribution. ANOVA was performed for the 3 parameters by layer for each experiment and was statistically significant for each experiment. A double–repeated measures ANOVA analysis (12 repeated measures in each site and 5 repeated experiments) comparing sites (End versus Mid versus Epi) was performed and showed that significant differences existed between sites (see Greenhouse-Geisser epsilon P values at the right of each parameter). Scheffé multiple range post hoc tests for the 3 parameters of the restitution curves across all 5 experiments were also statistically significant (P values between sites for mean±SD values at bottom of Table 1), except for the τ value between the Mid and End sites.

The longer CL associated with the development of alternans of ARI at Mid sites compared with the Epi site could be explained by (1) differences in restitution kinetics of ARI (larger ΔARI and longer τ at Mid sites) and (2) differences in the DIs associated with the onset of alternans at both sites. Figure 7 depicts the restitution curve of the Mid and Epi sites...
from needle electrode A shown in Figure 5 to graphically illustrate how the combination of these 2 factors would explain the onset of ARI alternans at Mid sites at 800 ms versus 600 ms for the Epi site, as shown in Figure 4A. At a CL of 800 ms, the DI preceding P1 was 316 ms at the Mid site, whereas the DI was longer, at 373 ms, at the Epi site. The DI of 316 ms corresponded on the restitution curve to a 57-ms shortening of ARI at the Mid site (Figure 7A) and with the onset of QT/T alternans at this site. On the other hand, the DI of 373 ms was associated with only a 22-ms shortening of ARI at the Epi site (Figure 7B). In other words, the value was close to the steady-state ARI at this site, and no ARI alternans was observed at the Epi site. At a CL of 600 ms, the DI at Mid sites was 116 ms and corresponded to a marked shortening of ARI (170 ms). The DI at the Epi site was 175 ms and was associated with a 68-ms shortening of ARI and the onset of alternans at this site. Because of the characteristics of restitution kinetics at Mid sites as well as the shorter DI at these sites compared with Epi sites, the critical degree of CL shortening required for the onset of alternans was achieved at a longer CL at Mid sites. The same 2 factors could also explain the increase in the magnitude of dispersion of ARI between Mid and Epi sites, especially at CLs equal to or shorter than those associated with the onset of alternans at Epi sites.

Control Data

Figure 8 illustrates the results of abrupt shortening of the CL in one of the 4 control experiments, and Figure 9 compares the control results with those obtained after the administration of AP-A in the same experiment. Figure 8 represents recordings obtained from a plunge needle electrode in the left ventricular free wall during abrupt shortening of the CL from 1000 ms to 600, 400, 300, and 250 ms, respectively. During S1 of 1000 ms, the ARI at Mid sites was 25 ms longer than that at the Epi site. Alternation of ARI was not observed at any site, even during the short cycles of 250 and 300 ms. At a CL of 250 ms, the ARI shortened after P1 and showed further shortening after P2. In 2 other control experiments, there was a brief (3 consecutive cycles) period of alternans of ARI at Mid sites of 11 to 20 ms at a CL of 250 ms. The Mid-Epi ARI dispersion at 1000 ms ranged from 16 to 27 ms (mean±SD, 18±8 ms) in the 4 control experiments and became gradually smaller at short CLs. Abrupt shortening of CL did not induce ventricular arrhythmias in any of the 4 control experiments. In 1 of the 2 experiments, the abrupt shortening of CL protocol was applied before and after AP-A, and ventricular arrhythmias were induced only after AP-A.

### Table 1. Exponential Parameters of Restitution Curves in the AP-A Model of LQTS

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No.</th>
<th>End</th>
<th>Mid</th>
<th>Epi</th>
<th>ANOVA</th>
<th>ΔARI, ms</th>
<th>End</th>
<th>Mid</th>
<th>Epi</th>
<th>ANOVA</th>
<th>τ, ms</th>
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<tr>
<td>1</td>
<td></td>
<td>442.4±13.6</td>
<td>463.2±10.3</td>
<td>426.4±9.6</td>
<td>P&lt;0.0001</td>
<td>272.1±12.5</td>
<td>285.2±9.9</td>
<td>262.9±13.2</td>
<td>P&lt;0.0001</td>
<td>138.7±7.1</td>
<td>156.8±8.4</td>
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<tr>
<td>4</td>
<td></td>
<td>476.0±37.9</td>
<td>534.3±47.8</td>
<td>443.5±13.3</td>
<td>P&lt;0.0001</td>
<td>294.5±39.8</td>
<td>334.1±43.8</td>
<td>284.0±20.1</td>
<td>P&lt;0.0001</td>
<td>298.6±10.3</td>
<td>344.1±17.1</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>444.9±28.1</td>
<td>471.5±12.6</td>
<td>418.2±27.6</td>
<td>P&lt;0.0001</td>
<td>258.4±22.6</td>
<td>273.5±11.6</td>
<td>245.5±13.6</td>
<td>P&lt;0.0001</td>
<td>168.6±8.9</td>
<td>182.8±7.2</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>476.2±10.2</td>
<td>509.7±22.9</td>
<td>443.8±17.3</td>
<td>P&lt;0.0001</td>
<td>306.2±26.7</td>
<td>329.5±26.4</td>
<td>284.9±24.2</td>
<td>P&lt;0.0005</td>
<td>270.4±21.7</td>
<td>311.9±21.4</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>545.9±24.1</td>
<td>578.8±44.4</td>
<td>445.5±19.9</td>
<td>P&lt;0.0001</td>
<td>326.1±16.3</td>
<td>375.0±41.3</td>
<td>271.6±20.7</td>
<td>P&lt;0.0001</td>
<td>323.1±31.7</td>
<td>372.7±27.7</td>
</tr>
</tbody>
</table>

For experiments 1, 4, 6, 7, and 8, n=12 each for End, Mid, and Epi. For mean±SD values (at bottom of table), n=60 each for End, Mid, and Epi. P values associated with mean±SD values (at bottom of table) are by Scheffe multiple range post hoc tests.
Spontaneous reentrant ventricular arrhythmias were observed in 4 of 9 AP-A experiments (44%) after abrupt shortening of CL associated with QT/T alternans and the increase in Mid-Epi dispersion of ARI (Table 2). Ventricular arrhythmias ranged from 1 or 2 reentrant beats in 1 experiment to sustained polymorphic VT requiring cardioversion in the 3 other experiments. Reentrant arrhythmias were consistently initiated only by the paced beats that followed the ones associated with greater Mid-Epi dispersion of ARI (P3, P5, and P7). This is illustrated in Figure 9, obtained from the same experiment in which recordings were obtained before and after AP-A. The figure shows surface ECG leads during abrupt shortening of a basic CL of 1000 ms to 400 ms. In Figure 9A, a single reentrant beat (R) followed P3; in Figure 9B, a single reentrant beat followed P5; and in Figure 9C, 2 reentrant beats followed P7. Figure 10 shows recordings from the same needle in the left ventricular free wall during control and abrupt shortening of the CL to 400 ms in panel A and after AP-A and abrupt shortening of CL to 500 ms in panel B and to 400 ms in panel C. The latter protocol induced 2 reentrant beats after P7. The Mid-Epi dispersion of ARI at 1000 ms during control was 25 ms and decreased gradually at shorter CLs.

Figure 8. Recording obtained from a plunge needle electrode in the left ventricular free wall from a control experiment showing the results of abrupt shortening of the pacing CL from 1000 ms to 600, 400, 300, and 250 ms. Shown are surface ECG lead aVF and 5 unipolar electrograms from Epi, Mid, and End sites. ARIs are shown in milliseconds for each site during basic drive (S1) and abrupt shortening of the paced CL (P). ARI alternans (defined as a >10-ms difference between 3 consecutive beats) was not observed at any site, even during the short CL of 250 ms. The Mid-Epi dispersion of ARIs at 1000 ms was 25 ms and decreased gradually at shorter CLs.

Q/T Alternans and Initiation of Reentrant Ventricular Arrhythmias

Spontaneous reentrant ventricular arrhythmias were observed in 4 of 9 AP-A experiments (44%) after abrupt shortening of CL associated with QT/T alternans and the increase in Mid-Epi dispersion of ARI (Table 2). Ventricular arrhythmias ranged from 1 or 2 reentrant beats in 1 experiment to sustained polymorphic VT requiring cardioversion in the 3 other experiments. Reentrant arrhythmias were consistently initiated only by the paced beats that followed the ones associated with greater Mid-Epi dispersion of ARI (P3, P5, and P7). This is illustrated in Figure 9, obtained from the same experiment in which recordings were obtained before and after AP-A. The figure shows surface ECG leads during abrupt shortening of a basic CL of 1000 ms to 400 ms. In Figure 9A, a single reentrant beat (R) followed P3; in Figure 9B, a single reentrant beat followed P5; and in Figure 9C, 2 reentrant beats followed P7. Figure 10 shows recordings from the same needle in the left ventricular free wall during control and abrupt shortening of the CL to 400 ms in panel A and after AP-A and abrupt shortening of CL to 500 ms in panel B and to 400 ms in panel C. The latter protocol induced 2 reentrant beats after P7. The Mid-Epi dispersion of ARI at 1000 ms during control was 25 ms and markedly increased to 121 ms after AP-A. In Figure 10B, after AP-A, abrupt shortening of CL to 500 ms resulted in marked alternans of ARI at Mid and End sites compared with the Epi site, but the Mid-Epi dispersion of ARI after even-paced beats (P3 and P5) was not significantly different from that of S1. Abrupt shortening of the CL to 500 ms did not initiate reentrant arrhythmias. On the other hand, shortening of the CL to 400 ms consistently induced 1 or 2 reentrant beats, as shown in Figure 9. However, it was not possible to calculate precisely the ARI at the short CLs associated with arrhythmias, especially at the critical sites associated with reentry, because of the superimposition of the depolarization complex on the QTI. Nevertheless, Figure 10C illustrates the development of conduction block during the short CL of 400 ms between the Epi and Mid/End sites during P5, P7, and P11, with delayed activation during P5 and P11. After P5, premature activation of the Epi site occurred consistent with reentrant excitation. Needle recordings from nearby sites demonstrated diastolic...
activation, bridging the interval between the delayed activation at Mid sites and spontaneous reactivation at the Epi site in this needle recording. The 2 Mid electrograms at the bottom of Figure 10C were recorded from a different site and illustrate the diastolic bridging of activation between the first and second reentrant beats. However, a complete reentrant circuit could not be accurately mapped from available recordings in this experiment.

Figure 11 shows surface ECG leads from 2 other experiments in which abrupt shortening of ARI initiated reentrant polymorphic VT. In panel A, shortening of CL from 1000 to 600 ms initiated VT after P3; in panel B, shortening the CL from 700 to 350 ms initiated VT after P5. Figure 12 illustrates tridimensional activation maps from the same experiment shown in Figure 11B. Figure 12, top, shows the activation map and selected electrograms of the control paced rhythm (S1) at a CL of 700 ms. Figure 12, bottom, illustrates the activation map of the P5 beat that initiated a reentrant tachyarrhythmia. Activation started at the pacing site in the septal region of section 3, and the activation wavefront circulated around arcs of functional conduction block between Epi and Mid sites in sections 4 and 5 before reactivating a subepicardial site in section 4 at the 220-ms isochrone. Note that abrupt shortening of the CL to 350 ms resulted in alternans of ARIs, which was more marked at Mid sites E to H compared with Epi sites B, C, I, and J. During the first 4 paced short cycles, the paced wave front was conducted to sites A to J. However, after the fifth short cycle, the paced wavefront was conducted to sites B, C, I, and J with short ARIs and was blocked at sites between C and F/G with the longest ARI. In this particular example, there was a slight (35-ms) alternation of the cardiac CL may explain the greater degree of ARI prolongation at sites D to H during P4 compared with P2, which was a factor in the spontaneous initiation of VT after P5.

**Discussion**

The present study investigated the phenomenon of QT/T alternans, which occurs during abrupt shortening of CL in a well-characterized model of LQT3. We have shown that QT/T alternans was associated with a greater degree of dispersion of repolarization (estimated as differences in ARIs) compared with longer CLs with longer QT but no alternans. The ARI dispersion was most marked between Mid and Epi zones in the left ventricular free wall. In the presence of a critical degree of ARI dispersion, propagation of the activation wave front during the basic impulse could be
blocked between these zones to initiate reentrant excitation and polymorphic VT.

Mechanism of Increased Dispersion of Repolarization During QT/T Alternans

Two factors contributed to the modulation of ARI during QT/T alternans, resulting in a greater magnitude of dispersion of ARI between Mid and Epi zones at critical short CLs compared with basic rhythm. These are (1) differences in restitution kinetics at Mid sites, characterized by larger ΔARI and a slower time constant (τ) compared with Epi sites and (2) differences in the DI that would result in different input to the restitution curve at the same constant CL. The longer ARI of Mid sites resulted in shorter DI during the first short cycle and thus a greater degree of ARI shortening. As shown in Figure 7, the combination of these 2 factors could explain the onset of ARI alternans at Mid sites at critical short CLs compared with Epi sites. The same 2 factors also explain the observation that the greater magnitude of dispersion of ARIs between Mid and Epi sites was seen at CLs equal to or shorter than those associated with the onset of alternans at Epi sites. Another factor that could contribute to the beat-to-beat modulation of transmural dispersion and QT/T alternans is the possibility that only M cells, because of their weaker net repolarizing current, are capable of developing alternans and that the alternans seen in Epi represented the electronic effect of the M cells.

Electrophysiological Mechanism of Arrhythmogenicity of QT/T Alternans

In contrast to uncertainties regarding the ionic mechanisms underlying the differences in restitution kinetics across the left ventricular wall in the dog, the present study provides strong evidence for an electrophysiological mechanism of reentrant arrhythmias associated with QT/T alternans in the AP-A model of LQTS. The results of the present study clearly demonstrate that QT/T alternans is associated with a greater degree of spatial dispersion of repolarization compared with slower rates associated with longer but constant QT/T intervals. The magnitude of spatial dispersion of QT/T can be such that conduction block and reentrant excitation could develop during a fixed rate associated with alternans. In the present study, reentrant excitation was only initiated by the odd-paced beats (P3, P5, and P7) that followed even-paced beats.

Figure 10. Recordings obtained from the experiment shown in Figures 8 and 9 that compare transmural electrograms from the same site in the left ventricular free wall before and after AP-A. Shown are ECG lead aVF and unipolar electrograms from Epi, Mid, and End sites. Panel A shows a recording during control and abrupt shortening of CL from 1000 to 400 ms. In panel B, recordings were obtained after AP-A during abrupt shortening of CL from 1000 to 500 ms that resulted in marked ARI alternans at Mid and End sites compared with the Epi site. Note that the Epi-Mid dispersion of ARIs during S1 increased from 24 ms during control to 121 ms after AP-A. However, in panel B, the degree of Epi-Mid dispersion of ARI after even-paced beats (P2 and P4) was not significantly different from S1, and no reentrant rhythms were induced. On the other hand, in panel C, shortening of the CL to 400 ms induced 2 reentrant (R) beats after P7. Note the development of conduction block during the short CL of 400 ms between Epi and Mid/End sites during odd-paced beats (P3, P5, and P7) and the occurrence of delayed activation at the Mid/End sites during P3, P5, and P7. After P7, the Epi site was prematurely activated. Diastolic bridging consistent with reentrant activation was seen in neighboring sites. The 2 Mid electrograms at the bottom of panel C were recorded from a different site and illustrate the diastolic bridging between the first and second reentrant beats. E indicates electrotonic deflections synchronous with the activation potentials in nearby electrograms.
(P₂, P₄, and P₆) that were associated with a greater degree of dispersion of ARI. The beat that initiated reentrant excitation encroached on the spatial dispersion of repolarization of the preceding beat, resulting in arcs of functional conduction block and circulating wave fronts to initiate the first reentrant cycle. Because of the heterogeneity of repolarization associated with QT/T alternans, reentrant excitation would be expected to be perpetuated in the form of fast polymorphic VT (Figures 11 and 12), underscoring the serious nature of the arrhythmogenic substrate associated with this phenomenon. Although in the present study a premature beat was not required to initiate reentry, this, of course, does not mean that premature depolarization at a similar coupling interval will be more arrhythmogenic in the presence of QT/T alternans than in its absence. Furthermore, in the present model, the spatial dispersion of repolarization was maximal between Mid and Epi sites. However, in other species, e.g., the guinea pig, dispersion of repolarization at the epicardial surface may play an important role.

**Clinical Correlates**

In the present study, the occurrence of VT was usually preceded by manifest QT/T alternans in the surface ECG. However, as shown in Figure 2, during cardiac CLs associated with minimally discernible alternans of the QT/T in the surface ECG, the magnitude of dispersion of ARIs between Mid and Epi sites could be large and could certainly provide the substrate for reentrant arrhythmias. This was usually seen at CLs associated with marked ARI alternans in Mid and End zones but slight or no alternans in Epi zones. On the other hand, once significant alternans at the Epi zone developed, it was usually associated with reversal of the ARI gradient between Epi and Mid/End zones. This was associated with alternans of the configuration of the intramyocardial QT wave as well as alternans of the QT/T wave in the surface ECG.

Our observations shed some light on the possible electrophysiological mechanism of VT that often follows QT/T alternans in the clinical LQTS. However, there are significant differences between the experimental model and the clinical observation. QT/T alternans is more stable and persists longer in the clinical setting and frequently does not require the degree of abrupt CL shortening used in the present study. The distinction is also important from the mechanistic point of view, since ionic currents that determine action potential alternans during an abrupt change in CL may be different from those that determine restitution.

**Study Limitations**

In addition to what is discussed above, the AP-A model of LQTS has other limitations that may affect the general applicability of the data to the clinical situation. For example, although the AP-A model is considered a suitable surrogate for the clinical LQT3, abnormalities of the Na⁺ channel inactivation are fixed and are not dependent on drug binding to the channel in LQT3. Although in the low-affinity binding of the AP-A to the Na⁺ channel at depolarized potentials, the model may not be suitable for analysis of the kinetics of restitution during successive short cycles. However, this will not significantly affect the restitution kinetics of the first short cycle. In some experiments, a basic CL of 1000 ms was not long enough to achieve a steady-state ARI at the Mid/End zone. These sites were not included in the analysis. Although a longer basic paced CL would have been preferable, such CLs were difficult to maintain because of the underlying idioventricular rhythm. Finally, we have already alluded to the differences in restitution kinetics in different species. Similarly, differences may exist in the presence and distribution of M cells. Thus, caution should be exercised during extrapolating some of our data to other animal species.
Figure 12. Top, Tridimensional activation map during control paced rhythm at 700 ms (S1) from the same experiment shown in Figure 11B. Activation began at the pacing site (indicated by star) in section 3. The total ventricular activation time was 80 ms, and there were no arcs of functional conduction block. Selected electrograms are shown on the right panel. There was no QT/T alternans at this CL at any site. Bottom, Recordings from the same experiment during abrupt shortening of the CL to 350 ms (P). The activation map of the P5 beat that initiated reentrant excitation is shown on the left. Selected electrograms along the reentrant pathway that demonstrate complete diastolic bridging are shown on the right. Note the development of QT/T alternans, which was more marked at Mid sites E to H compared with Epi sites B, C, I, and J. The reentrant wave front circulated around arcs of functional conduction block between Epi and Mid sites in sections 4 and 5 (represented by heavy solid lines) before reactivating a subepicardial site in section 4 at the 220-ms isochrone.
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