Role of Pectinate Muscle Bundles in the Generation and Maintenance of Intra-atrial Reentry

Potential Implications for the Mechanism of Conversion Between Atrial Fibrillation and Atrial Flutter

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Abstract—To determine the role of pectinate muscle (PM) bundles in the formation of intra-atrial reentry, 10 isolated canine right atrial tissues were perfused with Tyrode’s solution containing 1 to 2.5 μmol/L acetylcholine (ACh). The endocardium was mapped using 477 bipolar electrodes with 1.6-mm resolution. Reentry was induced by a premature stimulus (S2). Computer simulation studies were used to investigate the importance of regional myocardial thickness in reentry formation. A total of 40 episodes of reentry were induced; 28 episodes were stationary, and the remaining 12 were nonstationary. The stationary reentry was induced either immediately after the S2 stimuli (n=9) or after an initial period of irregular activations that lasted 1460±1077 ms (n=19). Of 28 episodes, 20 were initiated by conduction block along large PM ridges, leading to wave break and the initiation of reentry. The reentrant wave fronts remained stationary and rotated around these ridges as anchoring sites. During the transition from the initial irregular activations to stationary reentry, the electrogram morphology converted from “fibrillation-like” to “flutter-like” activity. In 8 episodes, initially stationary reentry converted to irregular activations because of interference with outside wave fronts (n=5) or spontaneous separation of waves from the ridges (n=3). Compared with stationary reentry, nonstationary reentry always occurred over an area without large PMs, and the mean life span was much shorter (102±151 versus 3.8±1.1 rotations, P<0.001). Computer simulation studies showed that a critical ridge thickness is needed for reentry to anchor, thereby converting fibrillation to flutter. We conclude that PM ridge forms an area where wave break occurs, allowing the initiation of reentry. It also provides a natural anchor to the reentrant wave front, lengthening the life span of reentry. The attachment and detachment of the reentrant wave front to and from the ridge determine “flutter-like” or “fibrillation-like” activity. (Circ Res. 1998;83:448-462.)

Key Words: pectinate muscle bundle ■ reentry ■ anchoring ■ atrial arrhythmia ■ source-sink relationship

Atrial fibrillation is a rapid and irregular rhythm, whereas atrial tachycardia (including atrial flutter) is a rapid but regular rhythm. Although these 2 rhythms are associated with completely different ECG manifestations, the conversion from one rhythm to the other has long been recognized.1 Watson and Josephson2 have demonstrated in humans that during electrophysiological study, the onset of atrial flutter was often preceded by a period of atrial fibrillation. Waldo and his colleagues studied the onset of atrial flutter in the canine pericarditis model3 and in humans.4 They discovered that the onset of atrial flutter was invariably preceded by a transitional rhythm, mostly atrial fibrillation. The conversion of atrial fibrillation to atrial flutter was associated with the development of a long line of functional conduction block.5 The mechanism underlying the formation of this long line of functional block remains unclear. Studies in ventricular tissues have demonstrated that drifting reentrant wave fronts (spiral waves) could become stationary by anchoring to epicardial arteries or other heterogeneities, leading to sustained and stable reentry.6,7 Computer simulation study8 revealed that pectinate muscle (PM) structures in the atria could also serve as sites for reentry to anchor, resulting in stable reentrant excitations. However, no experimental data exist to test this hypothesis. In the present study, we applied computerized mapping techniques to study the patterns of activation during “fibrillation-like” and “flutter-like” activity in isolated perfused canine right atria. Computer simulation techniques were used to investigate the effects of complicated atrial structures on the formation of reentry. The results were used to test the following hypotheses: (1) uneven thickness of

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the atrial tissue created by PM structures facilitates the development of intra-atrial reentry; and (2) anchoring of the reentry to PM results in a regular tachycardia, whereas detachment of the reentrant wave front from PM leads to “fibrillation-like” activity.

Materials and Methods

Experimental Preparation and Recording Electrodes

The research protocol was approved by the Institutional Animal Care and Use Committee of the Cedars-Sinai Medical Center and followed the guidelines of the American Heart Association. Ten mongrel dogs of either sex weighing between 18 and 27 kg were anesthetized with sodium pentobarbital (30 to 35 mg/kg IV), intubated, and ventilated with room air by a respirator (Harvard Apparatus). The chest was opened through a median sternotomy, and the heart was removed rapidly. The right coronary artery was immediately cannulated and perfused at 10 mL/min with oxygenated and warmed (36.5°C) Tyrode’s solution with a pH of 7.4. The Tyrode’s solution had the following ionic composition (mmol/L): NaCl 125, KCl 4.5, NaHPO₄ 1.8, CaCl₂ 2.7, MgCl₂ 0.5, NaHCO₃ 24, and dextrose 5.5, in triple-distilled deionized water. The right atrial appendage and free wall were then excised along the proximal portion of the right coronary artery. The distal portion of the right coronary artery was ligated, and branches to the residual right ventricular tissue were cauterized to enhance perfusion to the right atrium. All preparations had intact PM bundles. The tissue was then placed in a tissue bath and mounted on the mapping plaque with endocardial surface down (Figure 1A). Excess tissue was trimmed to the size of the recording plaque (Figure 1B, 3.2×3.8 cm), which was connected to a computerized mapping system. The recording plaque consisted of 509 bipolar electrodes. The interelectrode distance was 1.6 mm, and the interpolar distance was 0.5 mm. However, because of technical difficulties, data were acquired with the last 477 electrodes only. The data were acquired continuously for 8 seconds at 1000 samples per second with 18-bit accuracy. The signals were filtered with a high-pass filter of 0.5 Hz. Both the bath and the stock Tyrode’s solutions were continuously gassed with 95% O₂ and 5% CO₂.

A bipolar electrode with interpolar distance of 0.5 mm was used to record bipolar electrograms from the epicardium to document tissue response to pacing and premature stimulus. A pseudo-ECG was also registered with widely spaced bipoles, 1 at each end of the tissue preparation. The data were acquired by AXON TL-1 to 40 A/D acquisition hardware and Axoclamp-2A software (Axon Instrument Inc) and were digitized at 1 kHz with 12 bits of accuracy.

Study Protocol

A bipolar stimulating electrode was placed at either the left edge or the bottom of the epicardial surface to deliver baseline pacing (S₁) with twice diastolic threshold current at cycle lengths of 300 ms. The refractory period at these sites was determined by the extrastimulus method with twice diastolic threshold currents. Another pair of epicardial stimulation electrodes was placed 1.5 cm away from the S₁ site to give premature stimulation (S₂) to induce reentry (Figure 1B). The initial strength of S₂ was 5 mA. If repetitive activations were not induced, the strength of S₂ was increased at 5-mA steps until the induction of reentry or until 20 mA was reached. If the arrhythmia was not induced at baseline, 1 to 2.5 mmol/L acetylcholine (ACh) was added to the perfusate, and the same induction protocol was repeated. Once reentry was induced, endocardial mapping was performed. The data were then displayed on a computer screen. If the activation pattern was compatible with reentry using a PM bridge as part of the reentrant circuit, the bridge was transected with a blade to test whether the reentry was still inducible. In the present study, 3 tissues were tested by transection.

Data Analysis

The method for selecting the time of activation has been reported in detail previously. Briefly, the time of activation was taken at the time of the greatest slope (dV/dt) for each electrogram. The maximal dV/dt in the window of data analysis was first selected by the computer. The investigators had the option of choosing a threshold dV/dt value (a percentage of the maximal dV/dt) and a threshold interval (in milliseconds). The computer selected a time as the local activation if the dV/dt at that time exceeded the threshold value and if the interval between that time and the time of previous activation exceeded the threshold interval. Because each channel has a different signal to noise ratio, the threshold values selected varied from channel to channel at the investigator’s discretion. All electrograms were then manually edited. Once the times of activation were determined, they were displayed dynamically on the computer. The patterns of activation were then studied. The activation times were also used to construct conventional isochronal activation maps.

Definitions

A reentrant wave front was defined as a wave front that propagated around a central area (core) and reentered the site of origin. The location of the core was identified by dynamic display as the area encircled by the path of the tip of the reentrant wave front. The reentry was defined as the innermost edge of the reentrant wave front. If the tip was found to grossly deviate from its initial wave tip path, i.e., by more than 1 interpolar distance (1.6 mm) for greater than roughly 75% of its path, this reentrant wave front was considered to be nonstationary. Otherwise, it was considered stationary.

When the PM bundle was tightly attached to the atrial wall, we defined this structure as “ridge-like” structure. In contrast, the “bridge-like” structure defined a PM bundle that separated from the underlying atrial tissues (see below).

Histological Examination and Anatomical Correlation

At the conclusion of each study, the preparation was photographed before removal from the tissue bath. The tissue was then fixed in 10% neutral buffered formalin and processed routinely. The areas of slow conduction, conduction block, and the core of the reentrant wave front were correlated with anatomic macroscopic and histological findings. Cross sections were performed from the epicardium to endocardium. The cross sections were stained with hematoxylin-eosin to determine tissue thickness, myocardial fiber orientation, and the presence, if any, of tissue abnormalities. The atrial wall thickness was measured both at the PM and at the adjacent atrial free wall using an MCID image analyses system (Imaging Research Inc).

Computer Simulation Studies

We performed computer simulation studies to investigate the effect of ridges, simulating PMs, on the formation of reentry. We chose as our cell model the Luo-Rudy I model, which we modified by (1) lowering the maximum Ca²⁺ conductance from 0.09 to 0.05 mS/cm² to make the spiral waves meander chaotically rather than break up, in accordance with our observations in this type of tissue, and (2) increasing the maximum conductance of the time-dependent K⁺ current from 0.282 to 0.705 mS/cm² to shorten the single-cell action potential duration to mimic the effect of ACh. Our tissue model consisted of a grid of these cells, coupled resistively. The size of the grid, mirroring the experimental preparations, was 190×160×4 cells, corresponding to a tissue patch whose dimensions were 3.8×3.2×0.08 cm. To reflect tissue anisotropy, we set the diffusion constants in the x and y directions to be 0.0005 cm²/ms and in the z direction to be 0.0025 cm²/ms. This yielded physiologically accurate conduction values. Numerical integration of the equations was by the forward Euler method, using a space step equal to 0.02 cm in all directions and a variable time step ranging from 0.01 to 0.1 ms.

Functional reentry, in the form of spiral waves, was induced in the tissue model by cross-field stimulation: first a normal wave was stimulated, then a premature wave was initiated perpendicular to the first wave. The tip of the spiral wave was traced to determine the presence of meander. A pseudo-ECG was calculated by subtracting the average voltage in a 12×12 patch of cells near the upper right corner of the tissue from the average voltage in a 12×12 patch of...
cells near the lower left corner of the tissue. A ridge was then created in the center of the tissue by increasing the tissue thickness in a 50×20 cell region. We simulated multiple values of ridge thickness: 2, 3, 4, 6, and 10 cells.

An additional simulation was performed to investigate the effect of a bridge resembling an unattached PM bundle on reentry formation. A bridge was created that connected 1 side of the atrial tissue with the other. The bridge was 2 mm (10 cells) in diameter and 2.4 cm (120 cells) in length. The conduction velocity in the bridge tissue was twice that of the rest of the tissue. Premature stimulation was performed to determine whether the bridge can serve as an integral part of the reentrant circuit.

**Statistical Analysis**

All statistical analyses were performed using GB-Stat. Results were expressed as the mean±SD. Student’s t tests were used to compare the differences between stationary and nonstationary reentry in life span and cycle length. A value of *P*<0.05 is considered significant.
Results

Activation Pattern During Regular Pacing

Figure 2A shows a color-coded isochronal activation map during regular S1 pacing in a representative isolated canine right atrial tissue (see panels D and E). Regular pacing (S1) was applied at the left edge of the representative tissue (see panels D and E). Panel A shows a color-coded isochronal activation map during regular pacing, demonstrating the nonuniformity of the conduction velocity. Selected electrograms are shown in panels B and C. Panel D shows gross endocardial structures, including part of the crista terminalis (CT) and PM bundles in the atrial tissue. These PM bundles were either tightly attached to the atrial free wall (ridge-like structure between arrows) or discontinuous with the atrial free wall (bridge-like structures, green and red probes). The site for S1 pacing is marked by an asterisk. The transillumination of the same tissue shows the complex patterns of atrial fiber orientation and muscle thickness (panel E). To better correlate the endocardial activation patterns with the underlying anatomic structures, all the endocardial tissues in the present study were displayed by mirror images.

Figure 2. Activation pattern during regular pacing in an isolated canine right atrial endocardial tissue. Regular pacing (S1) was applied at the left edge of the representative tissue (see panels D and E). Panel A shows a color-coded isochronal activation map during regular pacing, demonstrating the nonuniformity of the conduction velocity. Selected electrograms are shown in panels B and C. Panel D shows gross endocardial structures, including part of the crista terminalis (CT) and PM bundles in the atrial tissue. These PM bundles were either tightly attached to the atrial free wall (ridge-like structure between arrows) or discontinuous with the atrial free wall (bridge-like structures, green and red probes). The site for S1 pacing is marked by an asterisk. The transillumination of the same tissue shows the complex patterns of atrial fiber orientation and muscle thickness (panel E). To better correlate the endocardial activation patterns with the underlying anatomic structures, all the endocardial tissues in the present study were displayed by mirror images.

Characteristics of Induced Reentrant Wave Fronts

At baseline (no ACh), only short runs of repetitive beats (<10 beats) could be induced in each tissue. During ACh perfusion (1 μmol/L in 3 tissues and 2.5 μmol/L in 7 tissues), the mean refractory period at the S1 pacing site was shortened from 117±17 ms (range, 90 to 140 ms), and a total of 40 episodes of reentry were initiated. Reentry was induced with an S2 at a mean S1-S2 coupling interval of 78±20 ms and at a mean current of 6.3±3.2 mA. The mean current threshold at S2 sites was 0.19±0.10 mA.
Stationary Reentry

In 28 of 40 episodes (70%), the reentrant wave front was stationary. These reentrant wave fronts were induced either immediately (within 200 ms) after the S₂ stimuli (n=9) or after an initial period (1460±1077 ms; range, 405 to 5475 ms) of irregular activations (n=19). During the transition from the initial irregular activations to stationary reentry, the electrogram morphology converted from “fibrillation-like” to “flutter-like” activity. Once stationary reentry was initiated, it became the source of activation for the entire isolated atrial tissue. The mean number of rotations (life span) was 102±151 (range, 10 to 540), and the mean cycle length was 116±22 ms (range, 90 to 155 ms). As will be shown below, 2 major patterns of activation were observed during stationary reentry. In 20 episodes, the reentrant wave front had a “spiral wave” appearance. In 8 episodes, it showed a wave front that propagated along the PM bridge between its atrial insertion sites. The wave front then spread from the insertion site in all directions to activate the remaining portion of the tissue. In all episodes, the PM bundles played an important role in reentry formation.

Initiation of Stationary Reentry: Role of PM Bundle

Figure 3 shows an example of immediate initiation of stationary reentry (same tissue as in Figure 2). In Figure 3A, an endocardial breakthrough (asterisk) occurred in the right lower part of the mapped area 180 ms after an S₂ stimulus. The breakthrough initiated stationary reentry by the occurrence of conduction block and wave break (B through H). Panel I shows the actual activations registered in panels A through H. A large PM with ridge-like structure was present at the line of block (panel X). As the reentrant wave front rotated, it also propagated up through the bridges and emerged as a bystander wave front (squares in panels H and Y). Diagrams in panels X and Y show the path and direction of the tip of the wave front (red lines and arrows); letters A through H indicate the approximate location of the tip of the wave front in the corresponding panels. In panel Z, each letter on the map (a through h) indicates the recording site of a corresponding channel (chn) in panel I. See text for details.
3.5 mm thick) was present at the line of block. The PM bundle was tightly attached to the atrial free wall in the center but became discontinuous with the atrial free wall over the right end, forming bridge-like structures (Figure 2D, green and red probes). The wave front then propagated around the line of block and initiated another wave front (solid circle) via the bridge-like structures (Figure 3B through 3D, and 3X). The wave front then rotated around the ridge-like structure (Figure 3E and 3F) and traveled under these bridges (Figure 3G and 3H), completing a clockwise reentrant circuit (Figure 3Y). Part of the reentrant wave front also propagated up through the bridges and emerged as a bystander wave front over the right lower part of the mapped area (squares in Figure 3H and 3Y). This bystander wave front may propagate toward the central part of the tissue and invade the core of the reentry, resulting in the separation of reentrant wave front from the PM (shown in Figure 7). Figure 3I shows the actual activations registered in Figure 3A through 3H. In Figure 3Z, each letter on the map (a through h) indicates the recording site of a corresponding channel (chn) in panel I. Panel I shows the actual activations during the period of the transition. The asterisks in panels H and I indicate the location (between sites a and b) of the endocardial breakthrough, which initiated stationary reentry.

Figure 4 shows an example of initiation of stationary reentry preceded by a period of irregular activations. During the period of the transition, the rhythm and morphology of local electrograms converted from “fibrillation-like” to “flutter-like” activity. In Figure 4A, multiple wave fronts (up to 6 waves) were observed during the initial period of irregular activations induced by an S2 stimulus. Panel B shows that an endocardial breakthrough (asterisk) occurred adjacent to a large PM bundle. The breakthrough failed to travel across the PM bundle, forming a line of block along it (panel C). The wave front then propagated around the line of block and initiated reentry (C through G). Finally, the reentrant wave front became the source of activation for the entire tissue. In panel H, each letter on the map (a through h) shows the recording site of a corresponding channel (chn) in panel I. Panel I shows the actual activations during the period of the transition. The asterisks in panels H and I indicate the location (between sites a and b) of the endocardial breakthrough, which initiated stationary reentry.

**PM Bundle Serves as Anchoring Site for Reentrant Wave Front: Ridge-Like Structure**

As shown in Figures 3 and 4, a total of 20 episodes of reentry in 7 tissues (2 to 5 per tissue) were initiated by conduction block along large PM ridges. These reentrant wave fronts remained stationary and rotated around these ridges as anchoring sites. Figure 5 (same episode as in Figure 3) shows an example of stationary reentry with a life span of 24 rotations. The pathway of the tip of the reentrant wave front circled in a clockwise direction (Figure 5A through 5E) around the ridge-like structure (marked by the rectangle) of a large PM bundle shown in Figure 2D. The reentrant wave front showed a “spiral wave” appearance (Figure 5F). Figure 5G shows the
location of channels around the ridge-like structure, and Figure 5H shows the actual electrograms registered by these channels during stationary reentry. Compatible with the electrograms located in the core of reentrant wave front,\textsuperscript{10,13} these electrograms showed either double potentials (arrows) or low-amplitude potentials (<0.4 mV, asterisks). Note that when the reentrant wave front rotated around the ridge, the conduction velocity was not uniform. Because of the presence of small PMs that inserted into the upper border of this large PM ridge, the conduction velocity from channels 239 to 244 was slow (13 cm/s). However, it became faster (47 cm/s) from channels 265 to 260. During reentry with a stationary core, the pseudo-ECG recording had the characteristics of a regular tachycardia with amplitude alternans (Figure 5I).

These findings support the idea that a PM bundle with ridge-like structure may act as an anchoring site for a reentrant wave front. Histopathologic studies showed no evidence of fibrosis or other abnormalities in the mapped tissues. The mean size of PM ridges of 7 tissues that served as anchoring sites for reentry was 3.5±0.6 mm wide (range, 2.5 to 4.5 mm) and 3.8±1.4 mm thick (range, 2.5 to 6.0 mm). The mean length of lines of block along these ridges was 10.5±0.9 mm (range, 9.0 to 11.5 mm). In contrast, the adjacent atrial free walls were thin (range, 0.4 to 1.2 mm).

**PM Bundle Serves as Part of Reentrant Circuit: Bridge-Like Structure**

In 3 tissues, stationary reentry used PM bundle as part of the reentrant circuit (8 episodes, 2 to 3 per tissue). Figure 6 illustrates an example. Figure 6A through 6C shows a wave front propagating from the left upper to the right lower part of the mapped area along the PM structure. On arrival at the end of the PM, its atrial insertion site, the activation spread in all directions into the main body of the tissue (Figure 6D and 6E, asterisks). The leading edge of the wave front then reentered the other end of the PM, completing a reentrant circuit (Figure 6F and 6G). In this episode, reentry was initiated immediately after the S\textsubscript{2} stimulus with a life span of 27
rotations. Another 2 episodes of reentry with a similar activation pattern were induced in the same tissue. Anatomic analysis verified that a PM bundle with bridge-like structure corresponded to this activation pattern. After the bridge-like structure was transected by a blade (Figure 6I), this form of reentry was no longer inducible. In Figure 6I, each letter on the tissue (a through h) shows the position of a corresponding channel (chn) in panel J. Panel J shows the actual activations of these reentrant wave fronts. The numbers indicate the activation times (ms) pointed out by arrows. During reentry, the pseudo-ECG recording revealed a regular tachycardia (panel K).

Conversion From “Flutter-Like” to “Fibrillation-Like” Activity due to Destabilization of Stationary Reentry

In 8 episodes, initially stationary reentry converted spontaneously from “flutter-like” to “fibrillation-like” activity. The stationary reentrant wave fronts were destabilized either by interference with outside wave fronts (5 episodes) or by spontaneous separation of waves from the anchoring sites (3 episodes). The mean duration of the “fibrillation-like” activities was $2993 \pm 1608$ ms (range, 1280 to 6460 ms).

Figure 7 (same episode as in Figures 3 and 5) illustrates an example of stationary reentry destabilized by outside wave fronts. In Figure 7A, a new wave front (asterisk) emerged at the right lower edge of the mapped area, when the leading edge of the reentrant wave front was in the left upper edge of the mapped area. As displayed in Figure 3H and 3Y, the new wave front most likely represented the activation from an adjacent bridge-like structure. The wave front propagated toward the central part of the tissue (Figure 7A and 7B) and invaded the core of the reentry (Figure 7C). After core
excitation, the stationary reentry was destabilized. In Figure 7D, an endocardial breakthrough (solid circle) occurred in the lower part of the mapped area 25 ms later and started a period of irregular activations. In Figure 7E, multiple wave fronts (up to 4 waves) were observed during this period. In Figure 7F, each letter on the map (a through h) indicates the recording site of a corresponding channel in Figure 7G. Figure 7G shows the actual activations during the period of the transition. The lower 5 channels are located around the core of the reentry (sites d through h). The upper 3 channels registered the activations at the right side of the mapped region (sites a through c). A wave front (arrow with asterisk) propagating from right to left excited the cells near site d and destabilized the reentrant wave fronts (arrows with squares). After core excitation by an outside wave front, the local electrograms showed the conversion from a regular rhythm with a similar electrogram morphology to an irregular rhythm with a beat-to-beat variation in electrogram morphology.

A second mechanism of destabilization of stationary reentry is the spontaneous separation of reentrant wave fronts from the anchoring sites. Figure 8 displays an example in which the stationary reentry was anchored to a PM ridge (3.5 mm wide and 2.5 mm thick). The line of block along it was 9 mm long. Figure 8A through 8D shows the reentrant wave front (the 33rd cycle) rotating around the anchoring site. The wave front then spontaneously detached from the anchoring site without evidence of outside interference (Figure 8E through 8F). Detachment converted the regular to an irregular rhythm with a beat-to-beat variation in electrogram morphology. Figure 8I and 8J, respectively, shows the actual activations during stationary reentry and immediately before the detachment. Note that oscillation of the cycle length of reentry with a maximal difference of 25 ms occurred before the spontaneous separation (Figure 8J). Cycle length oscillation was also demonstrated in the other 2 episodes of spontaneous separation. In all 3 episodes, the sites of separation were located at the ends of the PM ridges. When the wave front was making an acute turn (Figure 8G) and when the cycle length became short (from 110 to 85 ms in Figure 8J), spontaneous separation occurred.

Nonstationary Reentry

In the remaining 12 episodes (12/40, 30%), the central core of the reentrant wave front drifted while reentrant excitation continued. The mean cycle length was 68 ± 11 ms (range, 55 to 90 ms). Nonstationary reentry was observed during the period of irregular activations after an S2 stimulus. Figure 9 shows an example. Figure 9A through 9G shows a reentrant wave front with counterclockwise rotation and a drifting central core. Figure 9H shows the trajectory of the tip of the reentrant wave front. The drift of the core toward the left lower edge of the mapped region was apparent. As demonstrated previously, the electrograms located in the core registered low-amplitude potentials that varied from beat to beat, alternating between double potentials, electrical quiescence, and high-amplitude potentials (Figure 9I). These variations were caused by the drift of the core. During reentry with a drifting central core, the simultaneous pseudo-ECG recording revealed “fibrillation-like” activity (Figure 9J). In the same tissue, a clockwise reentrant wave front with a cycle length of 120 ms stabilized around a large PM ridge (2.5 mm thick).
wide and 3.0 mm thick) during a different episode. This finding suggests that the stability of the reentry may also be determined by the opportunity to anchor to a large PM.

As shown in Figure 9K, nonstationary reentry always occurred over an area (<2.0 mm in thickness) without large PMs. Because reentry was terminated either by interference with outside wave fronts (7 episodes) or by the drift of the core toward the tissue border (5 episodes), the life span of nonstationary reentry was short. The mean life span of reentry was 3.8 ± 1.1 rotations (range, 3 to 6) before termination. We found a similar pattern of nonstationary reentry in 5 tissues.

Compared with stationary reentry, the mean cycle length of nonstationary reentry (68 ± 11 ms) was significantly shorter (P<0.001). The mean life span (3.8 ± 1.1 rotations) was also much shorter (P<0.001). To control the effects of ACh on the cycle length of reentry, we compared the episodes of stationary (n=16) and nonstationary (n=12) reentry obtained from the same tissues (n=5) with same concentration of ACh. The mean cycle length of nonstationary reentry (68 ± 11 ms) was also significantly shorter than that of stationary reentry (120 ± 26 ms) (P<0.001).

**Computer Simulation Studies**

In computer simulations, reentrant wave fronts resembling spiral waves of excitation could be easily induced with cross-field stimulation (panel I in Figure 10). The spiral wave, however, was unstable and meandered toward the boundary, resulting in termination (H in panel I). The pseudo-ECG shows irregularly irregular “fibrillation-like” activity.

When a ridge-like structure was added to the preparation, the spiral wave could anchor to the structure depending on the thickness of the ridge. Panel II in Figure 10 shows the failure to anchor a chaotically meandering spiral wave when the ridge was 3 computational cells thick. The pseudo-ECG remains irregularly irregular. With a ridge of 4 computational cell layer thickness, reentry quickly anchored to the ridge as shown in panel III in Figure 10. The pseudo-ECG shows the conversion from “fibrillation-like” activity to flutter.

![Figure 8. Conversion from “flutter-like” to “fibrillation-like” activity caused by the spontaneous separation of a reentrant wave front from the anchoring site. In panels A through D, the reentrant wave front rotated around the anchoring site (blue oval area in panel G). However, the wave front was detached from the anchoring site spontaneously, converting the regular to an irregular rhythm (panels E and F). Panel G shows the path and direction of the tip of the wave front (red lines and arrows); letters A through F indicate the approximate location of the tip of the wave front in the corresponding panels. Panel H shows the sites (a through e) of the corresponding electrogram recordings in panels I and J. Panels I and J show the actual activations during stationary reentry and immediately before the detachment from the anchoring site.](image-url)
When a bridge-like structure was added to the simulated tissue sheet, stationary reentry could be induced. Panel IV in Figure 10 shows that the bridge-like structure was used as part of the reentrant circuit.

**Discussion**

A major finding in this study is that PM structure is important in the generation and maintenance of intra-atrial reentry. PM ridge forms an area where wave break occurs, allowing the initiation of reentry. PM bundles may not only serve as part of a reentrant circuit (bridge) but also provide a natural anchor to the reentrant wave front (ridge), lengthening the duration of the reentrant excitation. The attachment and detachment of the spiral wave to and from the PM ridge, respectively, determine whether the tissue exhibits “flutter-like” or “fibrillation-like” activity. Computer simulation studies showed that a critical thickness of the ridge is needed for reentry to anchor, thereby converting “fibrillation-like” to “flutter-like” activity. These findings may be important in the understanding of the basic mechanisms of atrial tachycardia and fibrillation and may explain the mechanisms of transition between atrial fibrillation and atrial flutter in humans.1,2,4

**Wave Break and Source-Sink Mismatch Induced by PM Ridge**

Gray et al15 reported that incomplete reentry and epicardial breakthrough patterns are often observed during atrial fibrillation in sheep hearts. These breakthrough patterns resulted from reentrant excitation through the PMs with a bridge-like structure. We observed the same phenomenon both in the tissue preparation and in computer simulation studies. However, we also observed that the PM ridge, which does not separate from the atrial free wall, significantly modulates the pattern of the arrhythmia. This represents a novel finding of our study. We propose that in addition to serving as a bridge, the PM may influence the safety factor of impulse propagation, resulting in wave break and the formation of reentry.
The safety factor of impulse propagation in cardiac tissue depends on the relationship between source (amount of current available upstream) and sink (the structure that determines current density downstream).18–21 Because the PM ridge that serves as an anchoring site (range, 2.5 to 6 mm thick) is much thicker than the adjacent atrial free wall (range, 0.4 to 1.2 mm thick), there is a source-sink mismatch when the impulse propagates from the atrial free wall into the PM ridge. During regular pacing, the wave fronts are large and the action potential has a rapid upstroke and long duration, overcoming the source-sink mismatch and exciting the PM ridge. However, during premature stimulation or during irregular and fast atrial rhythms, the diastolic interval of atrial cell shortens, resulting in reduced upstroke velocity, amplitude, and duration of the action potential.19 These atrial wave fronts therefore carry less current (smaller source) than the wave fronts generated by regular pacing at a slow rate. These small wave fronts might be blocked by the PM ridge, leading to wave break and the formation of reentry. Compatible with this hypothesis, a wave break at the junction between the PM ridge and the atrial free wall appears to be a consistent finding at the initiation of intra-atrial reentry.

Figure 10. Results of computer simulation. Panel I shows chaotically meandering spiral wave. A reentrant spiral wave was induced by a premature wave perpendicular to a normal wave in a 190×160×4 sheet of modified Luo-Rudy I cells. Top: A through G, Voltage snapshots drawn from a 1300-ms epoch of activity. Voltage values are color coded: red indicates highest voltage, blue lowest. H, Path of the tip of the spiral wave over the whole epoch, which ended when the wave meandered to the nonconducting boundary and terminated. Bottom: Pseudo-ECG for the whole epoch (tick marks are 200 ms apart). Note the irregularly irregular appearance. Panel II shows failure to anchor a chaotically meandering spiral wave. A spiral wave was induced by the same protocol as in panel I above, in tissue identical to that of panel I except that a ridge-like structure was added to the center of the tissue. The ridge was 50×20×3 cells. Top: A through H, Voltage snapshots of the spiral wave over about 2000 ms. Bottom: Pseudo-ECG for the epoch (tick marks are 200 ms apart). Note that the ECG remains irregularly irregular. Panel III shows conversion from fibrillation-like activity to flutter, as a chaotically meandering spiral wave attaches to a ridge. A spiral wave was induced as in panels I and II, in tissue identical to that of panel I, except that the ridge is now 4 cells thick, rather than 3 cells as in panel II. The spiral wave was initiated near the upper right corner of the tissue, then meandered for about 1000 ms before attachment. Top: Voltage snapshots spanning the 2000-ms epoch. A through D, Spiral meander in the first 1000 ms; E through H, the spiral wave anchors to the ridge. Bottom: Continuous pseudo-ECG for the epoch. The signal is irregular for the meandering epoch, then converts to a periodic tachycardia when anchored. All other features are as in panel II. Panel IV shows reentry through a bridge-like structure. A bridge-like structure, representing a PM whose body is separated from the underlying atrial tissue, has been added to the basic tissue model of panel I. The simulation was initiated with a unidirectional wave passing through the bridge (the thin horizontal black bar in the center of the tissue) from left to right. The wave then conducted from the right foot of the bridge outward into the main body of the tissue, creating concentric circles. A, The wave has spread radially to the left-hand side of the bridge, which it has excited. A and B, Excitation passed through the bridge from the left to right, while the rest of the wave expanded radially outward in the main body of the tissue and extinguishes on the boundary. C, The excitation has reentered the tissue at the right foot of the bridge, beginning another wave of excitation, propagating radially outward in the main body of the tissue. D through F, The wave propagates outward and completes a reentrant cycle (G). H, Path and direction of the wave front (red lines and arrows); letters A through G indicate the approximate location of the wave front in the corresponding panels.
In addition to source-sink mismatch caused by uneven thickness of the atrial tissue, PM may also contribute to the formation of reentry by serving as an anisotropic barrier. In animal studies, it has been shown that PM bundle plays a role in anisotropic propagation. However, the anisotropic ratio of atrial conduction may vary according to the experimental condition. The anisotropic ratio obtained from an isolated canine atrial muscle bundle was more than 5. When measured from a preparation containing both the PM structures and the thin atrial free wall tissues, the ratio was much less (range, 1.0 to 1.6). Our results are compatible with the latter report. In humans, Spach and Dolber studied the anisotropic property of the PM bundle in patients aged between 1 and 65 years. They found that there is a progressive loss of side-to-side electrical coupling with age. This results in increased nonuniform anisotropy and facilitates the development of reentry. Therefore, PM also may serve as an anisotropic barrier to induce wave break and reentry. The importance of PM in atrial arrhythmogenesis may increase in older patients.

PM Ridge as Site of Anchoring
The PM ridge also serves a site for reentry to anchor. While there are many PM ridges in the preparation, the anchoring occurred only at a large PM. These findings suggest that the size of the PM is critically important in determining whether reentry can anchor. This is consistent with the computer simulation studies that demonstrated that a critical thickness of the ridge (4-cell layer) was needed for the spiral wave to anchor. Once reentry anchored to the PM, the activation cycle length became more regular. However, its most important effect was to prolong the life span of reentrant excitation, sometimes up to hundreds of cycles without breaking. A second effect of spiral anchoring was to convert “fibrillation-like” to “flutter-like” activity, a sequence of events similar to that reported in humans by Waldo and Cooper.

During stable atrial flutter in the canine pericarditis model, a line of functional block with a mean length of 24 ± 4 mm was localized on the right atrial free wall. When the previously stable line of block decreased to a mean of 16 ± 3 mm, conversion to atrial fibrillation resulted. In our study, however, the mean length of the lines of block during “flutter-like” activity was estimated to be 10.5 mm. This discrepancy can be explained by the higher density of mapping electrode array used in the present study. Therefore, our results are not inconsistent with the observations made by others. Rather, our findings suggest the possibility that the transition from atrial fibrillation to atrial flutter in the in vivo canine study is associated with anchoring of the reentrant wave front to a large PM bundle.

While anchoring of the spiral wave resulted in regularization of the atrial activity from fibrillation to flutter, the spiral wave sometimes detached from the PM ridge because of outside interference or spontaneous separation, resulting in fibrillation. Cycle length oscillation was observed before the spontaneous separation of the spiral wave from the anchoring site. The separation occurred when the wave front was making an acute turn at the end of the PM ridge and when the cycle length oscillated to a short cycle. These findings imply that a small action potential (small source) arising from a premature activation may not have a source-sink ratio sufficient to complete the acute turn, leading to the spontaneous separation.

Relative Importance of Anatomic and Functional Characteristics in Reentry Formation
This study emphasizes the importance of anatomic structure for the formation of intra-atrial reentry. However, because ACh is required for the induction of reentry, our results are also compatible with the notion that functional characteristics of the tissue (primarily shortening of refractoriness) are also important for reentry formation. However, the anchoring of reentry to PM is an important and previously unrecognized factor in determining the characteristics of intra-atrial reentry. In the same tissue, the cycle length of stationary reentry (anchored to PM) was much longer than that of nonstationary reentry (not anchored to PM). This finding indicates that longer cycle length of stationary reentry results from the presence of a larger reentrant circuit (the involvement of PM).

We postulate that the shorter cycle length of atrial fibrillation compared with atrial flutter may be due to the same mechanism.

The importance of anatomic structure is also demonstrated by computer simulation studies. Unlike the canine atrial tissues that had fixed sizes of the PMs, we were able to vary the thickness of the ridge in the computer simulation. Using this method, we demonstrated that increasing the thickness of the ridge without changing functional characteristics of the tissue was sufficient to allow the reentrant wave front to anchor, thereby converting fibrillation to flutter. This finding again supports the idea that anatomic structure is important in determining the characteristics of intra-atrial reentry.

Conduction Velocity During Stationary Reentry
Similar to the findings reported by Feld and Shahandeh-Rad and Giroud et al., nonuniform conduction velocity was observed when reentry stabilized around a large PM bundle in all preparations. As shown in Figure 5, when the reentrant wave front propagated across the fiber orientation, conduction velocity was slow (13 cm/s). Similarly, in Figure 6, conduction delay occurred when the wave front propagated from the adjacent atrial free wall toward the PM bridge. Tissue anisotropy, wave front curvature, and source-sink mismatch due to uneven thickness of the atrial tissue all may play roles in the variation of conduction velocity during reentry.

Study Limitations
There are several important limitations in the present study. First, we used only a portion of the right atrium. Because the right atrium was opened and laid out flat, connections present in vivo were lost. Furthermore, the influences from the left atrium, septum, and rest of the right atrium were also lost. The electrical activations could be altered in vivo by the presence of electrical inputs from, and interaction with, these other structures. Second, the theoretical model used in this study may not accurately model the action potential charac-
teristics of the atrial cells. The Luo-Rudy model used in this study was developed based on the action potential characteristics of ventricular cells. The canine atrial cells have different repolarization currents from those of the ventricular cells. In addition, the effects of ACh on activation and repolarization were not included in the Luo-Rudy model. In this study, we modeled the effects of ACh by increasing the time-dependent $K^+$ current, $I_{k, A}$. A mathematical formulation of $I_{k, A}$ does not yet exist. Although $I_{k, A}$ has a voltage and time dependence different from $I_k$, we believe that this difference is not crucial for these results. This article considers the effects of different anatomic structures on the propagation of wave fronts. The presence of different repolarizing currents will affect such factors as the size of the obstacle necessary to anchor a wave and the degree of meander seen, as well as membrane threshold and other cable properties. However, the fundamental phenomena we are considering here, such as the occurrence of anchoring and the support of reentry by a ridge-like or bridge-like structure, are not likely to be affected by the differences in repolarizing currents. We believe that these are generic phenomena that occur in a wide variety of models. Even such simplified models as the Fitzhugh-Nagumo equations, as used in Foster et al., can qualitatively reproduce these phenomena.

Even with this limitation, we found remarkable similarities between the computer modeling and the experimental results. In computer simulation, the minimum thickness of the ridge for successful anchoring was $4 \times 4$ computational cell layer (including the thickness of the underlying grid, 4 computational cell layer). In other words, the thickness ratio between the ridge and the grid for anchoring was $\approx 2:1$. When the ratio was $< 2:1$ (eg, $3 \times 4$ computational cell layer), the spiral wave failed to anchor. In the atrial tissues, a ratio of $> 2:1$ in thickness was also observed between the PM ridges that served as anchoring sites and the surrounding atrial free walls. Therefore, we propose that our computer model provides gross supportive evidence for the mechanism by which reentry anchored to PM.

Conclusions

We conclude that a large PM ridge provides a natural substrate for the initiation of intra-atrial reentry (wave break) and prolongs the life spans of reentrant wave fronts (anchoring). The attachment and detachment of the spiral wave to and from the PM ridge determine “flutter-like” or “fibrillation-like” activity, respectively, in isolated canine atrial tissue.

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References


Role of Pectinate Muscle Bundles in the Generation and Maintenance of Intra-atrial Reentry: Potential Implications for the Mechanism of Conversion Between Atrial Fibrillation and Atrial Flutter

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