Two Types of Ventricular Fibrillation in Isolated Rabbit Hearts

Importance of Excitability and Action Potential Duration Restitution

Tsu-Juey Wu, MD; Shien-Fong Lin, PhD; James N. Weiss, MD; Chih-Tai Ting, MD, PhD; Peng-Sheng Chen, MD

Background—The combined effects of excitability and action potential duration (APD) restitution on wavefront dynamics remain unclear.

Methods and Results—We used optical mapping techniques to study Langendorff-perfused rabbit hearts. In protocol IA (n=10), D600 at increasing concentrations was infused during ventricular fibrillation (VF). With concentration increased to 0.5 mg/L, fast VF (dominant frequency, 19.1±1.8 Hz) was consistently converted to ventricular tachycardia (VT). However, increasing D600 further to 2.5 or 5.0 mg/L converted VT to slow VF (11.9±2.3 Hz, P=0.0011). In an additional 4 hearts (protocol IB), tetrodotoxin converted a preexisting VT to slow VF (11.0±1.4 Hz). Optical maps show wandering wavelets in fast VF, organized reentry in VT, and spatiotemporal periodicity in slow VF. In protocol II, we determined APD and conduction time (CT) restitutions during D600 infusion. CT was used as an estimate of excitability. At 0.1 mg/L, APD and CT restitutions were steep and flat, respectively. APD restitution became flattened when D600 increased to 0.5 mg/L, converting fast VF to VT. Further increasing D600 to 2.5 or 5.0 mg/L steepened CT restitution and widened the range of S1 pacing cycle lengths over which CT decreased, converting VT to slow VF.

Conclusions—Two types of VF exist in isolated rabbit hearts. Fast (type I) VF is associated with a steep APD restitution and wandering wavelets. Slow (type II) VF is associated with a flat APD restitution, a steep CT restitution, and spatiotemporal periodicity. Both excitability and APD restitution are important in VF maintenance.

Key Words: arrhythmia ■ fibrillation ■ mapping ■ ventricles

It is hypothesized that both action potential duration (APD) and conduction velocity (CV) restitution characteristics are sources of wavelength (WL) oscillations that lead to wave break.1 Because APD and CV oscillations are both associated with short diastolic intervals,1 it is difficult to test separately the relative importance of APD and CV restitutions in ventricular fibrillation (VF) maintenance. Methoxyverapamil (D600), an excitation-contraction uncoupler, not only inhibits slow Ca2+ inward current at low concentrations (1 μmol/L, equivalent to 0.52 mg/L) but also depresses fast Na+ inward current at higher concentrations (5 to 50 μmol/L, equivalent to 2.6 to 26 mg/L).2 The latter effect reduces excitability and CV. We hypothesize that low D600 concentrations flatten APD restitution by calcium channel blockade,3 whereas high D600 concentrations reduce excitability and steepen CV restitution by sodium channel blockade.

To estimate CV and excitability, we measured the inverse of conduction time (CT) between 2 epicardial points. Optical mapping techniques in Langendorff-perfused rabbit hearts were used to study the effects of different D600 concentrations on APD and CT restitution and on wavefront dynamics during VF. We also used tetrodotoxin (TTX), a sodium channel blocker, to determine the role of decreased excitability on VF/ventricular tachycardia (VT) transitions. The purpose of this study was to test the hypotheses that both excitability and APD restitution are important in VF maintenance.

Methods

Langendorff Preparation and Pseudo-ECG Recordings

The hearts of adult rabbits (3.4 to 4.8 kg) were excised under general anesthesia. The ascending aorta was immediately cannulated and perfused at 20 to 30 mL/min with oxygenated and warmed (36.5°C) Tyrode’s solution with a pH of 7.4. Then the heart was both perfused and superfused in a tissue bath made with transparent glass.
A pseudo-ECG was registered throughout the experiment with widely spaced bipoles, one at the left ventricular apex and the other at the high lateral wall of the right ventricle.

Optical Mapping
The optical mapping system has been described previously. The hearts were stained with di-4-ANEPPS. They were then excited with quasi-monochromatic light (500±40 nm) from a 250-W tungsten-halogen lamp. Fluorescent and scattered light was collected by an image-intensified charge-coupled device camera. The data were gathered at 3.75-ms sampling intervals, acquiring from 100×100 sites simultaneously over a 40×40-mm² area. The mapped area included parts of the right and left ventricular free walls. A pair of hook bipolar electrodes was inserted into the right ventricular outflow tract for pacing or the determination of effective refractory period (ERP).

Protocol I: Effects of D600 and TTX on VF/VT Activations

**Protocol IA (n=10)**
We used burst pacing (cycle lengths, 75 to 100 ms; currents, 5 to 10 mA) to induce baseline VF. Increasing concentrations of D600 (0.1, 0.5, 2.5, and 5.0 mg/L) were then infused sequentially (15 minutes for each) during VF to observe the change of rhythm. D600 was then washed out with drug-free Tyrode’s solution (washout period).

**Protocol IB (n=4)**
D600 (0.5 to 1.0 mg/L) was infused to convert baseline VF to VT. TTX (3 μmol/L) was then infused to observe the change of rhythm. After a 10-minute infusion, TTX was washed out with drug-free Tyrode’s solution.

Protocol II: Restitution Curves

**Protocol IIA (n=7)**
We used extrastimulus (S₂) at twice diastolic threshold current to determine ERP at baseline and at the end of 15-minute infusion of different D600 concentrations (0.1, 0.5, 2.5, and 5.0 mg/L). APD and CT⁻¹ restitutions were then determined by use of 12 different S₁ pacing cycle lengths (500, 400, 300, 250, 200, 180, 160, 150, 140, 130, 120, and 110 ms) in all hearts (S₁ pacing method).

In hearts 3 through 7, to minimize motion artifacts and to obtain baseline restitution data, an adjustable glass wall was used to compress and restrain the hearts during pacing. In these 5 hearts, we also determined APD restitution by using the S₁-S₂ method in addition to the above S₁ pacing method.

**Protocol IIB (n=3)**
We first determined APD and CT⁻¹ restitutions by the S₁ pacing method at baseline, 15-minute 0.1 mg/L, and 15-minute 5.0 mg/L D600 infusion sequentially. We then induced VF if it did not occur during the previous S₁ pacing. D600 was then washed out with drug-free Tyrode’s solution to obtain washout VT. After direct current cardioversion, APD and CT⁻¹ restitutions were again determined immediately by the S₁ pacing method.

**Protocol III: Control (n=3)**
To evaluate whether spontaneous VF/VT transition can occur without the presence of D600, we used drug-free Tyrode’s solution to

Figure 1. A through E, APD and CT⁻¹ determinations (data from heart 1 of protocol IIA). CT at ventricular walls was inhomogeneous during S₁ pacing (A). Mean value of CT⁻¹ along lines 1 to 4 was used to estimate CT⁻¹ restitution (B). Recordings from sites a through d were used for APD analyses (C). D and E, Examples of CT⁻¹ (line 2) and APD₁₀ (site a) determinations at different D600 concentrations and different S₁ pacing cycle lengths. F through H, Isochronal maps showing effects of different D600 concentrations on CT (data from heart 7 of protocol IIA). Asterisks in B, C, and F through H mark pacing site. LAD indicates left anterior descending artery; PCL, pacing cycle length.
perfuse 3 hearts continuously for 60 minutes. A pseudo-ECG was recorded at 5, 30, and 60 minutes, respectively, to document the rhythm.

**Data Analysis**

Optical mapping data were processed through several image-processing algorithms. After data processing, pixels at several sites were chosen for APD and CT−1 determinations (Figure 1).

**Construction of APD and CT−1 Restitution Curves**

**S1 Pacing Method**

As shown in Figure 1A, CT at the ventricular walls was inhomogeneous during S1 pacing. Therefore, we used the mean value of CT−1 along 4 different lines to construct CT−1 restitution (Figure 1B). For APD determination, pixels at 4 sites (sites a through d) of the mapped area were used (Figure 1C). APD was measured at 70% repolarization (APD70). Similarly, the mean value of APD70 at these 4 sites was used to estimate APD restitution. Figure 1, D and E, shows examples of CT−1 and APD70 determinations. APD and CT−1 restitution curves were then constructed by plotting means of APD70 (ms) and means of CT−1 (cm/s) against different S1 pacing cycle lengths. By use of the formula APD70/CT−1=WL (cm), WL restitution curves were also constructed.

**S1-S2 Method**

When APD restitution was tested with the S1-S2 method, the APD70 associated with S2 was used. An APD restitution curve was created by plotting the S2 APD70 against its diastolic interval. A restitution curve was then generated by exponential fit. This curve was also obtained at sites a through d in each heart.

**Determination of APD Dispersion**

APD dispersion (ms) was defined as the difference between maximum and minimum APD70 obtained from 100 pixels evenly distributed over the mapped area during S1 pacing.

**Fast Fourier Transform Analysis**

Fast Fourier transforms (FFTs) of pseudo-ECGs (4 seconds in duration) were used to determine the dominant frequencies at different stages of ventricular arrhythmias. In protocol IA, 4 stages of VF were defined: (1) baseline VF (no D600), the stable VF 5 minutes after the successful induction by burst pacing; (2) fast (type 1) VF, the VF with 15-minute 0.1 mg/L D600 infusion. In hearts 6, 9, and 10 (Figure 2A), however, VF-to-VT transition occurred during 0.1 mg/L infusion. Fast VF was defined as the period of VF immediately preceding VF-to-VT transition in these 3 hearts; (3) slow (type II) VF, the VF at 3 minutes after the transition from VT to an irregular and slow rhythm; and (4) washout VF, the VF during the washout period.

**Statistical Analysis**

Paired t tests and ANOVA with repeated measures were used for statistical comparisons. A probability value of P≤0.05 was considered significant. In case of multiple comparisons, Bonferroni-adjusted probability values were used to determine the significance.

**Results**

**Protocol IA**

**VF/VT Transitions During D600 Infusion**

As in Figure 2A, baseline VF was successfully induced by burst pacing in all 10 hearts. After 0.1 mg/L D600 infusion, the irregular rhythm was converted to VT within 12 minutes in 3 hearts (hearts 6, 9, and 10). In the remaining 7, fast VF was consistently converted to VT within 8-minute 0.5 mg/L infusion. Subsequently, during 2.5 (n=3) or 5.0 (n=4) mg/L infusion, VT was again converted to an irregular and slow rhythm (slow VF). Finally, washout VT (6 of 10 hearts) and VF (10 of 10 hearts) were obtained sequentially within 25-minute washout. Figure 3, A through I, shows examples of pseudo-ECG during VF/VT transitions. Note that as in Figure 3, C and F, VF/VT transitions always occurred abruptly. FFT analyses of pseudo-ECG (Figure 3, A’ through I’) show that there was a significant difference of the mean dominant frequency when baseline VF, fast VF, and washout VF occurred (17.9±1.9, 19.1±1.8, and 17.7±1.7 Hz, P=0.001). In the 7 hearts with transition to slow VF, the mean dominant frequency during fast VF was much higher than during slow VF (19.8±1.5 versus 11.9±2.3 Hz, P=0.0011).

**Wavefront Characteristics During VF and VT**

Optical mapping data showed that multiple wavefronts were present during both fast VF (Figure 4B) and slow VF (Figure 4D). However, wavefront characteristics in these 2 periods were distinctly different. Both wandering wavelets and organized reentry with a drifting core were present during fast VF. As observed previously for Wiggers’ stage II VF in vivo, wave-wave interaction, wave break, and generation of new
reentry occurred continuously. During slow VF, however, wave-wave interaction and generation of new reentry were rarely observed. In contrast, the mapped area always showed the presence of a single long wavefront or a single epicardial breakthrough, activating repetitively in a similar pattern (ie, spatiotemporal periodicity). These wavefronts propagated slowly and usually spontaneously ceased to propagate in segments, leading to wave break (Figure 4, D and D’).

After the transition from fast VF to VT, activation patterns were characterized by either stationary spiral waves (n=7, Figure 4C) or epicardial breakthroughs (n=3). The mean dominant frequency was 17.5±2.2 Hz after 15-minute 0.5 mg/L D600 infusion.

Protocol IB
Effects of TTX on VT
Baseline VF was first induced by burst pacing in all 4 hearts (Figure 5A). After the infusion of 0.5 to 1.0 mg/L D600, the irregular rhythm was consistently converted to VT within 25 minutes (Figure 5B). After TTX (3 μmol/L) infusion, the VT was again converted to an irregular and slow rhythm (TTX-induced slow VF) within 2 minutes (Figure 5, C and D). Washout VT and/or VF occurred within 10-minute washout (Figures 2B and 5E). There was a significant difference of the mean dominant frequency between baseline VF and TTX-induced slow VF (18.6±1.0 versus 11.0±1.4 Hz, P=0.0011). Figure 5, A’ through E’, shows typical examples. On the basis of the above findings, slow VF was a result of combined calcium and sodium channel blockades.

Wavefront Characteristics During TTX-Induced Slow VF
Wave-wave interaction and reentry formation were rarely observed. The mapped area always showed the presence of a
single long wavefront, activating repetitively in a similar pattern. These wavefronts also propagated slowly, leading to spontaneous wave break (Figure 5, F and F'). Figure 5, G and G', shows washout VT. There was a stable activation pattern with 2:1 conduction at site a, causing electrogram alternans.

Protocol IIA

**Effects of D600 on ERP and APD Dispersion**

In all 7 hearts, ERP shortened progressively \((P<0.0001)\) with increasing D600 concentrations. At an \(S_1\) pacing cycle length of 500 ms, ERPs were 147±14 (baseline), 124±14 (0.1 mg/L), 111±10 (0.5 mg/L), 94±11 (2.5 mg/L), and 91±11 ms (5.0 mg/L). However, D600 had no significant effect on APD dispersion \((P=0.25, \text{data from hearts 3 through 7})\).

**Dispersion of APD**

Dispersions of APD at an \(S_1\) pacing cycle length of 160 ms were 19±3 (baseline), 16±2 (0.1 mg/L), 16±3 (0.5 mg/L), 17±2 (2.5 mg/L), and 16±2 ms (5.0 mg/L).

**Effects of D600 on APD, CT\(^{-1}\), and WL Restitutions**

**S\(_1\) pacing method.** Effects of D600 on APD, CT\(^{-1}\), and WL restitutions were similar among all 7 hearts. Figure 6, A through C, shows an example. At baseline and 0.1 mg/L, APD restitution was steep but CV estimated from CT\(^{-1}\) was fast with flat restitution (Figure 6, A and B). Increasing D600 to 0.5 mg/L flattened APD restitution (Figure 6A). These changes are associated with fast VF-to-VT transition. Further increase of D600 to 2.5 and 5.0 mg/L slowed CV (estimated from CT\(^{-1}\)) and steepened CT\(^{-1}\) restitution. The range of \(S_1\) pacing cycle lengths over which CT\(^{-1}\) decreased was wider for 5.0 mg/L than for 2.5 mg/L (Figure 6B). These changes were associated with VT-to-slow VF transition. In addition, WL restitution curves continuously shifted downward with infusions of 0.1, 0.5, 2.5, and 5.0 mg/L (Figure 6C). This finding indicates the progressive shortening of WL at increasing D600 concentrations. However, VF occurred both at long WL (low D600 concentration) and short WL (high D600 concentration). Therefore, the absolute value of WL did not determine whether the rhythm was VF or VT. The Table summaries the mean APD\(_{\text{max}}\) and CT\(^{-1}\) in these 7 hearts at different D600 concentrations (including baseline data in hearts 3 through 7) and different \(S_1\) pacing cycle lengths.
**S1-S2 method.** In the 5 hearts with the S1-S2 method for APD restitution determination (as in Figure 6, D through F, D’ through F’), the slope of APD restitution was >1 with short diastolic intervals at baseline (range, 32 ± 6 to 17 ± 7 ms) and 0.1 mg/L infusion (range, 24 ± 4 to 12 ± 6 ms). Furthermore, maximum slope of APD restitution decreased progressively (*P*<0.0001) with increasing D600 concentrations. Maximum slopes were 2.46 ± 0.83 (baseline), 1.49 ± 0.42 (0.1 mg/L), 0.64 ± 0.24 (0.5 mg/L), 0.27 ± 0.17 (2.5 mg/L), and 0.22 ± 0.12 (5.0 mg/L).

**Effects of D600 on Spatial Heterogeneity of Restitutions**

**APD Restitution**

At baseline, the maximum slope of APD restitution was different among the 4 recording sites (site a, 2.60 ± 0.50; b, 3.08 ± 0.60; c, 1.66 ± 0.34; and d, 2.51 ± 1.10; *P*=0.040). However, this heterogeneity became insignificant after D600 infusion.

**CT−1 Restitution**

Although there was no conduction block during S1 pacing in all hearts studied, CT−1 decreased inhomogeneously at increasing D600 concentrations (Figure 1, F through H). “Maximum CT−1 reduction” was used to estimate the heterogeneity of CT−1 restitution. It was defined as the difference of CT−1 at the longest and the shortest S1 pacing cycle lengths (see Figure 6B). There was no significant difference of maximum CT−1 reduction along the 4 different lines at baseline, 0.1, and 0.5 mg/L. However, significant heterogeneity occurred at 2.5 mg/L (line 1, 22 ± 8; line 2, 18 ± 5; line

### APD70 and CT−1 (Protocol IIA)

<table>
<thead>
<tr>
<th>D600, mg/L</th>
<th>100</th>
<th>200</th>
<th>250</th>
<th>300</th>
<th>400</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>APD70, ms</th>
<th>100</th>
<th>120</th>
<th>140</th>
<th>160</th>
<th>180</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline*</td>
<td>144 ± 8 †</td>
<td>142 ± 7 †</td>
<td>136 ± 5 †</td>
<td>129 ± 5 †</td>
<td>114 ± 4 †</td>
<td>106 ± 6 †</td>
</tr>
<tr>
<td>0.1</td>
<td>136 ± 8 †</td>
<td>135 ± 8</td>
<td>128 ± 5</td>
<td>121 ± 6</td>
<td>109 ± 5</td>
<td>102 ± 6</td>
</tr>
<tr>
<td>0.5</td>
<td>121 ± 8 †</td>
<td>117 ± 8</td>
<td>109 ± 8</td>
<td>104 ± 7 †</td>
<td>96 ± 6 †</td>
<td>91 ± 5 †</td>
</tr>
<tr>
<td>2.5</td>
<td>111 ± 10 †</td>
<td>108 ± 9 †</td>
<td>103 ± 7 †</td>
<td>97 ± 7 †</td>
<td>91 ± 5 †</td>
<td>86 ± 4 †</td>
</tr>
<tr>
<td>5.0</td>
<td>102 ± 11 †</td>
<td>100 ± 11 †</td>
<td>94 ± 10 †</td>
<td>91 ± 9 †</td>
<td>85 ± 7 †</td>
<td>81 ± 7 †</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CT−1, cm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline*</td>
</tr>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>0.5</td>
</tr>
<tr>
<td>2.5</td>
</tr>
<tr>
<td>5.0</td>
</tr>
</tbody>
</table>

PCL indicates pacing cycle length.

*Baseline data from hearts 3 to 7.
† *P*<0.05, ‡ *P*<0.01, § *P*<0.001 by paired *t* test when compared with the data during 0.1-mg/L infusion.
| Data from 2 to 6 hearts without statistical comparison (missing data caused by VF induction).
pressed fast Na⁺ inward current and reduced excitability, converting a preexisting VT into an irregular and slow rhythm (Figure 5, C and D).

Indicators of CT⁻¹ Restitution for VF Maintenance
In Figure 6B, the steepness of CT⁻¹ restitution is slightly greater for 2.5 mg/L than for 5.0 mg/L. The range of S₁ pacing cycle lengths over which this curve descends, however, is wider for 5.0 mg/L. These findings suggest that the range over which this curve descends might be a better indicator than the steepness for VF maintenance.

Two Types of VF
In this study, activation patterns of slow VF were very different from those of fast VF. Fast VF (dominant frequency: 19.1±1.8 Hz) was characterized by wandering wavelets and organized reentry with short life spans. Slow VF (11.9±2.3 Hz), however, rarely showed wave-wave interaction or epicardial reentry. Rather, the mapped area always showed a single long wavefront or a single epicardial breakthrough emerging from the same region repetitively. As this wavefront propagated outward, it frequently developed wave breaks at some locations, suggesting fibrillatory conduction block. Chen et al⁷ previously reported that VF in rabbit hearts had a wide range of activation rates (dominant frequency, 7.0 to 20.1 Hz). Like us, they also observed significant spatiotemporal periodicity during slow VF (9.9 Hz).

Implications for Mechanisms of VF
Two major hypotheses have been proposed to explain cardiac fibrillation. One is the multiple-wavelet hypothesis,⁸ and the other is the focal-source hypothesis.⁹ In support of the multiple-wavelet hypothesis, computerized mapping studies have documented that VF is characterized by the presence of multiple wandering wavelets in the hearts of large animals.⁵,⁶,¹⁰ Both steep APD restitution and preexisting heterogeneities play synergistic roles in creating wave breaks that maintain VF.⁴,⁵,¹¹ Organized reentrant wavefronts (rotors) have short life spans because of their intrinsic dynamic instability as well as outside interference. In support of the focal-source hypothesis, Jalife et al¹² hypothesized that a fairly stable mother rotor may serve as a source of fast focal activation. Because of its fast rate, wavefronts emerging from the mother rotor develop conduction block. Unlike the multiple-wavelet mechanism, however, wave breaks are epiphenomena and are not essential for VF maintenance. These 2 hypotheses are thought to be competing rather than complimentary mechanisms of VF.¹³,¹⁴

On the basis of the results of this study, we propose that these 2 mechanisms are both important in understanding VF maintenance. At baseline, with a steep APD restitution and normal excitability, the mechanism of VF is compatible with the multiple-wavelet hypothesis. However, when excitability is depressed and CT⁻¹ restitution steepens, because of either drugs or ischemia,¹⁵,¹⁶ the focal-source (mother-rotor) mechanism may gain increasing importance. Under these conditions, complex patterns of electrical activation associated with fibrillatory conduction block promote the electrocardiographic appearance of VF.
Clinical Implications
Our findings may partially explain Wiggers’ 4 stages of VF. Wiggers’ stage I VF resembles VT. Because of a steep APD restitution, VT degenerates into multiple-wavelet (Wiggers’ stage II) VF. Drugs that flatten APD restitution may prevent this degeneration. As ischemia progresses, reduced excitability may convert fast (Wiggers’ stage II) VF into slow (Wiggers’ stages III and IV) VF. For the latter 2 stages of VF, flattening APD restitution alone might not be a useful intervention.

Acknowledgments
This study was supported in part by Yen-Tjing-Ling Medical Foundation, Taipei, Taiwan; by grants R01-HL-58533, R01-HL-58241, P50-HL-52319, and R01-HL-66389 from the National Institutes of Health, Bethesda, Md; and by the Laubisch, Kawata, and Pauline and Harold Price Endowments, Los Angeles, Calif.

References
Two Types of Ventricular Fibrillation in Isolated Rabbit Hearts: Importance of Excitability and Action Potential Duration Restitution
Tsu-Juey Wu, Shien-Fong Lin, James N. Weiss, Chih-Tai Ting and Peng-Sheng Chen

_Circulation_. 2002;106:1859-1866; originally published online September 9, 2002; doi: 10.1161/01.CIR.000031334.49170.FB
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. 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/106/14/1859

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/