Atrial Septopulmonary Bundle of the Posterior Left Atrium Provides a Substrate for Atrial Fibrillation Initiation in a Model of Vagally Mediated Pulmonary Vein Tachycardia of the Structurally Normal Heart

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Background—The posterior left atrium (PLA) and pulmonary veins (PVs) have been shown to be critical for atrial fibrillation (AF) initiation. However, the detailed mechanisms of reentry and AF initiation by PV impulses are poorly understood. We hypothesized that PV impulses trigger reentry and AF by undergoing wavebreaks as a result of sink-to-source mismatch at specific PV-PLA transitions along the septopulmonary bundle, where there are changes in thickness and fiber direction.

Methods and Results—In 7 Langendorff-perfused sheep hearts AF was initiated by a burst of 6 pulses (CL 80 to 150ms) delivered to the left inferior or right superior PV ostium 100 to 150 ms after the sinus impulse in the presence of 0.5 μmol/L acetylcholine. The exposed septal-PLA endocardial area was mapped with high spatio-temporal resolution (DI-4-ANEPPS, 1000-fr/s) during AF initiation. Isochronal maps for each paced beat preceding AF onset were constructed to localize areas of conduction delay and block. Phase movies allowed the determination of the wavebreak sites at the onset of AF. Thereafter, the PLA myocardial wall thickness was quantified by echocardiography, and the fiber direction in the optical field of view was determined after peeling off the endocardium. Finally, isochrone, phase and conduction velocity maps were superimposed on the corresponding anatomic pictures for each of the 28 episodes of AF initiation. The longest delays of the paced PV impulses, as well as the first wavebreak, occurred at those boundaries along the septopulmonary bundle that showed sharp changes in fiber direction and the largest and most abrupt increase in myocardial thickness.

Conclusion—Waves propagating from the PVs into the PLA originating from a simulated PV tachycardia triggered reentry and vagally mediated AF by breaking at boundaries along the septopulmonary bundle where abrupt changes in thickness and fiber direction resulted in sink-to-source mismatch and low safety for propagation.

Key Words: atrial thickness ■ reentry ■ mapping ■ electrophysiology ■ fiber direction

Atrial fibrillation (AF) is the most common arrhythmia in adults, and it affects about 2.2 million patients in United States alone.1 For many years, large numbers of studies have focused on the role of the atrial electrophysiological properties in AF mechanisms. Although the mechanisms of AF initiation are complex, a growing body of evidence has demonstrated that paroxysms of AF are initiated or reinitiated by ectopic foci emanating from the pulmonary veins (PVs).2-5 On the other hand, a few studies have suggested that the complex geometric arrangements of atrial muscle fibers are key to successful conduction of the sinus wave and to the onset of conduction delays and block in the atria.6-10

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For example, Rha et al11 used 3D noncontact endocardial mapping in AF patients to investigate the activation sequence of single spontaneous PV impulses entering the posterior left atrium (PLA). They observed that many of those impulses underwent slow conduction and breakup at the septopulmonary bundle (SPB) near the right superior PV (RSPV) ostium. In an additional study using the NavX system (St. Jude Medical Inc., St. Paul, Minn.), Chang et al12 suggested that the muscular bundles of the PLA may provide the substrate that allows AF/atrial flutter to be reinitiated after PV isolation catheter ablation.
On the basis of these studies, we tested the general hypothesis that electric wavefronts generated at high frequency in the PVs enter the PLA, where they encounter abrupt changes in muscle thickness and fiber direction as they move into the SPB. Thus, they may undergo conduction delay and wavebreak as a result of sink-to-source mismatch leading to reentry and AF initiation.

Methods

All animal experiments were carried out in accordance to the Guidelines for the Care and Use of Laboratory Animals of the National Institute of Health. Seven male sheep (17 to 28 kg) were anesthetized with sodium pentobarbital (35 mg/kg, IP) then heparinized (200 U/kg, IP). Hearts were excised through a midsternal incision, placed in cardioplegic solution and subsequently connected to a Langendorff apparatus, to be perfused at 200 mL/min with warm, oxygenated Tyrode’s solution (pH 7.4; 95% O2, 5% CO2, 37±1°C). A small incision was made in the left atrial appendage, and the PLA was exposed as described previously.13 Acetylcholine (0.5 μmol/L) was continuously perfused. A charge-coupled device camera (Red-Shirt Little Joe, Scimeasure, Decatur, GA) was used for high resolution optical mapping movies (1000FPS, DI 4-ANEPPS13). In 4 additional isolated sheep hearts, bipolar electrograms were recorded from the left atrial lateral wall (LALW), corresponding to a bipolar electrode placed either in the distal coronary sinus or at the left atrial appendage, right atrial appendage (RAA), and Bachmann bundle (BB).

To simulate PV tachycardia initiating AF, we designed a pacing protocol that coupled a 6-pulse burst to the sinus wave. This protocol allowed us also to explore the role of the SPB in cholinergic (ie, vagotonic) AF initiation. A bipolar electrode placed on the RAA was used as a sensor coupled to a stimulator. Another bipolar, the pacing electrode, was positioned inside the endocardial sleeve of either the right superior PV or the left inferior PV (LIPV) as shown in Figure 1. Once the sinus wave was detected by the sensing electrode, a count down timer (set between 100 and 150 ms) controlled the delivery of a burst of 6 pulses (interval 80 to 150 ms).

We recorded 5-second movies of paced waves followed by AF initiation. If AF did not spontaneously terminate, the heart was defibrillated using a Medtronic 5351 ECVD defibrillator (Minneapolis, Mnn.). After the experiment, hearts were fixed in formalin (7%); myofibril orientation were obtained from a field of view similar to that of the optical movies. Myocardial thickness of the following left atrial areas was assessed for each heart using calipers: PLA roof transition (immediately above the superior PV ostia), PLA base, inferior and superior PLA-septum transition, and septum itself.

Data Analysis

Optical Identification of the First AF Wave

We obtained an average of 4±2 movies of AF per animal. All movies were filtered in space and time (7 and 13 kernels, respectively14); phase movies were generated to determine location of singularity points, as described previously,15 and isochronal maps of each of the pacing-induced waves preceding AF onset were constructed. In addition, normalized average conduction delays were computed in each AF episode initiation for all consecutive paced waves in the entire PLA area. Using MatLab (Mathworks Inc.), we calculated local activation times (defined at maximal dV/dt) relative to the initiation time. The activation times were used to calculate local conduction velocity (CV) by measuring the distance between 2 activation time isochrones. Absolute values were then normalized to the maximal CV to allow comparison of relative delays between each paced wave. Once each paced wave was normalized to its maximum CV, the normalized waves were averaged to produce an average map, which was superimposed on the animal’s anatomy to show areas of preferential slowing. Briefly, each paced wave for a given AF episode was normalized to its maximal CV. The normalized waves were then averaged for all episodes initiated from the RSPV and for all episodes initiated from the LIPV. The first AF wave was defined as the first spontaneously appearing wave after the 6th paced wave when fibrillation ensued. If AF was initiated before the 6th paced wave, the first wave that was traveling from a different location and with a different direction was classified as the first AF wave. Once the first AF wave was identified, it was then classified as: (1) reentrant, defined as a wavebreak that gives rise to 2 singularity points, and at least one of them becomes the center of the rotation that initiates AF.16 In a phase map, a singularity point is the locus at which all phases of the action potential converge15; (2) A breakthrough, defined as a wave that appears as a point in the field of view and propagates radially thereafter; and (3) An incoming wave, defined as a wave entering from outside the field of view.

Identification of the First AF Wave: Bipolar Electrograms

In 4 additional sheep, we repeated the protocol with bipolar electrodes placed on the LALW, BB, and RAA. To discern if the 1st AF wave detected optically originated from the left atrium or else from other atrial areas, we analyzed the bipolar electrograms with reference to the optical waves during AF initiation.

To establish which deflection was the 1st AF wave in the atrial electrograms, we took advantage of the fact that 1:1 propagation in our optical field of view translated to 1:1 propagation in the electrograms. In all of our examples we observed decremental conduction and 1:1 propagation of the paced stimuli on the electrograms until the 1st AF wave was detected. For example, if AF onset was observed in the movie...
after 5 paced optical waves and the 1st optical AF wave appeared as an incoming wave, the 6th electrogram deflection after the onset of pacing was considered to correspond to the 1st AF wave. By associating in this manner the deflections to the paced waves of the optical movies, we were able to distinguish in the electrograms the difference between a paced wave and an AF wave. This association between electrogram recording and optical paced wave onset also enabled us to identify the precise sequence of activation of the 1st AF wave. The spike of the last paced impulse (SLP) was considered as a time-reference to identify the sequence of activation of the 1st AF wave at various atrial locations and the following measurements were obtained:

1. Time elapsed between SLP and the first AF electrogram at each location; ie, LALW, BB, and RAA.
2. Once the above parameter was obtained, we calculated the difference between the activation times of the following electrode location pairs: RAA-BB, RAA-LALW, and BB-LALW.

3. Time elapsed between SLP and the 1st frame at which the initiating AF wave appeared in the corresponding optical movie.

The above measurements enabled us to reconstruct quantitatively and reliably the sequence of activation of the 1st AF wave and to place it on the electrograms recordings of the corresponding AF initiation episode.

**Superposition of Optical Maps on PLA Anatomy**

To investigate the relationship between wave propagation dynamics and the anatomy of the region, including fiber orientation, color isochrone, phase and average CV maps from each heart were superimposed on the corresponding high-resolution grayscale pictures of the PLA endocardium. Anatomic landmarks such as the SPB, PVs ostia, septum, and fossa ovalis, as well as the location of the pacing electrode tip, were scaled to carefully align the anatomic pictures with the wave propagation maps.
Statistics
Statistical comparisons were made between the average myocardial thickness at 6 different locations in the PLA (N=7) and average electrogram activation times (N=4) using the statistical software package SPSS 15. All data are presented as the mean ± standard deviation.

To discern if there was a statistical difference between myocardial thickness measures we used Student paired t test with a Bonferroni correction. To discern if there was a statistical difference between electrogram activation times, Student paired t test with a Bonferroni correction was used.

Statement of Responsibility
The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
PLA has Sharp Changes in Fiber Orientation and Thickness
As illustrated in panel A of Figure 2, the PLA of the sheep heart has a well-defined SPB that is easily noticeable by a group of parallel fibers. These fibers run superiorly along the PLA, and then branch around the ostia of the PVs. In addition, similar to the human heart, the sheep PLA exhibits varying degrees of thickness with the superior PLA region being much thinner than the inferior. Quantification using caliper measurements demonstrated that the largest increase in myocardial thickness occurs in the area of transition between the PLA and the septum (location 3 in panel A), at the septal border of the SPB. As a result, we tested the hypothesis that the inferior part of the PLA will be significantly thicker than its superior counterpart. Specifically, we submitted that location 3 is thicker than all other locations, and that locations 5 and 6 are thicker than locations 1 and 2. As depicted in Figure 2E, myocardial thickness is significantly different where the PLA merges with the septum (location 3 in panel A; denoted by #, P<0.01 Student paired t test with a Bonferroni correction) compared with other locations in the PLA. Location 5 is thicker than locations 1 and 2, P<0.025 Student paired t test with a Bonferroni correction, denoted by *. Also, location 6 is significantly thicker than location 1 denoted by a “; P<0.025 Student paired t test with a Bonferroni correction, but not location 2.

Burst Pacing-Induced Wavebreak
In the absence of pacing, impulses generated spontaneously by the sinus node propagated without delay through the PLA regardless of its anatomy (Figure 3A). As shown in Figure 3B, typically, the sinus wave entered the LA through Bachmann’s bundle on top and proceeded rapidly through the SPB to activate the PLA. Notice the relatively uniform propagation with no
apparent conduction blocks or delays. In a subset of experiments, the sinus wave entered the optical field from the coronary sinus and appeared as a breakthrough in the vicinity of the right PVs (data not shown). In contrast, to sinus rhythm, the isochronal maps of burst-paced waves demonstrated nonuniform conduction, which is indicated by clearly demarcated lines of conduction delay and block (black arrows, Figure 3C and 3E). On superposition of the isochronal map with the corresponding anatomy, the areas of conduction block were seen to locate at the septal border of the SPB where the PLA and the septum meet (Figure 3D and 3F). Single pixel recordings (Figure 4) also indicate that the activation was delayed where an abrupt change in fiber direction and thickness is present.

In Figure 5, normalization of CV measured for each wave to the maximum CV allowed construction of average CV maps superposed on each heart’s respective anatomy (see Methods section). Three representative examples are shown. The site of burst pacing was different in each case. It was the RSPV in panels A, B, and C; the LIPV in panels D, E, and F; and consecutively RSPV and LIPV in panels G, H, and I. Overall, the data demonstrate that, regardless of anatomic variations and pacing site, the largest conduction delays occurred at the septal boundary of the SPB, where the PLA meets the septum (panels C, F, and I).

First Wavebreak Leads to Sustained AF

In more than one third of the cases (10/28 episodes), the first wavebreak that initiated AF occurred within our field of view. In 5 of 10 such cases, the first wavebreak originated at the PLA-to-septum boundary where wall thickness changed most abruptly; in 3 it occurred at the border of the SPB with the RSPV ostium, and in the remaining 2 it was found at the boundary between the SPB and the LIPV ostium where there was also an abrupt change in fiber orientation. Figure 6 shows 2 different first AF wavebreak locations in representative episodes from 2 different hearts. In one case (panels A through D, movie 1), the wavebreak occurred at the PLA-septum boundary. In the other (panels E through H, movie 2), the wavebreak occurred on the...
left of the SPB near the LIPV. In both cases, that wavebreak led to sustained AF. This is further illustrated by the respective pseudo ECGs in panels D and H, and by the bipolar recordings from the RAA, SVC, BB, and LAA recorded from the same episode illustrated in panels A through D.

**PLA-Septum Boundary is the Main Site of AF Initiation**

We classified the first AF waves into 3 different types: wavebreaks, breakthroughs, and incoming waves (see Methods section for definitions). Their precise site of appearance was superimposed with the corresponding PLA anatomy and composite schematics were constructed as illustrated in Figure 7 for the 28 episodes of AF initiation in 7 hearts. For clarity, the 28 episodes are separated into 2 different categories, those initiated from the left PVs (19/28, upper panels) and those initiated from the right PVs (9/28, lower panels). Notably, as shown by the top panels, 42% of the first AF wavebreaks initiated by LIPV impulses were directly observed at very specific locations of the PLA, mainly where the PLA meets the septum. The remainder of the first AF waves was detected as either breakthroughs (26%) or incoming waves (32%). In those cases, it was equally clear that the first detectable AF wave appeared primarily where the PLA transitioned to the septum. The same pattern was observed for RSPV initiated wavebreaks, as shown in the lower panels of Figure 7.

**Simultaneous Optical-Electric Recordings of AF Initiation**

Because in some instances the first wave of AF did not appear as a wavebreak but as an incoming wave, we conducted additional experiments in 4 sheep with bipolar electrodes placed at the LALW, BB, and RAA. Thus, with simultaneous optical and electric recordings we could refine our estimation of the location of the 1st AF wave onset (see Methods section). We analyzed 23 episodes of AF induction where the 1st wavebreak occurred outside the field of view. Figure 8A depicts a representative example of AF initiation recorded using LALW, BB, and RAA electrodes. After 6 paced waves, AF electrograms first appeared quasi-simultaneously at the LALW and BB before being recorded at the RAA after a substantial delay. As shown in Figure 8B through 8D, the 1st AF wave was consistently detected by the BB or LALW electrodes before the RAA electrode, and importantly after the 1st optical AF wave. Specifically, the BB and LALW electrograms appeared significantly later than 1st AF optical wave (Figure 8D; $P \leq 0.025$). Altogether optical movies and electrograms show clearly that the first AF wave appeared in the PLA before it was detected at the BB or LALW. As a result, regardless of whether it was detected as an initiating wavebreak, an early breakthrough or an incoming wave, the first AF wave located primarily at the PLA-septum transition on the right side of the SPB or else at the left boundary of the SPB where there was an abrupt change of fiber direction.

**Discussion**

**Main Results**

The most important results of this study are as follows impulses initiated at the PVs by rapid burst pacing are delayed and may lead to wavebreak at 2 major specific locations of the PLA: PLA-septum transition on the right side of the SPB where there are abrupt changes in muscle thickness and fiber direction; and left boundary of the SPB where there is an abrupt change of fiber direction. Although there was some variation in whether the first wave of AF was a wavebreak, incoming wave, or a breakthrough, they all occurred along the edges of the SPB.

Although it can be debated that ectopic foci could have occurred outside our optical field of view, especially when breakthroughs were observed as the 1st wave of AF, our data suggest (Figure 7) that in a substantial number of episodes, the 1st wave of AF resulted from wavebreak. To our knowledge, these results provide the first direct mechanistic validation of the hypothesis that AF initiation by focal repetitive activity from the PVs, as observed clinically, can occur after wavebreak and reentry formation. In addition, the results extend recent work in patients, by demonstrating the precise location along the SPB...
where preferential areas of conduction delay and wavebreak result in AF.11,12 By showing a direct relationship between the abrupt changes in thickness and fiber orientation that characterize the RSPV-PLA boundary on the septal side of the SPB and the incidence of block and reentry, our study exquisitely points to that location as the most likely to provide sink-to-source mismatch leading to wavebreak for AF initiation, although the sharp change of fiber direction at the left boundary of the SPB with the LIPV ostium also provides a substrate for wavebreak and reentry.

Wall Thickness and Fiber Direction Changes Promote Sink-to-Source Mismatch
As reported previously for the Purkinje-muscle junction,18 anatomic expansions are prone to conduction delays and block specifically in areas of abrupt electric current source-to-sink mismatch.19 As also demonstrated by Cabo et al,20 source-to-sink unbalance can also explain wave detachment from obstacles and vortex shedding leading to functional reentry. In this study, most of the wavebreaks that initiated AF appeared at the septal side of the SPB near the RSPV where the myocardial thickness dramatically expands. It is clear that the source current provided by certain PV impulses was insufficient to overcome the vast sink of the transition PLA-septum, which resulted in wavebreak, reentry, and AF. Of note, in 5 of the 7 initial animals (see Figure 7), the first detectable AF wave appeared in the area of greatest conduction delay during pacing. Then, regardless of whether the first AF wave was detected as a wavebreak, a breakthrough or an incoming wave, it localized primarily to the region of greatest thickness change. Fiber direction changes in the transition from the PV ostia to the SPB were also important in draining source current and reducing propagation safety (see Figure 3). Slowing of CV in areas of change in fiber orientation has been abundantly demonstrated in anisotropic cardiac tissues by many investigators. For example, studies have shown in the 3D ventricle that twisting anisotropy lessens the velocity of propagation.21-25 Similarly, in PV-atrial preparations, it has been observed that areas of fiber orientation change alter CV.7,8,26 In this regard, our experimental data agree with the clinical results of Markides et al9 who showed that fiber orientation changes in the SPB is a substrate for slowing conduction. As a result, our analysis of the entire PLA-septal anatomy shed important light into the previously under described role of myocardial thickness gradients at the PLA as a major determinant of conduction impairment and AF initiation by PV impulses.

Limitations
Although we focused on the conduction characteristics of the PLA, we did not examine the role of changes in action potential duration or refractoriness. It was recently reported in numeric simulations that dynamic heterogeneities in action potential duration could initiate AF.27 The model used in that study, however, did not incorporate realistic myocardial thickness or fiber orientation. Here, we explored a wide range of delays and
intervals that did not affect significantly the location of AF onset (data not shown). Nevertheless, AF being a complex nonlinear phenomenon, we cannot exclude a role of action potential duration changes in the data observed. We did not investigate the role of connexins or fibrosis distribution. Because we only mapped/recorded signals from a small part of the atrium, there is...
the possibility that for a small percentage of episodes, the 1st wavebreak might have occurred in the right atrium. In addition, we made an incision in the LAA, which may have affected the pattern of propagation and limited the site of the first wavebreak. Further, the close relationship between the electric activity observed and the anatomy of the PLA make the results applicable only to structurally normal hearts in the presence of acetylcholine. It would be important to extend this work to the ischemic and failing hearts, as well as to the heart that has been exposed to chronic AF.

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Disclosures

None.

References


CLINICAL PERSPECTIVE

Our work demonstrates that specific anatomic structures at the posterior left atrium-pulmonary vein junction are strong determinants of the fate of high-frequency impulses generated at the pulmonary veins. It shows also that the lateral boundaries of the septopulmonary bundle are preferential locations for conduction delay, wavebreak and AF initiation. This new knowledge should be useful in the development of new, more effective ablation strategies that take areas of abrupt changes in myocardial thickness and fiber direction into consideration. In this regard, it will be of great interest to investigate the specific role of the septopulmonary bundle, with its fiber orientation changes and myocardial thickness gradients in the chronically remodeled atria in patients with long-standing persistent or permanent atrial fibrillation.
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