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Role of Papillary Muscle in the Generation and Maintenance of Reentry During Ventricular Tachycardia and Fibrillation in Isolated Swine Right Ventricle

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Background—The role of papillary muscle (PM) in the generation and maintenance of reentry is unclear.

Methods and Results—Computerized mapping (477 bipolar electrodes, 1.6-mm resolution) was performed in fibrillating right ventricles (RVs) of swine in vitro. During ventricular fibrillation (VF), reentrant wave fronts often transiently anchored to the PM. Tissue mass reduction was then performed in 10 RVs until VF converted to ventricular tachycardia (VT). In an additional 6 RVs, procainamide infusion converted VF to VT. Maps showed that 77% (34 of 44) of all VT episodes were associated with a single reentrant wave front anchored to the PM. Purkinje fiber potentials preceded the local myocardial activation, and these potentials were recorded mostly around the PM. When PM was trimmed to the level of endocardium (n=4), sustained VT was no longer inducible. Transmembrane potential recordings (n=5) at the PM revealed full action potential during pacing, without evidence of ischemia. Computer simulation studies confirmed the role of PM as a spiral wave anchoring site that stabilized wave conduction.

Conclusions—We conclude that PM is important in the generation and maintenance of reentry during VT and VF. (Circulation. 1999;100:1450-1459.)

Key Words: death, sudden | electrophysiology | waves | procainamide | antiarrhythmia agents

The safety factor of impulse propagation depends on the relation between the source (amount of current available in the propagating wave front) and the sink (structure that determines current density when the wave front arrives).1-3 The papillary muscle (PM) is an electrically active tissue. For an electrical impulse to propagate from the ventricle to the PM, the electrical charge it carries (source) must excite both the ventricular muscle downstream and the PM that inserts into the ventricular tissue. In this scenario, the PM serves as an additional sink for impulse propagation. This additional sink may decrease the safety factor of impulse propagation and result in conduction block. Recently, we reported4 that procainamide exerts its antiarrhythmic drug action by preventing spontaneous wave breaks and proposed that this finding may explain the observation that procainamide converts ventricular fibrillation (VF) to ventricular tachycardia (VT) in some patients.5,6 The purpose of the present study was to test the following hypotheses in isolated swine right ventricle (RV): (1) PM serves as a site of conduction block that leads to reentry; (2) PM serves as a site of anchoring for reentry, resulting in a sustained VT; and (3) procainamide converts VF to sustained VT by preventing spontaneous wave breaks and by facilitating the anchoring of a single reentrant wave front to the PM.

Methods

In Vitro VF Model
Details of this model have been reported elsewhere.7 Briefly, farm pigs (34 to 65 kg) were used in the study. The ventricles usually fibrillated during excision, and fibrillation continued in the excised RV. The right coronary artery was cannulated and perfused with Tyrode's solution (37±0.5°C, pH 7.35) equilibrated with 95% O2 and 5% CO2 at a flow rate of 30 mL/min. The flow rate was roughly equivalent to 1 mL · g ventricular wt⁻¹ · min⁻¹. At the bottom of the tissue bath was a built-in electrode array containing 477 bipolar recording electrodes in 21 columns and 25 rows. A pseudo-ECG was recorded by a pair of bipolar electrodes with widely spaced (>3 cm) bipole. Transmembrane potential (TMP) was recorded with standard glass microelectrodes.8

Protocol 1: VT After Tissue Mass Reduction
Eleven RVs were included in this protocol. The chordae tendineae and a portion of the anterolateral PM were removed to facilitate the

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endocardial mapping (Figure 1, A and B). The PM site was thicker than the surrounding ventricular tissue (Figure 1C). This tissue block was subsequently called the “isolated RV.” Scissors were used to cut out a 1.5×3-cm or 1×4-cm portion of the fibrillating RV from the boundary of the tissue distal to the perfusion site. If VF continued, an additional tissue was cut out from the fibrillating RV. In 9 RVs, the cutting spared the PM. In 2 RVs, PM was removed during the first cut. This process continued until the VF either terminated or was converted to VT. The VT was then terminated by rapid ventricular pacing or by defibrillation shocks. Attempts were then made to reinduce VT by rapid electrical stimulation. Sustained VT was defined as a VT that lasted >30 seconds with largely uniform electrogram morphology.

Protocol 2: Effects of Procainamide Infusion
Six RVs were studied with this protocol. After endocardial mapping studies of baseline VF, the effects of procainamide 5 μg/mL on the activation patterns were evaluated. The patterns of activation were mapped 5 minutes, 10 minutes, and 15 minutes after the beginning of the infusion. The procainamide concentration was progressively increased up to 15 μg/mL or when VF converted to VT. Procainamide was then washed out with infusion of drug-free Tyrode’s solution, and the patterns of activation were again mapped.

Protocol 3: Effects of PM Resection
Four RVs with intact PM were studied with this protocol. The tissue was placed in the bath endocardial side up. After baseline patterns of epicardial VF activation had been obtained, the RV mass was gradually reduced by cutting until VF converted to VT. The VT was terminated by overdrive pacing. VF was then reinduced to determine whether it could spontaneously convert to VT. After that, PM was removed so that it was level with the endocardium. Attempts were then made to reinduce VF or VT by premature stimulations and by rapid pacing.

Protocol 4: TMP and Effective Refractory Period of the PM
In 5 RVs, TMP recordings were made during different pacing rates from the surface of the PM, from the base (cut surface) of the PM, and from the endocardium 10 to 12 mm away from the PM. The effective refractory period was determined from multiple sites in the PM and away from the PM at a pacing cycle length (CL) of 400 ms. Both S1 and S2 were given at the same site with twice the diastolic threshold currents and 5-ms pulse duration.

Computer Simulations
Our computer simulations were carried out with the Luo-Rudy Phase I ventricular myocyte cell model with some parameter modifications: (gNa)max was set to 10, (gI)max to 0.055, and (gK)max to 0.338 to yield an action potential duration of 250 ms and other physiologically realistic parameter values. This cell model was then coupled into sheets of various sizes (see figure legends). The resulting model was numerically integrated by use of a forward Euler procedure whose space step dx=dy=dz=0.02 cm and whose time step varied from 0.01 to 0.1 ms. All calculations were carried out on a DEC Alpha workstation.

Figure 1. Endocardial surface of right ventricular free wall. A, PM and chordae tendineae (CT). B, Endocardial surface after PM was partially resected. C, Cross sections of a PM; note that it formed a ridge that protruded from RV endocardial surface. RCA indicates right coronary artery. D, Longitudinal section of PM made at end of experiment. Note uniform fiber orientation parallel to long axis of muscle and presence of normal cells with no signs of injury or autolysis (hematoxylin and eosin stain; magnification ×64).
Data Analyses

Analyses of mapping data were performed according to our previously described algorithm. We defined anchoring of reentrant wave fronts to PM as a mode of activation when the tip of consecutive reentrant wave fronts followed a path corresponding to the boundary of the PM. The tip of the reentrant wavefront was the red dot closest to the core during the dynamic display of reentry.

For wavelet numbers, we first counted the total number of wavelets over the entire 8-second period and the average number of activations recorded on each channel over the same time period. We then divided the number of wavelets by the number of activations to obtain the average number of wavelets per activation. In addition, we also determined the maximum number of wavelets at any instant of VF.

All data are presented as mean ± SD, and Student’s t test was used to compare the means. ANOVA with the Newman-Keuls test was used when multiple comparisons were performed. Linear regression analysis was performed to determine the relationship between PM area and core size of reentrant wave front and the relationship between PM area and the CL of VT anchored to PM. A value of P < 0.05 was considered significant.

Histopathological Examination

At the conclusion of the experiments, the tissues were fixed in 10% buffered formalin. PM area (the area at the junction of the PM at the contact site with the ventricular wall) was measured by a planimeter. Transmural sections were taken to evaluate the thickness of PM and fiber orientation. The tissues were processed routinely for histopathological examinations.

Results

The baseline VF was characterized by a maximum of 4.6 ± 0.7 and a mean of 3.1 ± 0.6 wavelets per activation. With tissue mass reduction and with procainamide infusion, the maximum and mean numbers of wavelets decreased to 3.6 ± 0.5 and 2.0 ± 0.4 (P < 0.05) and 3.2 ± 0.4 and 2.1 ± 0.7 (P < 0.01), respectively, immediately before VF converted to VT. In protocols 1 and 3, when the RV (34.1 ± 6.8 g) was reduced to 16.1 ± 2.0 g, VF converted to VT. In protocol 2 (n = 6), procainamide concentrations associated with VF to VT conversion were 15 µg/mL (n = 3), 10 µg/mL (n = 2), and 5 µg/mL (n = 1). Maps showed that 77% of all VT episodes (34 of 44) were due to a single wave front that anchored to the PM. Anchoring of a single reentrant wave front was seen in 28 of 44 episodes (63.6%) of VT. In 6 episodes (13.6%), repetitive breakthrough pattern near the PM was associated with VT. In the remaining 10 episodes (23%), reentrant wave front was present but the core was not near the PM.
Protocol 1

Role of PM During VF

Reentrant wave fronts were observed in 49 episodes of VF in 17 tissues (average 3.0±1.2 episodes/tissue) at baseline. Of these reentrant wave fronts, 77.6% (38 of 49 episodes) transiently anchored to PM. With the tissue mass reduction, the number of rotations of an anchored reentrant wave front increased from 1.5±1.4 to 4.4±2.7 (P<0.05) before converting to VT. Figure 2 shows a reentrant wave front during VF rotated in a clockwise direction (white arrows, A through F) before drifting toward the right lower portion of the tissue (G). A new wave front (double pink arrows) appeared and induced separation of the tip of the original wave front (double white arrows) from the PM, forcing the latter wave front to meander toward the boundary (H through L) before termination (M and N). Panel O shows the path of the original wave front.

PM and Sustained VT

Figure 3 shows an example of reentrant wave fronts anchored to the PM, resulting in VT. Figure 3A shows the endocardium. B through E show reentrant wave front rotating in a counterclockwise direction around the PM. The core of reentrant wave front (red circle) corresponds roughly to the PM in A. The core area measured 25.5 mm², which was larger than the PM area (16.0 mm²). The tip of the reentrant wave front attached to the PM. F is a diagram showing all the recording sites (a through f) during reentrant excitation in a counterclockwise direction. G shows actual electrograms recorded from sites a through f in F. The reentrant wave front followed this trajectory around PM in a stable fashion. There was no beat-to-beat variation of the reentrant pathways.

In contrast to the area remote from the PM (a, b, c, and f), electrograms near the root of the PM, d and e, show double
potentials with a discrete sharp first potential (marked by asterisk) followed by a wider second potential. These double potentials were recorded mostly around the PM, as shown in H and I (electrograms b through e). This sharp first potential in these same electrodes also preceded broader or wider potential by 10 to 14 ms during S1 pacing (J, electrograms c through e). However, at certain sites near b (b') and d (d'), this relationship was reversed. The intervals between the 2 were 15 to 17 ms. In regions remote from the PM, double potentials either were absent (as in site k) or were not separated by discrete interactivation intervals (site l). The center of the root of PM remained electrically quiescent (electrograms f, g, and j), <10% of the amplitude recorded at the periphery (h), or exhibited 2:1 block (i) during sustained reentry. All these sites underwent full activation during regular pacing (Figure 4I, electrograms f through j). Figure 4L shows the distribution of double potentials during this episode of VT. The area registering double potentials extends from the edge of PM insertion to the surrounding portion of the endocardium. No double potentials were registered at the base of PM.

**Effect of Removal of the Tissue Containing PM on the Pattern of Activation**

In 2 RVs, the PM was placed outside the mapping electrode array, as shown in Figure 4. During sustained VT in the first tissue (A), the patterns of activation in the mapped region showed a planar wave (C through E). When the tissue including PM was cut away (G), VT was converted to polymorphic VT before spontaneous termination.
Maps showed a single reentrant wave front whose tip meandered toward the lower edge of the tissue (H through L) and terminated at the boundary (M). After this, repeated attempts failed to reinduce sustained VT. In a second tissue, cutting away the PM converted VF to VT, which then spontaneously degenerated to VF. After cardioversion, multiple attempts induced either nonsustained VT or VT that spontaneously degenerated to VF. These findings are compatible with the notion that removal of the tissue containing PM eliminated the site of stable stationary anchoring of reentrant wave front.

The mean PM area (17.9 ± 3.8 mm²) was significantly (P < 0.01, n = 11) smaller than the core size of reentrant wave front (28.8 ± 5.9 mm²). There was a positive correlation between the PM area and the core size (r = 0.8, P < 0.01) and between the core size and the CL of VT (r = 0.7, P < 0.05). These findings demonstrate that the core size and the CL of reentrant wave fronts are influenced by the size of the underlying PM to which reentry is anchored.

**Initiation of Reentry by a Premature Stimulus**

Figure 5A shows an example of TMPs and pseudo-ECGs when VT was induced by S₁-S₂ stimuli. The arrows in a through h in TMP recordings correspond to the time (ms) above each frame in C. A single premature stimulus (S₂) applied to the epicardium, near the center of the mapped tissue, induced VT. Figure 5B shows the tissue specimen. Figure 5C, a through c, shows static frames of dynamic display during S₁ pacing. Panels d through h show the impulse induced by S₂ blocked along the region indicated by 2 red lines, resulting in reentry. In all RVs in which VT was induced by a single premature stimulus, the sites of conduction block were located at the junction of the PM and the RV free wall.

**Protocol 2**

In all RVs, continuous infusion of procainamide resulted in the conversion from VF to sustained VT (Figure 6). Before conversion of VF to VT, procainamide increased the CL of VT from 76 ± 8 to 93 ± 14 ms (P < 0.05). Mean action potential duration at 90% and 50% repolarization (APD₉₀ and APD₅₀) increased from 63 ± 6 to 78 ± 11 ms (P < 0.05) and from 47 ± 3 to 61 ± 9 ms (P < 0.05), respectively. The action potential amplitude (61 ± 13 mV) and (dV/dt)max (33 ± 20 V/s) were not significantly changed after procainamide infusion.
(58±7 mV and 35±8 V/s, respectively). The CL of VT (193±76 ms) was not significantly different from the CL of VT seen in protocol 1. Maps showed that all episodes of VT that occurred during procainamide infusion were associated with a single reentrant wave front anchored to the PM. The core size was 22.5±8.1 mm², which correlated well (r=0.93, P<0.05) with the PM area.

After procainamide was washed out with drug-free Tyrode’s solution for 34±8 minutes, VT either spontaneously degenerated into VF (n=4) or was convertible to VF by rapid pacing (n=2). The maximum and mean numbers of VF wavelets after procainamide washout were 4.3±0.9 and 2.9±0.8, respectively (P=NS versus baseline VF).

Protocol 3
In 4 RVs, we studied the patterns of activation during VT and VF before and after PM resection. With intact PM, sustained VT was associated with anchoring of reentrant wave front to the PM. After termination of the VT, VF was inducible and there was spontaneous conversion of VF to VT. Pseudo-ECG during sustained reentry showed a uniform morphology and a constant CL, compatible with VT. After PM resection, sustained VT was no longer inducible. Even with further tissue mass reduction, sustained VT could not be induced.

Protocol 4
The effective refractory period at the PM was 236±19 ms, and at endocardial sites remote from the PM it was 248±20 ms (P=NS). Normal action potentials were registered on the PM (before cut) in 5 tissues and at the cut surface of the PM in 3 tissues studied (Table). Figure 7 shows TMP recordings from the base (cut surface) of the PM and at the endocardium at 2 different pacing rates (400- and 230-ms CLs). The sharp upstroke and the long duration of AP are incompatible with ischemia or cell damage.

Histopathological Studies
The isolated RVs, including the PM, were grossly normal at the end of the experiment (Figure 1D). There was no sign of injury or autolysis. The trichrome stain showed no evidence

<table>
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<th>Action Potential Characteristics</th>
<th>Top of PM Before Cut (n=46)</th>
<th>Cut Surface of PM (n=22)</th>
<th>Surrounding Endocardium (n=55)</th>
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<td>(dV/dt)_{max}</td>
<td>55.4±6.0 V/s</td>
<td>56.6±9.6 V/s</td>
<td>53.9±6.6 V/s</td>
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<td>APD_{90}</td>
<td>197.1±24.5 ms</td>
<td>209.3±23.0 ms</td>
<td>200.4±22.0 ms</td>
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*P=NS for all comparisons.
The source-sink mismatch may also explain the role of PM in anchoring the reentrant wave front, which facilitates the conversion from VF to VT in this model. It is known that an artificially created anatomic obstacle can determine the dynamic behavior of a functional reentrant wave front and that the effects of an obstacle on the reentrant wave front is determined in part by the size of the obstacle. An important finding in our study was that a naturally occurring anatomic obstacle, the PM, is apparently a large enough anatomic obstacle to anchor reentrant wave front.

Importance of Tissue Mass and Number of Wavelets
The source-sink mismatch alone cannot fully explain the results of the present study. We found that the anchoring of reentry alone is insufficient to convert VF to VT at baseline, because the anchored wave front is often terminated by the interference of other wavelets that exist during fibrillation. For VF to convert to VT, either the tissue size has to be reduced or the electrophysiological characteristics of the tissue have to be altered. We also recently demonstrated in the same animal model that tissue mass reduction results in a decreased number of wandering wavelets. Kwan et al also showed that during procainamide infusion, the number of wavelets in canine VF is significantly reduced compared with baseline. If the number of wavelets is reduced to a critical number either by mass reduction or by procainamide, the wave front anchored to the PM survives longer because of decreased interference. Eventually, it takes over the electrical activation of the entire ventricles, resulting in VT. This critical number of wavelets was <3.6 in the mapped area during tissue mass reduction and <3.2 in the mapped area during procainamide infusion. We propose that the reduced number of wavelets below a critical value is important in facilitating the spontaneous conversion from VF to VT in this model.

Conclusions
We conclude that a PM ridge provides a large electrical sink for reentrant wave fronts (spiral waves) to anchor, resulting in sustained VT. Whether or not this phenomenon underlies the mechanism of idiopathic VT in the normal ventricles or contributes to the proarrhythmic effects of procainamide and other antiarrhythmic agents is unclear.

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Figure 8. Simulation studies show that spiral waves anchor to PM-like structure after tissue size reduction. I, Failure to anchor in larger tissue patch even with PM-like structure. Tissue patch is $400 \times 300 \times 4$ computational cells, corresponding to $8.0 \times 6.0 \times 0.08$ cm. Ridge like that in III was placed in upper left part of tissue. Top, Voltage snapshots show spiral wave in A breaking up in B and remaining in sustained spiral breakup state. Outline of ridge can be seen anchoring spiral wave in B, C, and D, but spiral wave is broken up by other waves in E, and no discernible anchoring can be seen in next several seconds. Bottom, Pseudo-ECG for epoch shows continued fibrillation-like tracing. II, Simulation of spiral breakup followed by spiral wave extinction (quiescent tissue) in smaller piece of cardiac tissue without ridge. Top, Spatial maps of voltage. Spiral wave forms in A and B and breaks up into multispiral state in C and D. E and F, Encounters with nonconducting boundaries of tissue have extinguished all but 1 spiral. G, Boundaries have absorbed leading edge of wave, so tissue returns to quiescence in H. Bottom, Pseudo-ECG for epoch represented above. Simulation was $180 \times 200 \times 4$ computational cells, corresponding to $3.60 \times 4.00 \times 0.08$ cm. III, Anchoring of spiral wave by ridge, such as PM, in small piece of tissue. Tissue patch here is same as II, except that ridge has been added, extending vertically from center of tissue. Size of ridge is $40 \times 40 \times 4$ computational cells, corresponding to $0.8 \times 0.8 \times 0.08$ cm. Top, Snapshots of spatial distribution of voltage. Spiral wave initiated in A becomes anchored to ridge in B and remains stable there. Square edges of ridge can be seen outlined by wave. Bottom, Pseudo-ECG of epoch shows a rhythm quickly settling into tachycardia.

References


