Mechanism of Procainamide-Induced Prevention of Spontaneous Wave Break During Ventricular Fibrillation
Insight Into the Maintenance of Fibrillation Wavefronts

Young-Hoon Kim, MD; Masaaki Yashima, MD; Tsu-Juey Wu, MD; Rahul Doshi, MD; Peng-Sheng Chen, MD; Hrayr S. Karagueuzian, PhD

Background—Ventricular fibrillation (VF) is maintained by 2 mechanisms: first by reentry formation and second by spontaneous wave break or wave splitting. We hypothesized that spontaneous wave break results from a critical shortening of the action potential duration (APD) during VF and that its prevention by procainamide eliminates spontaneous wave break.

Methods and Results—The endocardial surfaces of 7 isolated, perfused swine right ventricles were mapped with a 3.2×3.8 cm plaque with 477 bipolar electrodes. Activation pattern during VF was visualized dynamically while simultaneously recording epicardial action potentials with a glass microelectrode. APD restitution curves were constructed during VF (dynamic) and during S1 S 2 protocols. At baseline, VF was maintained by 5.3±1 wavelets. Procainamide (PA) at 10 μg/mL decreased the number of wavelets to 3.5±1 (P<0.05). At baseline VF was maintained by spontaneous wave break and by new reentrant wave front formation. PA eliminated spontaneous wave break during VF while having no effect on reentry formation. PA increased the cycle length of the VF (148.5±41.2 ms vs 81±10 ms, P<0.01) and the core area of the reentry from 5.8 to 14.5 mm² (P<0.05). Dynamic APD restitution curve during VF showed that PA eliminated the initiation of activation with APDs shorter than 30 ms. The effects of PA on cellular properties and wave front dynamics were reversed during 60 minutes of drug-free perfusion.

Conclusions—Critically short APDs during VF promote spontaneous wave break. Their elimination with PA, however, maintains VF by generating new reentrant wave front. (Circulation. 1999;100:666-674.)

Key Words: fibrillation • waves • reentry • action potentials

W e recently have shown that during ventricular fibrillation (VF), 2 different mechanisms continuously generate “daughter” wavelets that maintain the VF.1,2 The first mechanism, wave-wave interaction, leads to the formation of a reentry.1,2 Reentry by wave-wave interaction occurs when 2 fronts intersect perpendicularly. One front traveling north-south, for example, blocks at the site of residual refractoriness left behind by another wave that had just traveled east-west.1,2 Simulation studies have replicated the mechanism of reentry formation by wave-wave interaction.3 The second mechanism that sustains the VF is spontaneous wave break (wave splitting).2,4 According to this scenario conduction block occurs at a specific site along the wave front while the remaining portions of the front continue to propagate. The localized block, wave break, causes splitting of the mother wave front into 2 daughter wavelets. The mechanism(s) of spontaneous wave break remains poorly understood. Simulation studies suggest that spontaneous wave break occurs at sites where wave length (product of action potential duration, APD, and conduction velocity, CV) is critically short.5,6 Short wavelengths often result from very short APDs that frequently occur during rapid rates of activation due to myocardial cell APD restitution.7,8 Block with short APD is also shown to occur in isolated atrial tissue.8 In the present study we hypothesized that the development of spontaneous wave break during VF is related to the incidence of critically short APDs and that prevention of activation with such short APDs with procainamide (PA) prevents spontaneous wave break.

Methods

Surgical Methods

Seven farm pigs (weight 27 to 35 kg) of either sex were anesthetized with sodium thiopental 20 mg/kg IV. The chest was opened with a median sternotomy, the hearts were removed, and the right ventricle (RV) was perfused through the right coronary artery as described previously.9 The RV was perfused with 37°C Tyrode’s solution gassed with 95% O₂ and 5% CO₂ at a flow rate of 20 mL/min. The composition of the solution was as follows (mmol/L): NaCl 125.0, KCl 4.5, MgCl₂ 0.5, CaCl₂ 0.54, NaH₂PO₄ 1.2, NaHCO₃ 24.0, and

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glucose 5.5, with a pH of 7.35. The isolated RV was placed with the endocardial side down in a tissue bath, and the entire tissue was also superfused with warmed (37°C) oxygenated Tyrode’s solution. Two bipolar electrodes were placed on the RV for continuous recording and pacing the tissue. A pair of defibrillation coil electrodes (CPI) were placed on either side of the tissue bath and were connected to a HVS-02 external defibrillator (Ventritex).

Computerized Mapping Studies
At the bottom of the tissue bath was a built-in electrode array containing 477 active bipolar recording electrodes in a 21-column and a 25-row plaque. The interelectrode distance was 1.6 mm, and the interpolar distance was 0.5 mm measured from center to center. The electrodes were connected to a computerized mapping system that we described previously. The patterns of activation were visualized dynamically on the computer screen, in which each electrode site was illuminated when an activation was recorded. Each site (dot) initially turned red, then pink, then yellow, then green, and finally purple before fading to background (black) color. The core size of the reentry was measured during dynamic display by tracing the inner most edge of the wave front (tip) after 1 complete rotation. The number of wave fronts was defined as the number of sites depolarized that were separated from each other by recovered tissue. The length of each wavelet was measured by counting the number of continuous adjacent sites undergoing depolarization and then by multiplying this number by 1.6 mm when

Effects of Procainamide on Action Potential and Wave Front Characteristics During VF and VT

<table>
<thead>
<tr>
<th>PA Concentration</th>
<th>APD&lt;sub&gt;90&lt;/sub&gt;, ms</th>
<th>DI, ms</th>
<th>CL, ms</th>
<th>WF, Average No.</th>
<th>Core, mm&lt;sup&gt;2&lt;/sup&gt;</th>
<th>RWF, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before PA</td>
<td>62.9±9.8</td>
<td>13.4±2.4</td>
<td>77.8±10.4</td>
<td>5.3±1.0</td>
<td>5.8±1.3</td>
<td>10</td>
</tr>
<tr>
<td>5 μg/mL</td>
<td>71.7±10.5</td>
<td>15.0±2.3</td>
<td>83.1±10.4</td>
<td>4.0±0.8</td>
<td>7.7±2.5</td>
<td>9</td>
</tr>
<tr>
<td>10 μg/mL</td>
<td>76.3±13.2*</td>
<td>18.5±4.4*</td>
<td>95.1±13.4*</td>
<td>3.5±1.0*</td>
<td>9.4±3.9</td>
<td>6</td>
</tr>
<tr>
<td>15 μg/mL</td>
<td>84.7±20.4*</td>
<td>21.2±3.8*</td>
<td>107.1±19.6*</td>
<td>1.3±0.6*</td>
<td>14.5±5.9*</td>
<td>6</td>
</tr>
<tr>
<td>VT</td>
<td>109.1±22.7†</td>
<td>39.8±19.3†</td>
<td>148.5±9.1†</td>
<td>1.0±0.0†</td>
<td>20.5±3.6*</td>
<td>7</td>
</tr>
<tr>
<td>Washout</td>
<td>64.8±11.0</td>
<td>12.5±8.6</td>
<td>77.3±15.6</td>
<td>4.9±0.9</td>
<td>6.1±1.9</td>
<td>7</td>
</tr>
</tbody>
</table>

<sup>APD<sub>90</sub></sup> indicates action potential duration at 90% repolarization; DI, diastolic interval; CL, cycle length; WF, wave front; PA, procainamide; VT, ventricular tachycardia; VF, ventricular fibrillation; and RWF, reentrant wave fronts analyzed for core measurement.

*P<0.05, †P<0.01 vs previous (ANOVA). Values are mean±SD.
propagation was parallel to the axes of the plaque and by 2.26 mm when it was diagonal to them. CV during VF was determined by analyzing 1 of the 2 interacting wave fronts that was parallel to the axes of the plaque and then by dividing the distance (number of consecutive electrodes multiplied by 1.6 mm) by the time taken to travel that distance.2

Recording of Transmembrane Action Potentials
Transmembrane action potentials were recorded from 1 randomly chosen epicardial site with a standard glass microelectrode filled with 3 mol/L KCl and digitized at 3.13 kHz with 12-bit accuracy (Axon Instruments, Inc).9,11

Study Protocol
In all isolated RV tissues, spontaneous VF occurred during the isolation procedure and persisted in the tissue bath. Several 8-second VF data were acquired while continuously recording transmembrane action potentials.

Protocol 1
In 4 tissues, VF was allowed to continue undisturbed, and PA (5 µg/mL) then was added to the perfusate and maintained for 30 minutes. Every 5 minutes during infusion of PA, transmembrane potential recordings and mapping studies were repeated. The concentration of PA was then progressively increased to 10 and 15

Figure 2. Effect of increasing concentrations of PA on core size of functional reentry during VF in isolated, perfused swine RV. A through D, Snapshots of activation dynamics at baseline (A) and at each concentration of PA (B through D). The number on each frame represents the time during which the snapshot was taken. Arrows in each frame show the path of the innermost tip of the front; last frame in each panel illustrates the trajectory of the tip after completing 1 full rotation. PA increases the area of the core and reentry CL in a concentration-dependent manner.
mg/mL until VF terminated or converted to monomorphic tachycardia (MVT). Reversibility of drug effect was evaluated 60 minutes after drug-free Tyrode’s perfusion.

Protocol 2
In 3 tissues, after acquisition of simultaneous action potential and activation map data during VF at predrug, baseline state, biphasic shocks of 1.5 to 3.0 J were used to defibrillate the RV. The RV was then paced with twice diastolic threshold current at 400-ms cycle length (CL) and the APD restitution curve constructed by the extrastimulus method. VF was then induced by rapid pacing, and the effects of PA were evaluated as in protocol 1.

Dynamic APD Restitution During VF
The APD restitution curves were constructed during VF (dynamic restitution) with the use of a custom-written program in which the selection of action potentials during VF was based on the criterion of $(dV/dt)_{\text{max}} > 5$ V/s. CL was defined by the temporal difference between consecutive action potential upstrokes. APD to 90% repolarization time (APD$_{90}$) was measured along with diastolic interval (DI), defined as the difference between CL and APD$_{90}$. A dynamic APD restitution curve was then constructed by plotting APD$_{90}$ versus DI. If an action potential occurred before 90% repolarization of the previous one, its DI was considered to be zero.

Statistical Analysis
Data are presented as mean±SD. Student’s $t$ test was used when appropriate. ANOVA with a Newman-Keuls test was used when multiple comparisons were performed. A value of $P<0.05$ was considered significant.

Results

Activation Wave Front Dynamics During VF: Effects of PA
All isolated RVs developed spontaneous VF during mounting in the tissue bath. At baseline, VF was characterized by the presence of 4 to 5 wavelets, with an average CL of 65.2±9.8 ms (Figure 1 and Table). Identification of reentrant wave fronts completing 1 full rotation allowed us to determine reentry CL and its core size (Table). At baseline, the average reentry CL was 82±10 ms, with a central core area of 5.8±1.3 mm$^2$ (Figure 2A and Table). PA decreased the number of wavelets, VF CL, and the core area of the reentry in a concentration-dependent manner (Figure 2 and Table). PA also increased the length of wave front continuity (2.48±0.62 cm vs 1.2±0.27 cm, $P<0.01$) (Figures 1, 2, and 4). CV during VF was decreased after PA in a concentration-dependent manner from 78±1.3 cm/s (control) to 50±14 cm/s (10 μg/mL) and to 46±9 cm/s (15 μg/mL) ($P<0.01$ for all comparisons), as in previous in situ studies. PA did not further reduce CV during VF even after 30 minutes of infusion with 15 μg/mL of PA, when VF converted to ventricular tachycardia (VT). All PA effects were reversible on 60 minutes of drug-free Tyrode’s perfusion (Table).
Wave Front Dynamics During VF: Effects of PA

At baseline, wave-wave interaction and spontaneous wave break were present. Figure 3 illustrates an example of reentry formation by wave-wave interaction at baseline. The wave front numbered 1, propagating east-west, leaves behind a refractory tail that becomes encroached by a second front (No. 2) propagating upward from the mid-right of the plaque. Wave break occurs at the tail of the wave front 1 (double horizontal lines in frame c). The broken wave front 2 then rotates around the site of block (frame d) in a counterclockwise direction and completes 1 full reentrant rotation with a period of 70 ms (frames e through h). Spontaneous wave break frequently occurred at baseline, as in the in situ canine VF.2 Figure 4A illustrates one such example. In this episode, 3 wavelets, a, b and c, are present during the VF. At time 1752 (frame b), while wave front a was propagating toward the bottom (2 single arrows), spontaneous block occurs in its center (frame c). However, the 2 split edges of the front, a1 and a2 (frames c and d), continue to propagate. Wavelet c at the top (frame a) also undergoes breakup into 2 wavelets, c1 and c2, which propagate in opposite directions (frame e). A breakthrough excitation occurs at site d (frame c), which propagates in 2 directions, d1 and d2 (frame d). Wave front b also breaks up spontaneously into b1 and b2 (frame b), which propagate outside the mapped area. During PA infusion (Figure 4B), absence of spontaneous wave break becomes conspicuously evident (Figure 4B). Elimination of spontaneous wave splitting was associated as expected with a significant reduction in the number of wavelets, a slowing of the VF CL, and longer wavelet continuity than baseline (2.48 ± 0.62 cm vs 1.2 ± 0.27 cm, *P* < 0.01) (Figure 1 and Table). PA, however, had no effect on the mechanism of reentry formation by wave-wave interaction. Reentry still occurred, albeit around a larger central core (Table and Figure 2) and with a longer CL than baseline (148 ± 21 ms vs 82 ± 10 ms, *P* < 0.01). Figure 5 illustrates one such episode. Two wavelets, 1 and 2, interact roughly perpendicular to each other (frame c), resulting in wave break at a point located at the lower edge of the double vertical lines in frame c. Propagation below this point proceeds while above it blocks.

Figure 4. Prevention of spontaneous wave break by PA. A, Baseline snapshot during VF illustrates the phenomenon of spontaneous wave break. In a, there are 3 wavelets identified in white as a, b, and c propagating in directions pointed by the 3 double arrows. Wavelet a, propagating downward (a, b, and c), spontaneously breaks into 2 wavelets, a1 and a2 (d). Wavelets b and c in b also break up spontaneously, each into 2 waves, denoted by b1, b2, c1, and c2, respectively. One breakthrough wavelet, identified as d, emerges in the mid-upper region in c, which then spreads in 2 directions, d1 and d2, pointed by the 2 single arrows in d. B, Effects of 10 μg/mL PA infusion. Note that wavelet a propagates without splitting at site where breakup occurred at baseline (A, frames c and d). Complete absence of spontaneous wave break and a decrease in the number of the wavelets having longer continuity are the major effects of PA. For the numbers on each frame, BEg, and double-headed red arrows, see Figure 3.
The site allowing propagation just next to the blocked site (lower edge of the double vertical lines in frame c) was activation 90 ms earlier. This interval reflects the refractory period during VF after PA.1,2 With this approach, the refractory period during VF was significantly ($P<0.01$) prolonged after PA (104±6 ms vs 60±5 ms).

PA and Conversion of VF to VT
Progressive reduction in the number of wavelets during VF eventually led to a single stationary reentrant wave front that was expressed as a stable monomorphic VT (MV) with a mean CL of 148±41 ms (Table). In all 7 tissues studied, PA converted VF to MVT. The concentration associated with conversion to MVT were 5 µg/mL (n=1), 10 µg/mL (n=3), and 15 µg/mL (n=3). In each case a minimum of 20 minutes or PA perfusion was necessary to cause the conversion.

Effects of PA on APD Restitution
PA-induced reduction in the number of wavelets was reflected by a progressive increase in the CL and the diastolic intervals between consecutive action potentials recorded during VF (Figure 6 and Table). Figure 6 illustrates concentration-dependent progressive increase in the CL and APD evolving to a stable and periodic activation. Activation maps during this periodic activity showed a single stationary spiral wave (Figure 1). To determine the basis of APD prolongation (rate-dependent effect caused by fewer wavelets from drug-induced cellular effects), we constructed dynamic and standard ($S_1 S_2$) APD restitution curves before and after PA. Figure 7 shows that PA increased the APD at all diastolic intervals and prevented the initiation of action potentials with duration <210 ms with the use of the $S_1 S_2$ method. Figure 8 illustrates examples of dynamic APD restitution curves during VF and PA effects. PA completely eliminated action potentials during VF with duration <30 ms (all concentrations) and significantly ($P<0.05$) reduced the incidence of action potentials with duration <50 ms in a concentration-dependent manner (Figure 8). APDs with <50 ms occurred 18.87±5.3% of the times at baseline, 9.7±7.9% with 5 µg/mL PA, to 5.5±5.1% with 10 µg/mL PA, and finally with 3.8±3.5% with 15 µg/mL PA (500 to 600 consecutive action potentials analyzed for each concentration in each isolated RV).

Reversal of PA Effects
All of the effects of PA on activation wave front dynamics and APD restitution were reversible within 60 minutes with
drug-free Tyrode’s perfusion (Figure 8B). In 4 isolated RVs, the MVT present at the start of PA washout spontaneously degenerated into VF. In the remaining 3 tissues, VF was readily induced by rapid pacing 1 hour after drug-free Tyrode’s perfusion. Rapid pacing failed to induced VF during the MVT while PA was present.

Discussion

The ability of PA to simultaneously prevent spontaneous wave break during VF and initiation of action potentials with critically short (<30 ms) duration constitute the major findings of the present study. Maintenance of VF after PA was not supported by spontaneous wave break. These effects of
PA caused the VF to be maintained with fewer but longer wavelets and at longer CLs.

Reentry and Wave-Wave Interaction
Although PA prevented spontaneous wave break during VF, it had no effect on reentry formation by wave-wave interaction. Generation of new reentrant wave fronts by wave-wave interaction may be a major mechanism that maintains the VF during PA infusion.

Mechanism of Spontaneous Wave Break
PA-induced complete elimination of action potential initiation with duration <30 ms provides a new insight into the mechanism of spontaneous wave break. The shortest wavelength with 30 ms APD and a CV of 20 cm/s (greatest slowing of CV with PA) corresponds to 6 mm. This suggests that during VF a segment of the wave front with a wavelength shorter than 6 mm would undergo breakup. Simulation studies have shown that when a segment of the front becomes critically short, it undergoes breakup. A segment of the front with a critically shortened wavelength carries insufficient depolarizing current strength (weak source) to depolarize recovering cells during VF (sink). Such a “mismatch” causes splitting (breakup) of the wave front at the point of diminished current source (Figure 4A). The amount of wavelength shortening necessary for breakup, however, depends, among other factors, on tissue recovery (restitution).

Other factors, such as tissue anatomy, including tissue thickness, cellular coupling, and tissue anisotropy, might also exert an influence on the causation of source-sink mismatch and subsequent wave break. However, the demonstration of PA-induced prevention of spontaneous wave break at a site where spontaneous breakup occurred before exposure to drug (Figure 4B) suggests that drug-induced prevention of APD shortening per se played a decisive role in causing spontaneous wave break in the present study. The occasional occurrence of APDs with duration between 50 and 30 ms during VF might reflect possible recordings made from a cell near the core of a meandering reentrant wave front known to be characterized with shortened APD.

In an additional isolated, perfused RV we recorded 620 consecutive action potentials during VF from the endocardial surface before and after PA. As in the epicardial recordings, PA at 10 μg/mL completely eliminated APDs, 30 ms on the dynamic APD restitution curve during VF.

Mechanism of PA-Induced Increase of VF Cycle Length
Our results show that PA might prolong VF CL by 2 different mechanisms. First, prevention of spontaneous wave break decreases the number of wavelets and VF thus becomes maintained with fewer wavelets, causing less frequent activation and therefore an increase of CL. The second mechanism of PA-induced slowing of VF might...
result from the larger central core of reentry formed during VF. Functional reentry around a larger core has a longer rotation period than reentry around a smaller core.23 Our data suggest that both mechanisms might act in concert to increase the VF CL after PA.

**PA and Conversion of VF to MVT**

PA-induced elimination of spontaneous wave break led to a progressive decrease in the number of wavelets, eventually ending up with a single stationary reentrant wave front. Such a dynamic scenario was associated with conversion of VF to MVT (Figure 1). The conversion of VF to MVT, however, was a concentration- and a time-dependent phenomenon. No conversion would occur in <15 minutes of continuous perfusion of PA, often requiring PA concentrations >5 μg/mL. Finally, the ability of PA to convert nonstationary to stationary reentry needs to be addressed. The ability of PA to “flatten” the APD restitution curves (dynamic and standard) by eliminating short APDs and large variations in consecutive APDs are prevented both in time and space (dispersion of repolarization). PA-induced elimination of these tempo-spatial gradients of APD and excitability14,23 known to promote nonstationarity24 may convert nonstationary to stationary reentry.

**Limitations of the Study**

One limitation of the study is that the effects of PA on VF mechanism(s) were evaluated in the isolated RV and during continuous perfusion with oxygenated Tyrode’s solution (no ischemia). Although these conditions do not mimicVF in situ setting,2 the ability afforded by this model to map virtually the entire isolated RV endocardial surface and to simultaneously record transmembrane action potentials made the determination of the mechanism of spontaneous wave break possible. Another limitation of the present study might be that activation maps and simultaneous action potential recordings were done on different surfaces of the RV. However, the demonstration that PA blocks spontaneous wave break on both epicardial and endocardial surfaces with concomitant demonstration of elimination of both epicardial and endocardial APDs <30 ms indicate that critically shortened APDs promote spontaneous wave break during VF in the isolated RV, as in simulation studies.6

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