Progressive Increases in Complexity of T-Wave Oscillations
Herald Ischemia-Induced Ventricular Fibrillation

Bruce D. Nearing, Richard L. Verrier

Abstract—T-wave alternans (TWA), an ABAB oscillation, has been postulated as the initial pattern in a stepwise progression to higher-order oscillations, culminating in sudden arrhythmic death. The present study is the first to provide experimental evidence to support this intriguing concept. Epicardial and endocardial ECGs from 12 dogs were monitored during 8-minute left anterior descending coronary artery occlusion with right atrial pacing at 150 bpm. TWA magnitude was measured by modified moving average beat analysis, and the complexity of T-wave oscillations was assessed by complex demodulation. In 6 animals with subsequent ventricular fibrillation (VF), TWA achieved a threshold of 5.00±1.30 mV in epicardial ischemic-zone electrograms, which then exhibited a stepwise increase in T-wave oscillation complexity to quadrupling (ABCDABCD, 3 cases) or tripling (ABCABC, 2 cases) and to more complex forms (5 cases) preceding VF (6 cases). In dogs without VF, peak TWA levels did not increase from baseline, measuring a maximum of 0.35±0.10 mV (P=NS), or only 7% the value of those with VF, and T-wave multupling was not observed (0 of 6 versus 5 of 6, P<0.005). Discordant TWA episodes, with T waves alternating out of phase, were associated with increased T-wave complexity and fibrillation in 4 of 6 dogs with VF but in none of the 6 dogs without VF (P<0.025). TWA appears to be the first step in an orderly progression of T-wave complexity, episodes of discordant TWA, and VF. This demonstrated increase in T-wave complexity points to a fundamental mechanistic link underlying the ability of TWA to predict lethal arrhythmias. (Circ Res. 2002;91:727-732.)

Key Words: sudden death • ventricular fibrillation • myocardial ischemia • T-wave alternans • repolarization

Mounting evidence has demonstrated during the past decade that T-wave alternans (TWA), magnitude, which can be quantified by spectral and nonspectral methods, is highly correlated with the likelihood of life-threatening arrhythmias1–17 under diverse clinical conditions, including myocardial infarction,1,12,13 heart failure,14 cardiomyopathy,15 long QT syndrome,16,17 and others. To establish with certainty that TWA represents an initial step in the onset of lethal arrhythmias requires the demonstration of higher-order T-wave complexity just before the onset of ventricular fibrillation (VF), the arrhythmia responsible for sudden cardiac death. Multiple T-wave oscillations have not been previously reported, probably because T-wave multupling represents an extremely unstable and evanescent state that occurs under intensely proffibrillatory conditions. In the present study, we provide, as far as we know, the first demonstration of the increasing complexity of T-wave oscillations preceding VF. The phenomenon consistently occurred during transition from normal rhythm to fibrillation after a high level of TWA had been reached in a large animal model of acute myocardial ischemia. The observation was made possible by the use of regional electrodes centered in the zone of ischemia and by the application of complex demodulation to quantify the complex oscillatory forms.

Materials and Methods
Adult mongrel dogs of either sex (n=12) were studied under a surgical plane of anesthesia according to protocols approved by the institutional animal care and use committee and standards set by the National Institutes of Health. The animals were preanesthetized with xylazine (0.24 mg/kg SC) and anesthetized with α-chloralose (150 mg/kg IV, with supplemental doses of 600 mg in 60 mL saline as required). The left anterior descending coronary artery was dissected free of surrounding tissue through a thoracotomy and was occluded by the application of complex demodulation to quantify
current of injury. Heart rate was maintained constant by right atrial pacing at 150 bpm. ECGs were continuously recorded during two coronary artery occlusion-release sequences, consisting of a 4-minute control period, an 8-minute occlusion period, and a 4-minute release period, separated by a 30-minute rest period. The results of the first, or preconditioning, occlusion were discarded according to standard practice. ECG data were low-pass–filtered at 50 Hz, sampled at 500 Hz per channel, and stored on optical disks. The single epicardial plaque electrode recorded from each animal with the largest magnitude of TWA was identified (termed the maximum lead) and used for analysis. TWA magnitude was measured by "modified moving average" analysis.6

Complex demodulation4 was used to discriminate TWA, T-wave tripling and quadrupling, and complex forms. This was accomplished by using complex exponentials at the alternating, tripling, and quadrupling frequencies and by measuring the area under the T wave. Data were prepared by computing a sum from the series of samples from 60 to 220 ms after the R wave and detrending to remove constant and gradual changes in the T-wave area. Multiplying by a complex exponential at the TWA frequency and then low-pass filtering to remove the high-frequency terms resulted in a measure of TWA magnitude as a function of time. This process was reiterated for tripling and quadrupling by using a complex exponential at the tripling and quadrupling frequencies, respectively. Tripling and quadrupling were termed present if the run was at least two cycles, constituting 6 or 8 beats, respectively, and the amplitude was >0.1 mV. The onset of nonrepeating T-wave patterns, termed complex forms, was identified by decreased complex demodulation results for tripling and quadrupling despite visual evidence of T-wave patterns. Calculating the area under the T wave further verified the increasing complexity of T-wave oscillations. Poincaré maps formed by plotting T-wave magnitude of alternate beats also illustrate the divergence of multiplying forms. To track episodes of discordant TWA in the epicardial 4-electrode plaque, the data were again detrended to remove constant and gradual changes in the area under the T wave. For each beat, the detrended areas under the T wave in two leads were multiplied. The product was negative if discordant TWA was present, because the factors were positive and negative. The product was primarily positive if a concordant or no TWA was present, because the beats from each lead simultaneously had positive or negative detrended areas. Each ECG lead was paired with every other ECG lead in all possible combinations to identify discordance. Discordant TWA was termed present if it persisted for at least 6 beats and could be verified by visual inspection. TWA levels were compared by 1-way ANOVA, with the Tukey correction for multiple comparisons (SAS, SAS Institute). Significant occurrence of higher-order periodicities was determined by χ2 analysis. ANOVA regression analysis was used to determine significant relationships between the extent of multiplying and mean TWA values. Values are mean±SEM, with a value of P<0.05 indicating significance.

**Results**

The epicardial plaque electrode lead with the maximum TWA (maximum lead) displayed an orderly progression in complexity of T-wave oscillations from baseline morphology to TWA (6 cases), to tripling (2 cases), or to quadrupling (3 other cases) and then to complex forms (all 5 cases with tripling or quadrupling) and to VF (6 cases) in the 6 animals in which myocardial ischemia induced spontaneous VF (representative experiment, Figure 1; group data, Figures 2 and 3). TWA >0.10 mV occurred at 2.34±0.24 minutes after the start of the coronary artery occlusion, increased to 5.00±1.30 mV, and lasted for 1.37±0.30 minutes (~82 seconds) until the next level of complexity, namely, tripling (2 cases), quadrupling (3 cases), or VF (1 case). Quadrupling occurred at 3.48±0.12 minutes after the start of the coronary artery occlusion and lasted for 0.39±0.07 minutes (~23 seconds) before more complex forms occurred. Compared with quadrupling, tripling occurred slightly later (~23 seconds) after the start of coronary artery occlusion, at 3.86±0.03 minutes, and lasted for 0.44±0.14 minutes (~26 seconds) before more complex forms occurred. Complex forms were observed in the 5 dogs with tripling or quadrupling at 4.04±0.12 minutes and lasted for 0.46±0.15 minutes (~28 seconds) before VF occurred at 4.49±0.07 minutes after the start of the coronary artery occlusion. The sixth dog experienced VF at 3.7 minutes without prior evidence of multiplying or complex forms. Thus, the onset of VF occurred at a mean of 4.36±0.14 minutes in the entire group of 6 animals. The higher-order oscillations are inherently unstable and evanescent, lasting <30 seconds. The ischemia-induced T-wave multiplying followed a significant increase in TWA in the group that proceeded to fibrillation (from 0.13±0.02 mV at baseline to 5.00±1.30 mV, P<0.05). The maximum TWA level in the group without VF was not significantly elevated during ischemia (from 0.14±0.02 mV at baseline to 0.35±0.10 mV, P=NS), achieving only 7% of the magnitude of the group with VF (0.35±0.10 versus 5.00±1.30 mV), although preocclusion baseline TWA values did not differ between the groups.

In addition, episodes of discordant TWA were identified when any of the waveforms of the 4 electrodes on each
epicardial plaque were observed to alternate out of phase with the others. In 4 of the 5 dogs with tripling or quadrupling and complex forms before VF, discordant TWA appeared either at the onset of multupling (1 case) or during complex forms (3 cases), preceding the onset of VF by 18 ± 8 seconds (Figure 2). The onset and offset of discordance were visible in the affected lead. Thus, higher-order periodicities or discordant TWA in animals without VF. In the 6 animals in which myocardial ischemia did not trigger VF, no sustained increase in TWA (registering 0.14 ± 0.02 mV at baseline to 0.35 ± 0.10 mV, P = NS), multiple T-wave oscillations, complex forms, or discordant TWA occurred.

The regional specificity of TWA and multupling was evident in morphological diversity in simultaneous ECG tracings (Figure 4). Neither multupling nor discordant TWA was observed in the endocardial leads at any time, even in the 6 animals that experienced VF. In these leads, the ischemia-induced increase in TWA magnitude was delayed and diminished compared with epicardial TWA values, averaging 1.84 ± 0.29 mV (LV) and 0.87 ± 0.16 mV (right ventricle). LV blood pressure recordings exhibited no alternation or multupling, indicating that the TWA and multupling patterns observed were not induced mechanically by changes in the pressure pulse.

**Discussion**

The present study of the advancing complexity of T-wave oscillations after alternans during the transition from normal rhythm to myocardial ischemia-induced VF provides evidence that TWA may be fundamentally linked to VF. Complex demodulation of T-wave amplitude revealed discrete patterns (Figures 1 and 3), registering the increasing complexity of oscillations. All 6 of the animals that experienced fibrillation exhibited high levels of alternation, and 5 showed an orderly stepwise progression to multiple oscillations shortly before (<1 minute) the onset of the terminal rhythm. In essence, the progression to fibrillation constituted escalation of a deadly staircase of complex repolarization patterns (Figure 2). The animals that did not experience VF exhibited a significantly lower level of TWA (Figure 2). Discordant TWA, a sign of increased heterogeneity of repolarization, accompanied transitions to increased T-wave complexity and VF (Figure 2). Heart rate changes do not appear to be a critical factor in the observed changes, inasmuch as this parameter was held constant by right atrial pacing.

T-wave multupling appeared in a progressive sequence during the transition from normal rhythm to myocardial ischemia–induced VF. TWA always preceded either tripling or quadrupling, and the multupling patterns culminated in complex forms suggesting not random variation but an aperiodic multistate pattern that rapidly degenerated into fibrillation.

The existence of tripling, which occurred slightly later after the start of coronary artery occlusion than did the quadrupling pattern, indicates yet a higher level of complexity. Its appearance is significant in that it implies that the system is capable of supporting T-wave patternning of all periodicities, not only TWA, quadrupling, and higher-order periods but also a pseudorandom sequence, such as complex forms. T waves in three dogs followed a period-doubling route from baseline to VF, namely, TWA, T-wave quadrupling, complex T-wave forms, and VF. Such bifurcated routes have been widely observed in nature, including cardiac physiology, and can be generated by simple mathematical models. T waves in two dogs followed a different sequence, namely, TWA, T-wave tripling, complex T-wave forms, and VF. The simultaneous existence of these different routes to VF emphasizes the complexity and nonlinearity present in the cardiac system. Importantly, these different sequences existed side by side with normal repolarization patterns, which continued to occur outside the ischemic zone (Figure 4). This difference in repolarization patterns gives rise to a large degree of dispersion that is inherently unstable and presages VF.

**Significance of Multupling**

We are unaware of any reports of T-wave multupling preceding VF in the numerous studies of myocardial ischemia in intact large animals in the scientific literature. Period multupling in terms of heart rate frequency or action potential morphology has been reported in elegant studies in cardiac
cells, isolated tissues, and amphibian hearts, but no relationship with the onset of arrhythmias has been demonstrated. Transition to VF has been observed in only a few experiments by investigators who described the consistent prior occurrence of cycle-length alternans. Ritzenberg et al. observed subharmonics in heart rate frequency and multupling in the QRST waveform in canines when norepinephrine injection provoked sinus tachycardia of 200 bpm. Because sympathetic nerve stimulation and behavioral stress increase the magnitude of TWA and the incidence of VF during myocardial ischemia, it is possible, if not likely, that the catecholamine contributed to the increasing waveform complexities in the single animal in which VF was triggered. However, these data were not analyzed. Moreover, heart rate frequency subharmonics and action potential morphology relate to the R wave of the ECG and are thus distinct from TWA, which reflects electrical instability concentrated during cardiac repolarization, which underlies the T wave of the ECG and coincides with the vulnerable phase of the cardiac cycle from which VF emerges.

Figure 3. Discrimination of T-wave complexity based on beat-by-beat measurement of the total area between the ST segment and the isoelectric line (from 60 to 220 ms after the R wave at a paced rate of 150 bpm) in the maximum lead of the 4-electrode plaque. The area is plotted separately vs time of occlusion for all 12 animals. Positive and negative values indicate areas above and below the isoelectric line, respectively. An average T-wave amplitude for any given beat can be determined by dividing its area by 160 ms. The area is plotted in black when alternans or multupling was absent. During TWA, the A beat is plotted in red, and the B beat is plotted in yellow. During T-wave tripling, the A beat is plotted in green; the B beat, in turquoise; and the C beat, in dark blue. During T-wave quadrupling, the A beat is plotted in green; the B beat, in turquoise; the C beat, in dark blue; and the D beat, in magenta. During complex T-wave forms, each beat is plotted individually as an orange square. The separation between the lines shows the amplitude of the oscillation. A, In the 6 dogs with VF, the area oscillated between 2 states (alternans, panels A through F). In the 2 dogs with tripling, the area oscillated among 3 states (panels B and F). In the 3 dogs with quadrupling, the area oscillated among 4 states (panels A, D, and E). These 5 dogs exhibited complex forms (panels A, B, D, E, and F). The separate points (orange squares) of the complex forms did not organize into lines but appear to represent a more complex state. B, In the 6 dogs without VF (panels G through L), the area oscillated between 2 states in 4 dogs (alternans; panels G, I, J, and K), but the amplitude was lower, and neither multupling nor complex forms appeared. TWA was absent from the other 2 canines without VF (panels H and L).
Internally consistent. Multupling and VF always ensued when a high level of TWA was reached. In fact, the magnitude of TWA was 14-fold higher in those animals that experienced VF than in those that did not. Moreover, the complexity of multupling progressed in an orderly manner that was quantifiable by complex demodulation. Because none of the 6 animals that did not experience fibrillation exhibited either high degrees of TWA or multupling in any electrode throughout the entire period of coronary artery occlusion, it is unlikely that multupling is an epiphenomenon unrelated to the development of VF. It remains unknown whether conduction block may have played a role in the development of multupling. However, this phenomenon occurred during the early phase of ischemia, within 3 to 4 minutes after occlusion, before the time interval generally required for the development of significant conduction abnormalities. Carson et al. used an epicardial plaque array with 61 electrodes, found no evidence of significant conduction abnormalities during a 6-minute occlusion period and attributed ischemia-induced TWA to alternation of action potential configuration. Watanabe et al confirmed and extended these observations by using both unipolar and bipolar transmural electrodes to determine that TWA during the first 4 minutes of occlusion was not associated with conduction block. After a longer period of myocardial ischemia, conduction block became a significant factor in TWA. Notwithstanding this evidence, it remains possible that during the last few seconds during transition from TWA to multupling, transitory conduction block may occur.

The ionic basis for T-wave multupling is unknown. Because calcium appears to play a significant role in TWA, as evidenced by (1) oscillation of this ion in concert with repolarization alternans during myocardial ischemia, (2) suppression of TWA by calcium channel-blocking agents and the sarcoplasmic reticulum reuptake inhibitor ryanodine, and (3) complex patterns of calcium oscillations during overload of this ion, altered calcium handling is a candidate mechanism in multupling.

**Discordant TWA**

Discordant TWA, in which the T wave alternates out of phase in adjoining electrogram sites, is thought to reflect a state of extreme electrical instability that is presumably due to heightened levels of dispersion of repolarization. We observed that discordant TWA was strongly associated with an increase in T-wave complexity from concordant TWA to multupling or during complex forms preceding the onset of VF. Previous multisite mapping studies have reported that the activation site of ischemia- and reperfusion-induced VF occurs near the border between areas with discordant TWA. These observations suggest that discordant TWA is an important factor in T-wave multupling and VF.

**Summary and Conclusions**

Our novel observation (ie, when T-wave oscillations in an ABAB pattern reached a certain high magnitude, a stepwise change in complexity to tripling [ABCDABCD] or quadrupling [ABCDABCD] ensued, with episodes of discordant TWA culminating in VF) suggests that ischemia-induced TWA is a precursor that is mechanistically linked with VF. This pattern contrasts with the possibility that TWA could have been a continuous process in which alternans magnitude increased until the onset of VF. The present documentation of a stepwise progression in the transition from TWA to VF may provide important insights into the fundamental mechanisms responsible for this lethal arrhythmia.

**Acknowledgments**

This study was supported by NIH grant R01 HL-63968 from the National Heart, Lung, and Blood Institute, Bethesda, Md. The authors thank S.S. Verrier for editorial assistance.

**References**

References


Progressive Increases in Complexity of T-Wave Oscillations Herald Ischemia-Induced Ventricular Fibrillation

Bruce D. Nearing and Richard L. Verrier

Circ Res. 2002;91:727-732; originally published online September 26, 2002;
doi: 10.1161/01.RES.0000038887.17976.33

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/91/8/727

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research 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 Research is online at:
http://circres.ahajournals.org/subscriptions/