T wave alternans in an in vitro canine tissue model of Brugada syndrome

Hiroshi Morita,1 Douglas P. Zipes,1 John Lopshire,1 Shiho T. Morita,1 and Jiashin Wu1,2

1Krannert Institute of Cardiology, Indiana University School of Medicine and 2Department of Biomedical Engineering, Indiana University-Purdue University Indianapolis, Indianapolis, Indiana

Submitted 29 November 2005; accepted in final form 16 February 2006

TWA in ECG are not known. The purpose of the present study was to assess these relationships by optically mapping the epicardial and transmural distributions of AP in an in vitro canine right ventricular tissue model of Brugada syndrome. We tested the hypothesis that the dynamic instability and heterogeneity of APs in the epicardium but not in the midmyocardium or endocardium of right ventricular wall can lead to T wave alternans (TWA) in Brugada syndrome.

METHODS

Arterially perfused right ventricular tissue preparations. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication no. 85–23, revised 1996). We prepared tissues with procedures similar to those used previously (31–33, 36–38). In brief, we harvested hearts from 28 anesthetized (pentobarbital sodium at ∼30 mg/kg body wt) adult male mongrel dogs (25–30 kg) and immediately retrogradely perfused the hearts through the aorta with a cardioplegic solution (Tyrode’s solution, see below, with 15 mmol/l KCl, 4°C) that washed out the blood and pentobarbital sodium and protected the hearts during the subsequent period of tissue isolation. We isolated tissues of two different sizes, ∼2.5 × 2.5 × 1.0 cm3 (free wall preparations, n = 18) and ∼0.8 × 2.5 × 1.0 cm3 (transmural preparations, n = 10), from the same region (0–35 mm from the pulmonary valves) in the right ventricular free wall to evaluate the epicardial and transmural distributions of APs, respectively. Only one preparation was harvested from each heart. Each preparation contained either two (free wall preparations) or one (transmural preparations) branch of the right coronary artery (diameter: ≥1 mm). We inserted separate perfusion and pressure-monitoring cannulas in the arteries, ligated major arterial leaks with silk sutures, and trimmed underperfused tissue from the preparations. The isolated tissues were mounted in a warmed chamber with the recording surface (the epicardium or cut-exposed transmural surface) up, perfused with Tyrode’s solution (in mmol/l: 128.0 NaCl, 4.69 KCl, 22.0 NaHCO3, 0.65 NaH2PO4, 0.50 MgCl2, 11.1 dextrose, and 2.0 CaCl2, and gassed with 95% O2–5% CO2, pH 7.4) at an arterial pressure of 40–50 mmHg at 36.5 ± 0.5°C, and immersed in the perfusion efflux.

Tissue preparations were perfused continuously and stained with di-4-ANEPPS (C3H5N2O2S; ∼4 μmol/l in perfusate: Molecular Probes, Eugene, OR), which is a membrane-potential-sensitive fluorescent dye having no known electrophysiological effects and used widely in optical mapping studies. Sufficient time was allowed for the tissues to recover from the cardioplegic and isolation processes, until their full stabilization [with consistent arterial resistance, contractions, action potential durations (APDs), velocity of conduction, and ECG for >10 min]. We evaluated the healthiness of the preparations as we have done previously (31–33, 36–38). Well-perfused preparations had a vivid reddish color, low-noise optical signals with normal APD, and strong contractions upon stimulation, in contrast to the short APD (or inactivation), dull color, weak contractions, or noisy optical signals (poor perfusion and dye staining) of ischemic preparations. Healthy

Address for reprint requests and other correspondence: J. Wu, Krannert Institute of Cardiology, Indiana Univ. School of Medicine, 1800 N. Capitol Ave., Indianapolis, IN 46202 (e-mail: jiaswu@iupui.edu).

http://www.ajpheart.org 0363-6135/06 $8.00 Copyright © 2006 the American Physiological Society

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
T WAVE ALTERNANS IN A MODEL OF BRUGADA SYNDROME

preparations were immobilized with cytochalasin D (20–30 μmol/l; Sigma Chemical, St. Louis, MO), which depolymerizes cytoskeletal actin filaments and reduces the Ca\textsuperscript{2+} sensitivity of myocardial filaments (5) without affecting the shape of the canine ventricular AP, as we verified previously (4, 36). The immobilized tissue had no recordable pulsation in arterial pressure and no visible contraction during stimulation. Tissue immobilization eliminated motion artifacts in optically recorded APs and facilitated the identification of the AP dome and early afterdepolarizations. We verified that these procedures produced stable tissues having transmural dispersion of APD and conduction velocity (36) similar to in vivo observations (3, 17).

Electrophysiological recording. We paced the tissue preparations from the endocardium at a cycle length (CL) of 2,000 ms (2 ms duration, 2 × diastolic current threshold, with a bipolar electrode). Two silver electrodes were placed in the bath, 5–10 mm away from the epicardium and endocardium, to register the transmural ECG. An optical mapping system (36) with a 256-element (16 × 16) photodiode camera (C4675; Hamamatsu) collected the fluorescence signals from a 19.5 × 19.5 mm\textsuperscript{2} observation area (1.1 × 1.1 mm\textsuperscript{2} /element) on the tissue surface and converted it to 256 channels of electrical signals (APs). A custom data acquisition system recorded the APs, ECG, and arterial pressure at 1,000 samples · channel\textsuperscript{−1} · s\textsuperscript{−1}.

Drugs and study protocols. Data were recorded at pacing CLs of 3,000, 2,000, 1,000, 600, and 400 ms, after the preparations were fully immobilized and stabilized (baseline recording). Brugada-type ECG (J-ST elevation with negative T wave) and TWA were induced with the perfusion of pinacidil (2.5–5 to 10–15 μmol/l; Sigma Chemical), pilsicainide (5–10 to 13 μmol/l; Dai-Ichi Sunry Pharma, Tokyo, Japan), and terfenadine (0–5 μmol/l; Sigma Chemical; see Ref. 2).

Electrophysiological recording. We paced the tissue preparations from the endocardium at a cycle length (CL) of 2,000 ms (2 ms duration, 2 × diastolic current threshold, with a bipolar electrode). Two silver electrodes were placed in the bath, 5–10 mm away from the epicardium and endocardium, to register the transmural ECG. An optical mapping system (36) with a 256-element (16 × 16) photodiode camera (C4675; Hamamatsu) collected the fluorescence signals from a 19.5 × 19.5 mm\textsuperscript{2} observation area (1.1 × 1.1 mm\textsuperscript{2} /element) on the tissue surface and converted it to 256 channels of electrical signals (APs). A custom data acquisition system recorded the APs, ECG, and arterial pressure at 1,000 samples · channel\textsuperscript{−1} · s\textsuperscript{−1}.

Drugs and study protocols. Data were recorded at pacing CLs of 3,000, 2,000, 1,000, 600, and 400 ms, after the preparations were fully immobilized and stabilized (baseline recording). Brugada-type ECG (J-ST elevation with negative T wave) and TWA were induced with the perfusion of pinacidil (2.5–5 to 10–15 μmol/l; Sigma Chemical), pilsicainide (5–10 to 13 μmol/l; Dai-Ichi Sunry Pharma, Tokyo, Japan), and terfenadine (0–5 μmol/l; Sigma Chemical; see Ref. 2).

**Table 1. Occurrences of Brugada-type ECG (J-ST elevation with negative T wave), macroscopic TWA, and RTWC in 18 free wall and 10 transmural tissue preparations**

<table>
<thead>
<tr>
<th></th>
<th>All Tissues</th>
<th>Free Wall</th>
<th>Transmural</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brugada ECG</strong></td>
<td>28</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td><strong>TWA</strong></td>
<td>19</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td><strong>RTWC</strong></td>
<td>14</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>Brugada ECG</th>
<th>TWA</th>
<th>RTWC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinacidil, μmol/l</td>
<td>5.3 ± 3.7</td>
<td>9.1 ± 5.3</td>
<td>8.6 ± 5.3</td>
</tr>
<tr>
<td>Terfenadine, μmol/l</td>
<td>3.1 ± 2.5</td>
<td>4.2 ± 1.9</td>
<td>4.6 ± 1.3</td>
</tr>
<tr>
<td>Pilsicainide, μmol/l</td>
<td>6.0 ± 3.3</td>
<td>9.2 ± 4.0</td>
<td>8.9 ± 4.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. ECG, electrocardiogram; TWA, T wave alternans; RTWC, random T wave change; CL, cycle length.
eliminated macro-TWA (CL: 800 ± 219 ms, range: 1,000–600 ms, n = 5). Further increase in the pacing rate (CL: 529 ± 168 ms, range: 600–400 ms, n = 7) eliminated random T wave changes. Severe bradycardia (CL: 3,000 ms, n = 7) also diminished macro-TWA. All macro-TWA occurred within a window of activation CL of between 600 and 3,000 ms.

**Transmural dispersion of APs.** Macro-TWA and random T wave changes occurred in 7 and 6 of the 10 transmural preparations, respectively, all in association with changing dome and duration of APs only within the first 2- to 3-mm depth from the epicardium, whereas the midmyocardial and endocardial APDs had no detectible alternans (Figs. 2, A and C, and 3). The deep T waves were associated with a prominent dome and prolonged duration of epicardial APs, which inverted the transmural gradient of APD and increased the transmural dispersion of repolarization (e.g., Fig. 3D). In contrast, the shallow T waves were associated with epicardial APs having lower amplitude dome or no dome and less transmural dispersion of repolarization (e.g., Fig. 3E). Therefore, the alternating APs, which occurred only in the epicardium, caused macro-TWAs in the transmural preparations. The alternans recorded in the AP and ECG correlated precisely, supporting the conclusion that the AP alternans was generated by the electrical activity in the ventricular muscle and not by any possible residual contractility in the immobilized tissue.

**Epicardial dispersion of APs.** Macro-TWA and random T wave changes occurred in 12 and in 8 of the 18 free wall preparations, respectively. We observed coexistence of adjac-
cent regions having APs with alternating domes (Epi 1), without a dome (Epi 2), and with constant domes (Epi 3) in the epicardium during TWA and during random T wave changes in these preparations (Figs. 2, B and D, and 4). The deep and shallow T waves were associated with the appearance and disappearance of the AP dome and longer and shorter APDs, respectively, in the Epi 1 region during macro-TWA and random T wave changes (e.g., Fig. 4, A and B), and alternating transmural dispersion of APDs (Table 4).

Ventricular arrhythmias during macro-TWA and random T wave changes. We induced TWA/random T wave changes (during 896 deep T wave beats) in 19 preparations. All these TWA/random T wave changes were associated with alternans in the domes and durations of epicardial AP and in the dispersion of repolarization. In contrast, we observed only 333 occurrences of phase 2 reentry (15) during TWA/random T wave changes in 17 (11 free wall and 6 transmural) of these preparations, more frequently during the deep T wave beats than during shallow T wave beats (16 vs. 3 preparations). These observations suggested that alternating epicardial APs caused both TWA and phase 2 reentry in this preparation, since alternating epicardial APs occurred during all TWA and phase 2 reentry and since phase 2 reentry was only weakly associated with (in <40% of) TWA. Both the enhanced phase 2 dome and increased dispersion of repolarization contributed to spontaneous phase 2 activation and reentry in the deep T wave beats (e.g., Fig. 5). We also observed phase 2 activations that produced large T waves but failed to conduct or complete a reentry loop in six free wall and two transmural preparations (e.g., Fig. 6). Ventricular tachycardia or fibrillation occurred spontaneously after TWA in 10 epicardial and 3 transmural tissues (e.g., Fig. 1C).

DISCUSSION

New observations. This study has provided a mechanism to explain TWA in Brugada syndrome based on the spatial heterogeneities and temporal alternans in the domes of the APs and in AP. These observations help expand and update the existing knowledge about TWA in Brugada syndrome obtained previously with only three (2 epicardial and 1 endocardial) floating microelectrodes (12, 39). This study has also provided a possible mechanism for the initiation of ventricular arrhythmias during deep T wave beats in TWA, which has been reported to occur in patients with Brugada syndrome (9, 19, 20, 28). We observed that macro-TWA occurred in association
with alternating transmural dispersion of repolarization (as the consequences of alternating APs within the first 2- to 3-mm depth from the epicardium) at the pacing rates of 30–100 beats/min (e.g., Fig. 3). We also observed the coexistence of adjacent epicardial regions having APs with alternating domes, with nonalternating domes, and without domes (e.g., Fig. 4) and frequent initiation of ventricular arrhythmias and phase 2 reentry in the regions having prominent AP domes at the time of deep T wave beats (e.g., Fig. 5).

In contrast to the previously suggested origin of TWA in Brugada syndrome from the alternating success and failure of phase 2 reentry (12), our experiments suggested that phase 2 reentry was not the cause of macro-TWA but instead was the consequence of AP alternans. We observed that macro-TWA/random T wave changes (in 896 deep T waves) correlated directly with alternating epicardial APs (with longer epicardial APD during deep T waves, e.g., Figs. 2–4) and partially with phase 2 reentry (333 occurrences, e.g., Fig. 5). Therefore, the increased dynamic heterogeneities in the dome and duration of AP caused macro-TWA, phase 2 reentry, and ventricular arrhythmias.

**Table 4. Alternating transmural dispersion of APD during TWA in 7 preparations**

<table>
<thead>
<tr>
<th>T Waves</th>
<th>Transmural Dispersion of APD, ms</th>
<th>Longest APD, ms</th>
<th>Shortest APD, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep</td>
<td>43.6 ± 24.8 (Epi: 7)</td>
<td>273 ± 37.2</td>
<td>237.6 ± 25.9 (Mid: 6, Endo: 1)</td>
</tr>
<tr>
<td>Shallow</td>
<td>16.6 ± 9.3 (Epi: 2, Endo: 5)</td>
<td>239.6 ± 25.2</td>
<td>223.0 ± 25.6 (Epi: 3, Mid: 3, Endo: 1)</td>
</tr>
</tbody>
</table>

Values are means ± SD. APD, action potential (AP) duration. Alternating dome and duration of epicardial APs caused alternans in transmural dispersion of APD. The no. of preparations having the longest or shortest APDs in the epicardium (Epi), midmyocardium (Mid), and endocardium (Endo) are indicated in parentheses.

**Fig. 4.** Epicardial heterogeneity of AP alternans during TWA (A, CL: 2,000 ms) and during random T wave changes (B, CL: 600 ms) in a free wall preparation having ECG characteristics of Brugada syndrome (in μmol/L: 10 pinacidil, 5.0 terfenadine, and 10 pilocarpine). The maps of APD differences between the deep and shallow T wave beats (APD_{deep} – APD_{shallow}), APs at the Epi 1 sites (○), and ECGs are shown at left in A and B. Superimposed ECGs and APs of the deep and shallow T wave beats are shown at right in A and B. Alternating APs between those with and without domes (Epi 1) corresponded to the deep and shallow T waves and coexisted with stable APs without (Epi 2) or with (Epi 3) domes in the epicardium. Increasing pacing rate converted TWA (A) to random T wave changes (B). Top and right sides of the mapping region were next to the tricuspid valve and right ventricular outflow tract.

**In vitro ventricular model of Brugada syndrome.** We used pilocarpine, pinacidil, and terfenadine to reproduce the major characteristics of TWA in Brugada syndrome (13, 16, 30), including S-T elevation, bradycardia-dependent TWA with alternating depth of T waves, deep phase 1 repolarization, large phase 2 dome and reentry, and frequent occurrence of ventric-
patients with Brugada syndrome, in whom TWA occurred at normal (57–100 beats/min) and exercise-increased (>95 beats/min) heart rates, and could be eliminated by isoproterenol or by rapid atrial pacing (8, 9, 19–21, 24, 27, 28).

Alternans in $I_{Na}$ can subsequently lead to alternans in phase 1 repolarization, in phase 2 dome, and in APD. Reducing $I_{Na}$ (by pilsicainide) slows the rate of depolarization and facilitates a deep phase 1 repolarization, especially when coupled with the pilsicainide-augmented $I_{Na}$. The changes in depolarization and phase 1 repolarization modulate the slower-activating membrane ionic currents, thus affecting the phase 2 dome and phase 3 repolarization. Therefore, alternating $I_{Na}$ can lead to alternans in the dome and duration of AP and produce TWA as a

Fig. 5. An example of phase 2 reentry in the epicardium of a free wall preparation having ECG characteristics of Brugada syndrome (in μmol/l: 10 pinacidil, 5.0 terfenadine, and 10 pilsicainide, CL: 600 ms). A. left, shows ECG, AP in the Epi 1 region (site 1), and the epicardial distribution of activation time (in ms). The ECG and APs at sites 1-4 are enlarged in A, right. Regions having alternating domes and regions without and with AP domes (Epi 1–3) are separated by the lines of open squares in the map of activation time. B: epicardial distributions of membrane potential (lighter density representing higher potential) at 3 (a), 78 (b), 107 (c), and 152 (d) ms after the onset of phase 2 reentry. Phase 2 activation conducted counterclockwise (demonstrated by the movement of lighter density in B) from site 1, to sites 2, 3, and 4, and then reentered site 1 (along the dashed arrow line in the map and as indicated on the APs in A).

Fig. 6. Examples of blocked phase 2 conduction during TWA in a free wall preparation (A; in μmol/l: 10.0 pinacidil, 5.0 terfenadine, and 13.0 pilsicainide, CL: 2,000 ms) and in a transmural preparation (B; in μmol/l: 2.5 pinacidil, 5.0 terfenadine, and 5.0 pilsicainide, CL: 2,000 ms) having ECG characteristics of Brugada syndrome. A: free wall example. Activation times (in ms) within the epicardial mapping region are shown in a. APs at the Epi 1–3 sites (●) and ECG are shown in b. The sequential maps in c show the distribution of membrane potential (lighter density representing higher potential) at 23, 67, 92, and 160 ms after the onset of phase 2 activation. Prominent AP dome appeared at the 2nd beat in Epi 1 (b). Phase 2 activation conducted from the border of the Epi 1 region to the Epi 2 and Epi 3 regions made a figure-eight pattern around the Epi 1 region and then was blocked at the opposite border (still in refractory because of longer local APDs) of the Epi 1 region (a and c). The blocked phase 2 reentry produced a large biphasic T wave in the second beat in the ECG (b). B: transmural example. ECGs (top) and transmural distributions of APs (separated by the grid, bottom) during shallow (left) and deep (right) T wave beats. There was no extrasystole at the shallow T wave beat (left). The large AP domes initiated nonconducted phase 2 activation (see arrows) in the epicardium and produced a deep T wave in the ECG (right).
consequence. Terfenadine further enhanced the phase 2 dome of AP and promoted the occurrence of reentry and ventricular arrhythmias.

Our observations of APD alternans within the 2- to 3-mm depth from the epicardium (e.g., Fig. 3) correspond well with the known transmural gradient of \( I_{Na} \) density, which is highest in the epicardium (11), thus supporting the role of \( I_{Na} \) in APD alternans. The alternating epicardial APD caused corresponding alternans in the transmural gradient of APD and in the T wave (e.g., Fig. 3 and Table 4). Progressively deeper (more negative) phase 1 repolarization can cause an initial delay and amplitude augmentation of the AP dome (possibly by delaying the activation of the membrane \( Ca^{2+} \) current, \( I_{Ca\text{,L}} \), with a low but suprathreshold phase 1 potential), then sudden abolition of the dome (phase 1 repolarization becoming subthreshold for \( I_{Ca\text{,L}} \)), and corresponding prolongation and sudden abbreviation of APD. The observed coexistence of adjacent epicardial regions having APDs with domes, without domes, and with alternating domes (e.g., Fig. 4) could be because of regional variations in \( I_{Na} \) alternans and in sequential downstream alternans of \( I_{Na} \) and \( I_{Ca\text{,L}} \). Thus the higher epicardial density of \( I_{Na} \) contributes to both transmural and epicardial dispersions of repolarization in this model of Brugada syndrome.

Differences in macro-TWA between long QT and Brugada syndromes. TWA has been reported previously in isolated ventricular models of long QT syndrome during rapid pacing (7, 25). Excessive midmyocardial prolongation of APD increased the transmural dispersion of repolarization and generated alternans in APD, in intracellular \( Ca^{2+} \), and in T waves when new activations were initiated before the full recovery from prior activations in left ventricles having long QT syndrome (25). Therefore, the major mechanism of TWA in long QT syndrome is APD alternans in the left ventricular midmyocardium during tachycardia. In contrast, TWA in this experimental model of Brugada syndrome is caused by AP alternans in the right ventricular epicardium (e.g., Fig. 4) that is bradycardia-dependent and can be eliminated by increasing heart rate.

Limitations. The present study created an isolated ventricular tissue model of Brugada syndrome with pharmacological agents. Although these agents reproduced the major ECG characteristics of Brugada syndrome, differences between the drug actions and clinical channelopathies exist, and multiple mechanisms that lead to arrhythmias in clinical Brugada syndrome may be present. Although optical mapping enabled simultaneous recording from 256 sites, each channel of AP represented the combined activations of all surface cells within the 1.1 × 1.1 mm² area covered by a single photodiode. Both the aggregated cellular activations in each channel of AP and statistical representations of the endocardial, epicardial, and midmyocardial APs could cause averaging/filtering effects that reduce the depth of phase-1 notch, smooth the contour of AP, and reduce the dispersion of APDs. Compared with intact hearts, the isolated sections of right ventricular free wall are smaller and simpler, and thus could only host a subset of potential electrophysiological mechanisms, although this simplicity provided excellent experimental preparations for testing our hypothesis. In addition to the major regional differences in the dome and duration of AP, as demonstrated in the current study, ECG characteristics of Brugada syndrome can also be generated by conduction delay as reported due to altered \( I_{Na} \) and local fibrosis in the right ventricular outflow tract of a human heart (10). Therefore, the mechanism of TWA demonstrated in this model of Brugada syndrome is just one of the possible explanations of TWA in Brugada patients. Finally, we did not record activity in the left ventricle. Although the left ventricle is not thought to contribute to arrhythmias in Brugada syndrome, we cannot draw conclusions about what may be happening there.

GRANTS

This research was supported by Award 0455517Z from the American Heart Association Midwest Affiliate and by the Herman C. Kranert Fund, Indianapolis.

REFERENCES


20. Schwartz PJ and Malliani A.


