Cellular and Ionic Basis for T-Wave Alternans Under Long-QT Conditions
Wataru Shimizu, MD, PhD; Charles Antzelevitch, PhD

Background—T-wave alternans (TWA), an ECG phenomenon characterized by beat-to-beat alternation of the morphology, amplitude, and/or polarity of the T wave, is commonly observed in the acquired and congenital long-QT syndromes (LQTS). This study examines the cellular and ionic basis for TWA induced by rapid pacing under conditions mimicking the LQT3 form of the congenital LQTS in an arterially perfused canine left ventricular wedge preparation.

Methods and Results—Transmembrane action potentials from epicardial, M, and endocardial cells and 6 to 8 intramural unipolar electrograms were simultaneously recorded together with a transmural ECG and isometric tension development. In the presence of sea anemone toxin (ATX-II; 20 nmol/L), an increase in pacing rate (from a cycle length [CL] of 500 to 400 to 250 ms) produced a wide spectrum of T-wave and mechanical alternans. Acceleration to CLs of 400 to 300 ms produced mild to moderate TWA principally due to beat-to-beat alternation of repolarization of cells in the M region. Transmural dispersion of repolarization during alternans was exaggerated during alternate beats. Acceleration to CLs of 300 to 250 ms caused more pronounced beat-to-beat alternation of action potential duration (APD) of the M cell, resulting in a reversal of repolarization sequence across the ventricular wall, leading to alternation in the polarity of the T wave. The peak of the negative T waves coincided with repolarization of the M region, whereas the end of the negative T wave coincided with the repolarization of epicardium. In almost all cases, electrical alternans was concordant with mechanical alternans. Torsade de pointes occurred after an abrupt acceleration of CL, which was associated with marked TWA. Both ryanodine and low [Ca\textsuperscript{2+}] completely suppressed alternans of the T wave, APD, and contraction, suggesting a critical role for intracellular Ca\textsuperscript{2+} cycling in the maintenance of TWA.

Conclusions—Our results suggest that TWA observed at rapid rates under long-QT conditions is largely the result of alternation of the M-cell APD, leading to exaggeration of transmural dispersion of repolarization during alternate beats, and thus the potential for development of torsade de pointes. Our data also suggest that unlike transient forms of TWA that damp out quickly and depend on electrical restitution factors, the steady-state electrical and mechanical alternans demonstrated in this study appears to be largely the result of beat-to-beat alternans of [Ca\textsuperscript{2+}]. (Circulation. 1999;99:1499-1507.)

Key Words: torsade de pointes ◼ waves ◼ action potentials ◼ long-QT syndrome

T-wave alternans (TWA), an ECG phenomenon characterized by beat-to-beat alternation of the morphology, amplitude, and/or polarity of the T wave, is often associated with the acquired and congenital long-QT syndromes (LQTS). It is an important prognostic indicator in that it is commonly observed just preceding episodes of torsade de pointes.\textsuperscript{1-3} Although beat-to-beat alternation of repolarization somewhere in the heart is presumed to underlie TWA, the precise cellular and ionic basis has not been elucidated. The present study uses an arterially perfused canine left ventricular wedge preparation\textsuperscript{4-8} to assess the cellular and subcellular mechanisms of TWA observed under long-QT conditions.

Methods

Arterially Perfused Wedge of Canine Left Ventricle
Mongrel dogs weighing 20 to 25 kg were anticoagulated with heparin and anesthetized with pentobarbital (30 to 35 mg/kg IV). The chest was opened with a left thoracotomy, and the heart was excised and placed in a cardioplegic solution consisting of cold (4°C) or room temperature Tyrode’s solution containing 8.5 mmol/L [K\textsuperscript{+}]. Transmural wedges with dimensions of \(\sim 2\times1.5\times0.9\) cm to \(3\times2\times1.5\) cm were dissected from the left ventricular anterior wall. The tissue was cannulated through a small (diameter \(\sim 100\) μm) native branch of the left descending coronary artery and perfused with cardioplegic solution. Unperfused tissue, readily identified by its maintained red appearance (erythrocytes not washed away) was carefully removed with a razor blade. The preparation was then

Received August 14, 1998; revision received October 30, 1998; accepted November 5, 1998.
From Masonic Medical Research Laboratory, Utica, NY.
Correspondence to Dr Charles Antzelevitch, Masonic Medical Research Laboratory, 2150 Bleecker St, Utica, NY 13501-1787. E-mail ca@mmrl.edu
© 1999 American Heart Association, Inc.
Circulation is available at http://www.circulationaha.org
Arterially Perfused Left Ventricular Wedge

Figure 1. Schematic of arterially perfused left ventricular wedge preparation showing placement of 7 deep intramural unipolar electrodes in endocardial (Endo), M (5 sites; M1–M5), and epicardial (Epi) sites relative to the position of the transmembrane floating microelectrodes and transmural ECG. Isometric tension development was also measured.

 placed in a small tissue bath and arterially perfused with Tyrode’s solution of the following composition (mmol/L): 129 NaCl, 4 KCl, 0.9 NaH₂PO₄, 20 NaHCO₃, 1.8 CaCl₂, 0.5 MgSO₄, and 5.5 glucose, buffered with 95% O₂ and 5% CO₂ (37 ± 1°C). The perfusate was delivered to the artery by a roller pump (Cole Parmer Instrument Co). Perfusion pressure was monitored with a pressure transducer (World Precision Instruments, Inc) and maintained between 40 and 50 mm Hg by adjustment of the perfusion flow rate. The preparations remained immersed in the arterial perfusate, which was allowed to rise to a level 2 to 3 mm above the tissue surface when possible. To facilitate impalement with the floating microelectrode, in some experiments the bath solution was brought to a level just shy of the top of the wedge and the chamber was covered to the extent possible to avoid a temperature gradient between the top and lower segments of the preparation. Preparations displaying significant ST-segment elevation or depression were excluded from the study.

Recording of Transmural ECG and Transmembrane Action Potentials

The ventricular wedges were allowed to equilibrate until electrically stable (usually 1 hour) and stimulated with bipolar silver electrodes insulated except at the tips and applied to the endocardial surface. A transmural ECG was recorded with the use of 3 mol/L KCl-Agar electrodes (1.1 mm inner diameter). The electrodes were placed in the Tyrode’s solution, bathing the preparation 1.0 to 1.5 cm from the epicardial and endocardial surfaces of the preparation, along the same vector as the transmembrane recordings (Epi: “+” pole) (Figure 1). The electrical field of the preparation as a whole was measured with the use of this technique. Thus the electrocardiographic registration represents a pseudo-ECG of that part of the left ventricle. To differentiate it from local electrogram activity, we refer to it as an ECG in the remainder of the text.

Transmembrane action potentials were simultaneously recorded from the epicardial, M, and endocardial sites with the use of 3 to 4 separate intracardial floating microelectrodes (DC resistance: 10 to 20 MΩ, 2.7 mol/L KCl). Epicardial and endocardial action potentials (APs) were recorded from the epicardial and endocardial surfaces of the preparations at positions approximating the transmural axis of the ECG recording. M-cell APs were recorded at the site along the same axis at which APD was longest, usually in the deep subendocardium (Figure 1).

APD was measured at 90% repolarization (APD₉₀). Activation time (AT) was measured as the interval between the stimulus artifact and the upstroke of the AP. Transmural dispersion of repolarization (TDR) was defined as the difference between the longest and the shortest repolarization time (AT+APD₉₀) of transmembrane APs recorded across the wall. The QT interval was defined as the time between QRS onset and the point at which the final downslope of the T wave crossed the baseline. Graphic correlation of transmembrane and ECG activity was achieved by dropping a dotted line from the point of full repolarization of each AP (APD₉₀approximated by eye) or from the time maximum of the first derivative (Vmax) of the T wave of the unipolar electrograms (shortest in Epi/Endo and longest in the M region) to the ECG trace.

Recordings of Unipolar Electrograms

Six to 8 unipolar electrodes were used to measure the activation-recovery interval (ARI) in the deeper layers of the wedge (Figure 1). Unipolar electrodes consisting of silver wire (120 μm diameter), Teflon insulated except at the tip, were introduced halfway into the wedge from the cut transmural surface so that the extracellular recording sites subtended those of the transmembrane recordings. Each electrode was referenced to the bath ground (silver chloride electrode). Caution was exercised to ensure that the position of the bath ground did not influence the morphology of the unipolar electrogram. Each unipolar recording was differentiated, and the ARI at each site was measured as the interval between the time maximum of the first derivative (Vmax) of the QRS deflection and the Vmin of the T wave. AT was measured as the interval between the stimulus artifact and the Vmin of the QRS. Validation of the use of this technique for the approximation of APD at transmural sites within canine ventricular myocardium was provided in previous studies with the use of the perfused wedge preparations. 5,8 as well as in vivo studies by El-Sherif and coworkers. 9 The viability and electrical stability of the wedge preparations have been detailed elsewhere. 4–8,10

Recordings of Isometric Contractility

In some preparations, isometric contractile force was simultaneously recorded together with transmembrane, intramural, and ECG activity (Figure 1). One end of the wedge preparation was fixed to the bath with stainless steel pins; the other end was attached to a Grass force-displacement transducer (Grass Instruments, Astro-Med, Inc) to record isometric contractile force.

All amplified signals were digitized, stored on magnetic media and WORM-CD, and analyzed with the use of Spike 2 (Cambridge Electronic Design, Cambridge, UK-CED).

Study Protocols

Sea anemone toxin (ATX-II; 20 nmol/L) was used to augment the late sodium current (INa) and produce long-QT conditions similar to those caused by the defect in SCN5A, which is responsible for the LQT3 syndrome. 9 The validity of such pharmacological models as surrogates for the congenital syndromes was previously demonstrated in myocyte, 11 wedge, 5 and in vivo studies. 9

T-wave alternans was induced by reducing the pacing cycle length (CL) from 2000 to 1000, 800, 500, 400, 350, 300, and 250 ms. In some cases, CL was decreased from 500 to 400, 350, 300, and 250 ms (20 to 30 seconds at each CL).

To assess the role of acceleration-induced Ca²⁺ loading on TWA, we displaced Ca²⁺ release from the sarcoplasmic reticulum (SR) by using ryanodine (1 μmol/L) (n = 6). In another 6 preparations, extracellular Ca²⁺ was reduced to 50 μmol/L to deplete SR calcium.

Control measurements were generally obtained after 1 hour of equilibration. The ATX-II data were collected for a period of up to 1 hour starting 1 hour after addition of the drug. Ryanodine and low [Ca²⁺]₅ data were recorded after 10 minutes of exposure to the intervention.

Statistics

Statistical analysis of the data was performed with the use of a Student’s t test for paired data or ANOVA coupled with Scheffé’s test, as appropriate. Data are expressed as mean ± SD values, except for those shown in the figures, which are expressed as mean ± SEM values. Significance was defined as a value of P < 0.05.
Results

Correspondence Among Transmural ECG, Transmembrane AP, and Intramural Unipolar Electrograms

Figure 2 illustrates the correspondence among the transmembrane, unipolar, and ECG recordings in absence (A) and presence (B) of ATX-II (20 nmol/L). Each panel shows 7 intramural unipolar electrograms recorded from endocardial (Endo), M (5 sites; M1–M5), and epicardial (Epi) regions, transmembrane action potentials recorded from M (M2) and epicardial sites together with a transmural ECG at a BCL of 2000 ms. Numbers before and after depolarization of each unipolar electrogram indicate AT and ARI. Numbers before and after upstroke of each action potential indicate AT and APD90. Numbers associated with each ECG denote QT interval and those at bottom of each ECG denote transmural dispersion of repolarization. Horizontal lines in each unipolar electrogram show time maximum of the first derivative (Vmax) of T wave.

T-Wave Alternans During Rapid Pacing

Figure 3 illustrates the TWA induced by abrupt acceleration of pacing rate. TWA usually was not observed under control conditions (Figure 3A) but could be easily induced after 20 nmol/L ATX-II (Figure 3B). TWA was subtle at a CL of 400 ms and became progressively more prominent with acceleration to briefer CLs (350 and 300 ms). At a CL of 250 ms, TWA consisted of beat-to-beat changes in the polarity of the T wave. In most preparations, TWA alternans included alternans of T-wave polarity occurred at CLs of 300 to 250 ms.

Figure 4 illustrates the cellular basis for alternans in the amplitude of the T wave. The T-wave amplitude and QT interval differed in the successive beats principally because of beat-to-beat alternation of APD90 and ARI of cells in the M region. In contrast, epicardial cells showed very little beat-to-beat change of APD90 and ARI. The extent to which endocardium contributes is difficult to discern because this tissue is in close electrical contact with the M region. Because repolarization of the M cell with the longest APD90 (M2 in this preparation) marks the end of the T wave, the QT (QTend) interval alternates beat to beat, whereas the QTpeak interval showed very little beat-to-beat change as the result of small change of APD90 and ARI of epicardium. AT of each electrogram and of each AP was constant in each successive beat.

Figure 5 illustrates the cellular basis for alternans in the polarity of the T wave. The magnitude of the beat-to-beat alternation of APD90 and ARI in the M region is much more pronounced than that of the epicardial and endocardial cells. Transmural repolarization was as previously described (epicardium was the first to repolarize and the M region was the last) when the T wave was positive (first and third beats in Figure 5). When in alternate beats repolarization gradients reversed (the M region repolarized first and epicardium last), the T wave became negative (second beat in Figure 5). In all cases, repolarization of the endocardial cells was intermediate between that of the M and epicardial cells. AT remained constant in successive beats.
Figure 6 shows composite data of beat-to-beat change in the QT interval, APD90 of the 3 cell types, and TDR evaluated for 3 successive beats under steady-state conditions at CLs of 350 and 250 ms. At both CLs, beat-to-beat alternation of the APD90 was more pronounced in the M cells than in epicardial and endocardial cells, resulting in a significant beat-to-beat change in the QT interval and TDR. At a CL of 250 ms, the APD90 of the second beat (N1) is shorter in the M cells than in the epicardial and endocardial cells, creating a negative TDR (Figure 6B). It is noteworthy that the magnitude of the maximal TDR is not reduced, and in some cases increased, at the shorter CL of 250 ms, even though the QT interval and APD90 of the 3 cell type abbreviate (Figures 4, 5, 6A, and 6B).

Effects of Ryanodine and Low Extracellular Ca\textsuperscript{2+} on T-Wave Alternans

Ryanodine (1 μmol/L) slightly abbreviated the APD90 of the 3 cell types as well as the QT interval (P=NS) and did not alter TDR at a basic CL (BCL) of 2000 ms in the continued presence of 20 nmol/L ATX-II (Table 1). Low extracellular Ca\textsuperscript{2+} (50 μmol/L) further prolonged the APD90 and the QT interval (P<0.0005) but did not change TDR at a BCL of 2000 ms in the presence of ATX-II (Table 2).

Both ryanodine and low [Ca\textsuperscript{2+}], in the continued presence of ATX-II completely suppressed pacing-induced TWA. Figure 7 illustrates the effects of ryanodine and low [Ca\textsuperscript{2+}], to suppress the beat-to-beat alternation of transmembrane and ECG activity. As in Figure 5, alternating negative T waves in the presence of ATX-II alone are the result of early repolarization of the M region and late repolarization of epicardium (Figures 7A and 7C). Ryanodine and low [Ca\textsuperscript{2+}], totally suppress the beat-to-beat alternation of APD90, QT interval, and TDR (Figures 7B and 7D). It is noteworthy that the magnitude of the maximal TDR is reduced after ryanodine or low [Ca\textsuperscript{2+}]. Composite data from 3 successive beats recorded under steady-state conditions (CL=250 ms) are shown in Figures 6C and 6D. The maximal TDR is reduced after either ryanodine or low [Ca\textsuperscript{2+}].

Induction of Torsade de Pointes

Torsade de pointes was observed during rapid pacing in 3 out of 12 preparations in the presence of ATX-II alone. Torsade de pointes was induced by an abrupt acceleration of pacing CL to 250 ms, which was associated with marked TWA (Figure 8A). Figure 8B shows the initiation of torsade de pointes during simultaneous recordings of 6 unipolar electrograms from endocardial, M, and epicardial regions and a transmural ECG. Unipolar electrograms in the M region (M1, M2, and M3) display marked alternation of ARI. Torsade de pointes was observed during rapid pacing in 3 out of 12 preparations in the presence of ATX-II alone. Torsade de pointes was induced by an abrupt acceleration of pacing CL to 250 ms, which was associated with marked TWA (Figure 8A). Figure 8B shows the initiation of torsade de pointes during simultaneous recordings of 6 unipolar electrograms from endocardial, M, and epicardial regions and a transmural ECG. Unipolar electrograms in the M region (M1, M2, and M3) display marked alternation of ARI. Torsade de pointes was observed during rapid pacing in 3 out of 12 preparations in the presence of ATX-II alone. Torsade de pointes was induced by an abrupt acceleration of pacing CL to 250 ms, which was associated with marked TWA (Figure 8A). Figure 8B shows the initiation of torsade de pointes during simultaneous recordings of 6 unipolar electrograms from endocardial, M, and epicardial regions and a transmural ECG. Unipolar electrograms in the M region (M1, M2, and M3) display marked alternation of ARI. Torsade de pointes was observed during rapid pacing in 3 out of 12 preparations in the presence of ATX-II alone. Torsade de pointes was induced by an abrupt acceleration of pacing CL to 250 ms, which was associated with marked TWA (Figure 8A). Figure 8B shows the initiation of torsade de pointes during simultaneous recordings of 6 unipolar electrograms from endocardial, M, and epicardial regions and a transmural ECG. Unipolar electrograms in the M region (M1, M2, and M3) display marked alternation of ARI. Torsade de pointes was observed during rapid pacing in 3 out of 12 preparations in the presence of ATX-II alone. Torsade de pointes was induced by an abrupt acceleration of pacing CL to 250 ms, which was associated with marked TWA (Figure 8A). Figure 8B shows the initiation of torsade de pointes during simultaneous recordings of 6 unipolar electrograms from endocardial, M, and epicardial regions and a transmural ECG. Unipolar electrograms in the M region (M1, M2, and M3) display marked alternation of ARI. Torsade de pointes was observed during rapid pacing in 3 out of 12 preparations in the presence of ATX-II alone. Torsade de pointes was induced by an abrupt acceleration of pacing CL to 250 ms, which was associated with marked TWA (Figure 8A). Figure 8B shows the initiation of torsade de pointes during simultaneous recordings of 6 unipolar electrograms from endocardial, M, and epicardial regions and a transmural ECG. Unipolar electrograms in the M region (M1, M2, and M3) display marked alternation of ARI.
pointes was induced after a third paced (captured) beat (P3) whose propagation was markedly delayed or blocked in the M region (between M1 and M2), presumably setting the stage for reentry. In contrast, torsade de pointes was never observed at relatively short CLs, causing only subtle or no TWA in the presence of ATX-II. Torsade de pointes was never observed in control or after ryanodine or low \([\text{Ca}^2+]_o\) in the presence of ATX-II.

Correlation Between Electrical and Mechanical Alternans

To further evaluate the role of intracellular \(\text{Ca}^2+\) loading in TWA, we correlated electrical alternans with the mechanical alternans measured by using isometric tension techniques. Under control conditions, neither electrical (T wave and APD) nor mechanical alternans were observed at any pacing CL (Figure 9A). In the presence of ATX-II, TWA mainly caused by beat-to-beat alternation of the M cell APD was always accompanied by a mechanical alternans (Figure 9B). In most preparations (>90%), the longer AP was associated with a larger contraction, whereas the subsequent shorter AP coincided with the smaller contraction (concordant alternans) (Figure 9B, 10A, and 10C). Both ryanodine and low \([\text{Ca}^2+]_o\) completely suppressed the beat-to-beat alternation of contraction as well as that of the T wave, QT interval, and APD (Figures 10B and 10D).

### Discussion

#### Cellular Basis for T-Wave Alternans

T-wave alternans is a well-known ECG phenomenon often associated with the development of cardiac arrhythmias, particularly in the setting of the acquired and congenital LQTS.1–3,14 In tissue and single-cell studies, alternans of APD is a well-recognized event that usually occurs during rapid rates of stimulation or after an abrupt abbreviation of CL.15–20 Although linked to spatial (transmural, interventricular) and temporal (beat-to-beat) heterogeneity of repolarization, the precise cellular mechanism underlying TWA is not known. The present study uses the arterially perfused wedge preparation to probe the relation between transmembrane AP activity (or intramural ARI) and transmural ECG activity.

### TABLE 1. Effect of ATX-II and Ryanodine on QT Interval, APD\(_{90}\), and Dispersion of Repolarization

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control</th>
<th>ATX-II, 20 nmol/L</th>
<th>ATX-II, 20 nmol/L + Ryanodine, 1 (\mu)mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>QT</td>
<td>308±8</td>
<td>569±21*</td>
<td>544±46</td>
</tr>
<tr>
<td>APD(_{90}) (Endo)</td>
<td>265±9</td>
<td>499±28*</td>
<td>450±48</td>
</tr>
<tr>
<td>APD(_{90}) (M Cell)</td>
<td>280±6</td>
<td>541±19*</td>
<td>514±49</td>
</tr>
<tr>
<td>APD(_{90}) (Epi)</td>
<td>223±6</td>
<td>365±15*</td>
<td>343±44</td>
</tr>
<tr>
<td>Dispersion of RT</td>
<td>48±5</td>
<td>165±21*</td>
<td>160±21</td>
</tr>
</tbody>
</table>

Endo indicates endocardial cell; Epi, epicardial cell. *P<0.0005 vs control. BCL=2000 ms.

### TABLE 2. Effect of ATX-II in the Absence and Presence of Low Extracellular Calcium on the QT Interval, APD\(_{90}\) and Dispersion of Repolarization

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control</th>
<th>ATX-II, 20 nmol/L</th>
<th>ATX-II, 20 nmol/L + Low ([\text{Ca}^2+]_o), 50 (\mu)mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>QT</td>
<td>306±8</td>
<td>528±33*</td>
<td>754±31†</td>
</tr>
<tr>
<td>APD(_{90}) (Endo)</td>
<td>266±9</td>
<td>466±38*</td>
<td>672±38†</td>
</tr>
<tr>
<td>APD(_{90}) (M Cell)</td>
<td>275±7</td>
<td>496±36*</td>
<td>714±29†</td>
</tr>
<tr>
<td>APD(_{90}) (Epi)</td>
<td>219±6</td>
<td>337±32*</td>
<td>539±33†</td>
</tr>
<tr>
<td>Dispersion of RT</td>
<td>48±6</td>
<td>148±22*</td>
<td>164±21</td>
</tr>
</tbody>
</table>

Endo indicates endocardial cell; Epi, epicardial cell. *P<0.0005 vs control; †P<0.0005 vs ATX-II. BCL=2000 ms.
during TWA induced under long-QT conditions. The data indicate that TWA occurring in the presence of ATX-II is in large part secondary to beat-to-beat alternation of the repolarization of cells in the M region, consistent with the findings of Chinushi and coworkers.21 Smaller alternation of the APD of epicardium and endocardium results in dynamic modulation of the transmural voltage gradients that contribute to inscription of the T wave. It is noteworthy that in most preparations, small beat-to-beat alternation of APD in the M region precedes visually detectable TWA in the ECG (data not shown). Recent studies have reported that microvolt TWA, detected by digital signal-processing techniques, can serve as a noninvasive marker of vulnerability to ventricular arrhythmias.12,13,22 Our data demonstrating beat-to-beat undulation of transmural dispersion of repolarization attending TWA support the prognostic value of this parameter.

The data also elucidate the cellular basis for the negative T wave commonly observed during marked TWA. The M-cell AP is the last to repolarize in beats manifesting a positive T wave. Voltage gradients between M and epicardium contribute to the positive excursion of the T wave, whereas the gradient between M and endocardium limit the magnitude of the T wave and contribute to its descending limb.8 This situation is reversed in beats that manifest a negative T wave. The M cell is now the first to repolarize and the voltage gradients between M and epicardium and endocardium and M are reversed, leading to inscription of a negative T wave.

Role of T-Wave Alternans in Development of Torsade de Pointes
Torsade de pointes is an atypical polymorphic ventricular tachycardia most often associated with QT prolongation in both congenital and acquired LQTS. Although the precise mechanism of torsade de pointes has not been established, recent in vivo studies,9,23 perfused wedge studies,4–6 and clinical observations made with monophasic AP recordings have presented evidence in support of the hypothesis that an early afterdepolarization–induced, triggered response initiates torsade de pointes but that the arrhythmia is maintained by a reentrant mechanism. The present study demonstrates that maximal TDR increases during marked T-wave alternans when compared with that observed in the presence of ryanodine, low [Ca2+]o, or at longer CLs at which TWA is subtle or absent. Of note, acceleration-induced TDR is amplified despite an abbreviation of the average QT interval and APD90. The large fluctuations of TDR and M-cell APD are more pronounced during the first few beats after an increase in pacing rate, consistent with the findings of Verduyn et al.23 Indeed, abrupt acceleration of rate induces episodes of torsade de pointes, usually after the first or third beat at the abbreviated CL, since TDR attending these beats is most pronounced (Figure 8). Torsade de pointes could only be induced at the shorter CL (250 ms), which was associated with marked TWA in the presence of ATX-II, but was never observed at any pacing CL yielding subtle or no TWA. These results suggest that the exaggerated TDR attending marked TWA leads to a wider than usual vulnerable window, during which programmed stimulation or pacing can precipitate a reentrant arrhythmia such as torsade de pointes. In the absence of alternans, the vulnerable window is too small to induce torsade de pointes. Previous studies with the wedge have shown that at relatively slow rates, extrastimulation-induced torsade de pointes is a function of TDR, which determines the temporal width of the vulnerable window.5 With abbreviation of CL from 2000 to 350 ms, the vulnerable window is expected to narrow progressively, making torsade de pointes more difficult or noninducible. Further abbreviation of the CL (250 ms) restores the ability of the preparation to develop torsade de pointes in response to programmed stimulation by virtue of the fact that TDR and the vulnerable window are amplified in alternate beats during the period of TWA.
Ionic Basis for T-Wave and Mechanical Alternans

Sundry mechanisms have been proposed to explain the ionic basis for TWA, including beat-to-beat changes in intracellular levels of Ca\(^{2+}\), I\(_{\text{K}}\), K\(^{-}\) accumulation in the extracellular clefts, and Na\(^+/Ca^{2+}\) exchange current.\(^{15-20,26}\) There is growing evidence that electrical alternans of the AP is intimately coupled to mechanical alternans and that intracellular Ca\(^{2+}\) released from the SR plays a pivotal role in the maintenance of both.\(^{27}\) The simultaneous elimination of both electrical and mechanical alternans after block of the SR with ryanodine and depletion of SR calcium with low [Ca\(^{2+}\)]\(_{\text{o}}\) (Figures 6, 7, and 10) provides further support for the hypothesis that beat-to-beat changes in the level of [Ca\(^{2+}\)]\(_{\text{i}}\) modulate the repolarizing currents in the heart and thus contribute to TWA.

Sustained TWA was never observed without mechanical alternans, and when they occurred, the two were almost always concordant (ie, the longer M-cell AP was associated with the larger contraction) (Figures 9 and 10). The time course of repolarization of the M cells differs from that of epicardium and endocardium, causing a small dispersion of repolarization and refractoriness across the ventricular wall in control and a much larger TDR under long-QT conditions.\(^{28}\) A weaker, slowly activating delayed rectifier potassium current (I\(_{\text{Ks}}\))\(^{29}\) and larger late I\(_{\text{Na}}\)\(^{30}\) contribute to the longer APD of the M cell in the dog. The weaker net outward current active during the plateau phase also contributes to the greater response of M cells to agents that prolong APD, such as an ATX-II, and is likely to be responsible for the greater sensitivity of the M cell to fluctuation in [Ca\(^{2+}\)]\(_{\text{i}}\].

The available data suggest that electrical alternans in some cases is coupled to mechanical alternans and that intracellular Ca\(^{2+}\) released from the SR plays a pivotal role in the maintenance of both.\(^{27}\) The simultaneous elimination of both electrical and mechanical alternans after block of the SR with ryanodine and depletion of SR calcium with low [Ca\(^{2+}\)]\(_{\text{o}}\), (Figures 6, 7, and 10) provides further support for the hypothesis that that beat-to-beat changes in the level of [Ca\(^{2+}\)]\(_{\text{i}}\) modulate the repolarizing currents in the heart and thus contribute to TWA.

Sustained TWA was never observed without mechanical alternans, and when they occurred, the two were almost always concordant (ie, the longer M-cell AP was associated with the larger contraction) (Figures 9 and 10). The time course of repolarization of the M cells differs from that of epicardium and endocardium, causing a small dispersion of repolarization and refractoriness across the ventricular wall in control and a much larger TDR under long-QT conditions.\(^{28}\) A weaker, slowly activating delayed rectifier potassium current (I\(_{\text{Ks}}\))\(^{29}\) and larger late I\(_{\text{Na}}\)\(^{30}\) contribute to the longer APD of the M cell in the dog. The weaker net outward current active during the plateau phase also contributes to the greater response of M cells to agents that prolong APD, such as an ATX-II, and is likely to be responsible for the greater sensitivity of the M cell to fluctuation in [Ca\(^{2+}\)]\(_{\text{i}}\].

The available data suggest that electrical alternans in some cases is coupled to mechanical alternans and that Ca\(^{2+}\) release from the SR plays a pivotal role in the maintenance of both. Mechanical alternans is thought to occur when the next paced beat encroaches on the time required for reuptake of calcium into the network SR and transport to the junctional SR for rerelease.\(^{31}\) Thus mechanical alternans is induced when the pacing CL is briefer than the interval required for calcium release, reuptake, and transport to the junctional SR. Sustained TWA occurs only when the pacing rate is rapid enough to cause mechanical alternans.

How is mechanical alternans related to electrical alternans? If mechanical alternans is secondary to alternans in the availability of SR calcium ready for release, then it must also reflect alternation in [Ca\(^{2+}\)]\(_{\text{i}}\]. Relatively high [Ca\(^{2+}\)]\(_{\text{i}}\) can augment outward I\(_{\text{Ko}}\) outward calcium-activated chloride current (I\(_{\text{CaCl}}\)), and inward electrogenic Na\(^+/Ca^{2+}\) exchange current (I\(_{\text{Na-Ca}}\)). In concordant alternans, it seems reasonable to speculate that the larger contraction is accompanied by the...
larger increase in [Ca^{2+}], which in turn augments the 3 currents discussed above. Predominance of I_{Na,Ca} over I_{Ks} and I_{Cl(Ca)} would reduce net repolarizing current and prolong the M-cell AP. The smaller rise in [Ca^{2+}], attending the next beat would activate less I_{Na,Ca}, resulting in less inward current to maintain the plateau and a briefer APD. This scenario would be more likely to occur in M cells, in which I_{Ks} is intrinsically small. Discordant alternans would be expected when calcium-induced augmentation of outward current predominates. The larger [Ca^{2+}], would be expected to increase outward I_{Ks} and/or I_{Cl(Ca)} more than inward I_{Na,Ca}, thus abbreviating APD. The small rise in [Ca^{2+}], attending the next beat would be expected to activate less I_{Ks} and/or I_{Cl(Ca)}, resulting in a more prolonged APD. Although these hypotheses remain to be more fully evaluated, they are consistent with recent work demonstrating conversion of concordant alternans to discordant alternans in isolated feline myocytes by lowering the temperature of the superfusate.20 Because the temperature coefficient (Q_{10}) of I_{Na-Ca} is much larger than that of the ionic currents, the contribution of I_{Na-Ca} is deemphasized.

We think it important to point out that the mechanism of sustained (steady-state) TWA observed in study may be different from the transient TWA and APD prolongation observed during and/or immediately after pacing or at slower rates. Transient T-wave changes and APD prolongation during and/or immediately after an increase in rate is likely due to a transient increase of I_{Na,Ca} secondary to intracellular Ca^{2+} loading as well as to other restitution factors. Larger fluctuations of TDR and M-cell APD are generally seen in our study during the first few beats after an increase in pacing rate, congruent to the findings of Verdun et al.23 Thus electrical alternans secondary to restitution parameters quickly damps, whereas that secondary to alternation of [Ca^{2+}], is generally sustained at fast rates.

Limitations of the Study
Our interpretations of the data are based on the assumption that the activity recorded from the cut surface of the perfused wedge preparation is representative of cells within the respective layers of the wall throughout the wedge. Such validation was provided in 2 previous studies with the perfused wedge preparation8,9 as well as in the present study.

The extent to which ATX-II-induced augmentation of I_{Na} mimics the SCN5A defect responsible for the LQT3 syndrome is difficult to measure. Slowed or incomplete inactivation of I_{Na} by whatever means33 leads to a large late I_{Na} during the plateau phase and a prolonged APD. ATX-II similarly exerts its action to prolong APD by augmenting late I_{Na}. Previous studies have demonstrated the ability of the pharmacological model to closely mimic features of LQT3, including a prolonged QT interval, late-appearing T waves, a steep QT-rate relation, induction of torsade de points, and a high sensitivity to Na^{+} channel blockers.5,21 We believe that these qualitative similarities validate the ATX-II model as a surrogate for LQT3.

Acknowledgments
This study was supported by grant HL-47678 from the National Heart, Lung, and Blood Institute (C.A.) and grants from Medtronic Japan (W.S.), the American Heart Association (W.S.), and NYS and Florida Grand Lodges F&AM. We gratefully acknowledge the expert technical assistance of Judy Hefferon, Di Hou, and Robert Goodrow. We are grateful to Dr. J.M. Di Diego for assistance with the experimental protocols involving recordings of unipolar electrograms and contractile force.

References


Cellular and Ionic Basis for T-Wave Alternans Under Long-QT Conditions
Wataru Shimizu and Charles Antzelevitch

Circulation. 1999;99:1499-1507
doi: 10.1161/01.CIR.99.11.1499
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/99/11/1499

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/