Arrhythmogenic Substrate of the Pulmonary Veins Assessed by High-Resolution Optical Mapping

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Background—It has recently been recognized that atrial fibrillation can originate from focal sources in the pulmonary veins (PVs). However, the mechanisms of focal atrial fibrillation have not been well characterized. We assessed the electrophysiological characteristics of the PVs using high-resolution optical mapping.

Methods and Results—Coronary-perfused, isolated whole-atrial preparations from 33 normal dogs were studied. Programmed electrical stimulation was performed, and a 4-cm² area of the PV underwent optical mapping of transmembrane voltage to obtain 256 simultaneous action potentials. Marked conduction slowing was seen at the proximal PV, compared with the rest of the vein, on both the epicardial (31.3±4.47 versus 90.2±20.7 cm/s, P=0.001) and endocardial (45.8±6.90 versus 67.6±10.4 cm/s, P=0.012) aspects. Pronounced repolarization heterogeneity was also noted, with action potential duration at 80% repolarization being longest at the PV endocardium. Nonsustained reentrant beats were induced with single extrastimuli, and the complete reentrant loop was visualized (cycle length, 155±30.3 ms); reentrant activity could be sustained with isoproterenol. Sustained focal discharge (cycle length, 330 to 1100 ms) was seen from the endocardial surface in the presence of isoproterenol; each focus was localized near the venous ostium.

Conclusions—The normal PV seems to have the necessary substrate to support reentry as well as focal activity. Although reentry occurred more distally in the vein, focal activity seemed to occur more proximally. (Circulation. 2003;107:1816-1821.)

Key Words: atrium • fibrillation • electrophysiology • mapping

In the past few years, several investigators have demonstrated the presence of focal activity in the pulmonary veins (PVs) that is responsible for atrial tachycardia and atrial fibrillation (AF). In some cases, these foci are very rapid, and sustained activity induces and maintains AF, such that ablation of the foci terminates and eliminates AF (“focal driver”). In other patients, single or multiple extrasystoles from the PV foci induce self-sustaining AF, and ablation of these foci eliminates the reinitiation of AF (“focal trigger”). Clinical experience from several centers suggests that these foci may be responsive to autonomic manipulation, eg, with isoproterenol, which often increases the rate and frequency of ectopic beats.

The mechanisms of either focally driven AF or focal triggers have not been identified. Moreover, why the PVs are such a fertile substrate for atrial tachycardias and focal AF is not understood, nor has the electrophysiology of the veins been well characterized. In this study, we used high-resolution optical mapping to further define the electrophysiological characteristics of the PV and determine potential mechanisms of focal AF.

Methods

Preparation

We studied 45 PVs in 33 dogs (LBL Kennels, Reelsville, Ind). For comparison, the left atrial appendage (LAA) was mapped separately. The isolated atrial preparation is similar to that described previously. The heart and surrounding lung were removed, and the excised heart was perfused with cold cardioplegic solution. Two or more centimeters of each PV was retained intact with the rest of the left atrium.

A previous study from our laboratory demonstrated that the blood supply to the PVs was via the left circumflex coronary artery; the left circumflex coronary artery was therefore perfused with oxygenated, modified Tyrode’s solution (37°C) to maintain a perfusion pressure of 40 to 60 mm Hg. The voltage-sensitive dye Di-4-ANEPPS was perfused through the preparation, and the tissue was immobilized with either 2,3-butanedione monoxime (15 mmol/L) or cytochalasin-D (25 µmol/L). Measurements of action potential duration (APD) were performed on maps obtained with cytochalasin-D after washout of 2,3-butanedione monoxime.

Either the epicardial or the endocardial surface of the PVs was exposed to the optical mapping camera (the latter by inverting the vein inside-out) to visualize the vein from the ostium to the transition between myocardial and venous tissue. This anatomic transition was clearly visible in all veins on both surfaces as a region in which atrial muscle invaginates into the smooth muscle of the PV. Recent...
studies have shown that the length of the myocardial sleeve in dogs (from the ostium to the tip of myocardial sleeve) is 0.4 to 2 cm; the entire PV was therefore within the optical mapping field of view (2×2 cm).

Optical Mapping
Our optical mapping system has been described in detail previously and consists of a 16×16 (256-element) photodiode camera allowing a field of view of 4 cm². Fluorescent optical maps were acquired at 1000 Hz during programmed electrical stimulation.

Stimulation Protocol
One bipolar sensing electrode and one unipolar pacing intramyocardial electrode were placed in the posterior left atrium between the PVs. Optical maps were obtained during decremental overdrive pacing at cycle lengths of 500 to 200 ms and during single atrial extrastimulation at 2 different cycle lengths (500 and 300 ms).

Data Analysis
Raw fluorescence data were viewed as a movie of normalized fluorescence intensity, which reveals activation within the field of view. Quantitative data were obtained from optically derived action potentials (APs) for each of the 256 pixels of the photodiode array. Activation time and action potential duration at 80% repolarization (APD₈₀) were measured for each paced cycle length. Activation time was calculated at the maximum rate of rise of the fluorescent AP (dF/dt). APD₈₀ was the time difference between the activation time and 20% maximal fluorescent signal (peak of the optical AP). Isochronal maps of activation were constructed for each map. Conduction vectors were calculated for each point by the method described by Salama et al. The area of slowest conduction within the vein was identified as the region with maximum crowding of isochrones; the length of the region of slow conduction was calculated as the number of pixels within this region along the direction of the activation wave front.

Sympathomimetic Drug Infusion
Isoproterenol (10⁻⁷ to 10⁻⁶ mol/L) was infused via the coronary perfusate after baseline programmed stimulation had been performed. After steady state was achieved, the PVs were mapped again in the presence of isoproterenol, and the same stimulation protocol was repeated.

Statistical Analysis
All values are reported as mean±SD. Two-way comparisons were made by a 2-tailed t test. Comparisons with ≥3 variables were made by ANOVA. A value of P≤0.05 was considered significant. Dispersion of repolarization was assessed by the coefficient of variation (SD/mean) of the APD₈₀.

Results
The atrial musculature contracted vigorously when perfused with warm modified Tyrode’s solution in response to atrial pacing, with a threshold <2 mA at 2-ms stimulus duration. Stable optical signals were obtained in all specimens for several hours after initiation of perfusion without deterioration of signal quality.

Figure 1 shows high-resolution optical APs obtained from the epicardial aspect of a PV during atrial pacing. The APs at the distal aspect of the PV had a slower upstroke (phase 0) compared with the proximal aspect. Similar differences in AP upstroke were found on the endocardial surface between the proximal and distal PVs. The optical Vₘ₅₅ (dF/dt) at the distal vein was 93.1±45.6, compared with 195.7±46.3 at the proximal vein (P<0.001).

Figure 2 shows repolarization heterogeneity across PV and left atrium. APD₈₀ at drive cycle lengths of 500, 400, and 300 ms are shown for proximal and distal PV for both endocardial (endo) and epicardial (epi) aspects and LAA. APD₈₀ is longest at PV endocardium (endo->epi->LAA, *P≤0.05 at S₁=500 and 400 ms; at S₁=300 ms, epi->LAA, endo->LAA, *P≤0.05).

Figure 3 shows isochemical map of epicardial PV obtained during pacing at 500 ms. Scale is shown at right, with white being earliest activation and dark gray/black being latest activation. Pacing is occurring from left atrium (outside of field of view), which is at upper right of field of view. There is marked crowding of isochrones at junction of proximal vein and distal vein, signifying slow conduction in this region of PV. Arrow marks direction of wave-front propagation.
In addition to AP upstroke, repolarization times were also significantly different between the vein and the surrounding atrium, as shown in Figure 2. APD\textsubscript{80} at a drive cycle length of 500 ms was significantly greater at the endocardial (187±110/150 ms) and the epicardial (161±20.1 ms) aspects of the PV than at the LAA (125±16.5 ms; \(P=0.002\) and \(P<0.0001\), respectively). In addition, APD\textsubscript{80} was significantly (\(P=0.009\)) longer in the endocardium than the epicardium. This relationship was maintained across all pacing cycle lengths, and normal physiological rate adaptivity of APD\textsubscript{80} was present at both the epicardial and endocardial PVs (Figure 2).

Dispersion of repolarization, as measured by the coefficient of variance of APD\textsubscript{80}, was greater at the distal PV than the proximal vein at 500 ms, although the difference was significant only on the endocardial aspect (0.098±0.05 versus 0.042±0.01, \(P=0.01\)). When the epicardial and endocardial aspects were compared with each other, dispersion was greater at the proximal epicardial vein than the proximal endocardial vein (0.084±0.04 versus 0.042±0.01; \(P=0.019\) at \(S_1=500\)).

**Heterogeneous Conduction in PV**

Characteristic conduction patterns were found during impulse propagation from the left atrium into the PV. Figure 3 shows a typical activation map of the PV (epicardial aspect) during atrial pacing at a cycle length of 400 ms. The isochronal lines demonstrate significant conduction delay in the proximal vein compared with the adjacent left atrium. Conduction vector maps confirmed slower conduction velocities at the proximal PV. The area of slowest conduction was noted in the proximal PV and measured 0.50±0.06 cm, with conduction velocity in this region being significantly slower than in the rest of the vein (31.3±4.47 versus 90.2±20.7 cm/s, \(P=0.001\)). Similarly, areas of markedly slow conduction were seen on the endocardial aspect of the proximal PV, measuring 0.70±6.90 versus 67.6±10.4 cm/s, \(P=0.012\). The areas of slowest conduction in the epicardium were slower than the regions of slowest conduction in the endocardium (31.3±4.47 versus 45.8±6.9 cm/s, \(P=0.01\)).

Progressive conduction slowing was found at faster paced cycle lengths at both the epicardial and endocardial aspects of the PV, as shown in Figure 4. No such conduction slowing was observed in the LAA. With more rapid atrial pacing (\(\leq300\) ms), variable conduction block could be seen at the proximal aspect of every PV. In all veins, 2:1 conduction was observed at atrial pacing cycle lengths of 200 to 300 ms (Figure 5).

**Figure 4.** Decremental conduction in PV. Graph of conduction in PVs and left atrium at drive cycle lengths of 500, 400, and 300 ms. There is progressive slowing of conduction velocity, with a decrease in pacing cycle length, on both epicardial (epi) and endocardial (endo) aspects; *\(P<0.05\) at proximal and distal aspects of epicardial vein and at distal aspect of endocardial vein. No such conduction slowing is noted at LAA.

**Figure 5.** Optically derived action potentials during pacing at 250 ms, with 2:1 block in PV. Array demonstrates 4 action potentials per pixel in proximal vein at paced cycle length. In more distal portion of vein (lower left side of array), there are only 2 action potentials at half cycle length of pacing (2:1 block). Arrow marks direction of wave-front propagation.

**Figure 6.** Isochronal maps of activation and contour map of APD\textsubscript{80} for same region of epicardial vein during same drive train. A, Isochronal map was obtained during atrial pacing at 500 ms. There is significant slowing of conduction at proximal vein, as shown by crowding of isochrones; arrow marks direction of wave-front propagation. B, APD\textsubscript{80} for same impulse. Longest APD\textsubscript{80} is noted in distal vein, 0.5 cm distal to region of slowest conduction in proximal vein.
Substrate for Reentry
Progressive conduction slowing was noted within the vein with extrastimulus testing, especially at coupling intervals <300 ms. With more closely coupled extrastimuli (<250 ms), there was progressive failure of stimulus propagation in specific regions of the proximal PV as the extrastimulus approached APD_{80} of that segment of the PV (Figure 6A). Figure 6B shows a comparison of activation time and APD_{80} in a PV at a pacing cycle length of 500 ms. The area of slowest conduction (crowded isochrones) lies 0.5 cm proximal to the area of longest APD_{80} in the proximal PV. Marked regional differences between activation and repolarization times were found in all veins, with a mean distance of 0.44±0.072 cm between the region of longest APD_{80} and the region of slowest conduction.

Conduction block at the proximal PV was accompanied by reentrant beats in 60% of the preparations. In every case, propagation failed as the extrastimulus coupling interval approached APD_{80}. This area of conduction block (ie, wave front approached before APD_{80}) was small and was always contiguous to but distinct from the region of slow conduction in the vein. This led to unidirectional block of the wave front but continued propagation of the impulse in the rest of the vein through the zone of slow conduction. Conduction was slow enough to allow recovery of the blocked region, and thus reentry was initiated. An example is shown in Figure 7. In 50% of all cases of reentry, the complete reentrant loop could be mapped within the optical mapping field of view (2×2 cm), with a reentrant cycle length of 155±30.3 ms. This reentry was seen only at the epicardial aspect of the PV.

In the presence of isoproterenol (10^{-6} mol/L), the reentry could be sustained (cycle length at baseline versus on isoproterenol, 155±30.3 versus 145.8±38.4 ms, P=0.27). Isoproterenol did not cause a significant change in conduction velocity in the PV (100.9±9.9 cm/s at baseline versus 92.1±11.8 cm/s on isoproterenol, P=0.27). However, APD_{80} was shortened by 15.5±7.7% in the presence of isoproterenol (P=0.03).

Focal Activity
In the presence of isoproterenol (10^{-6} mol/L), sustained, spontaneous rhythms were observed in 66% of the preparations during endocardial mapping (cycle length range, 330 to 1100 ms). Focal discharge was defined as a point source originating from within the field of view. All foci were seen at or just proximal to the venous ostium, 0.84±0.4 cm proximal to the region of slow conduction in the PV. In 50% of these “focal” discharges, burst atrial pacing was necessary to initiate the spontaneous activity. In general, the rate increased with burst pacing but then gradually decreased to <1000 ms. There was no evidence of diastolic depolarization or triggered activity at the site of earliest activation on the optical map during any focal discharge. Figure 8 shows a typical PV discharging at a rate of 1100 ms; with burst atrial pacing, the rate increased transiently to 300 ms. In 1 animal, discrete foci were discovered in 2 different veins, with 1 focus being significantly faster than the other (330 versus 1000 ms). The faster focus was transient and was inducible only with burst atrial pacing. In contrast, the slower focus fired continuously (in the presence of isoproterenol) but seemed to be transiently suppressed in presence of the faster focus.

Discussion
In this study, we have demonstrated distinct electrophysiological characteristics of the PV using high-resolution optical mapping. The PV seems to possess a substrate for microreentry, although focal activity was also noted.

Conduction was found to be significantly slower at the proximal PV than in the rest of the left atrium, with decremental conduction and variable entrance block observed at faster atrial pacing rates. Marked repolarization heterogeneity was also discovered. Regions of slow conduction in the
 vein lay contiguous to regions with longer APD_{80}, thus creating a substrate for leading-circle reentry within the PV. Reentry was demonstrated repeatedly in this region in response to extrastimulus testing.

Focal discharge was also demonstrated on the endocardial aspect of the PV in the presence of isoproterenol; focal activity was always discovered proximal to the area of slow conduction and was slower than the reentry recorded more distally in the PV.

Recently, Chen et al\(^8\) demonstrated pacemaker activity and triggered afterdepolarizations in isolated PV cardiomyocytes; the distribution of ionic currents in PV cardiomyocytes, such as \(i_{\text{CaL}}\), \(i_f\), and \(i_{\text{K1}}\) (with \(i_{\text{K1}}\) being reduced in cells with pacemaker activity), suggested some similarities with sinus nodal cells. The slower phase 0 in the normal PV found in both their study and ours may result either from alterations in ion channels or from an elevated resting membrane potential. However, in the study by Chen et al of isolated cells, the influence of tissue structure and electrotonic interactions were not evaluated, and the precise mechanisms that underlie the genesis of PV foci were not postulated. In fact, Verheule et al\(^9\) recently reported that cellular size and morphology, ultrastructure, and gap junction distribution of the myocardial sleeved within PVs are similar to those elsewhere in the atrial myocardium and are distinctly different from those in specialized areas such as the SA and AV nodes. In our study, no focal activity was demonstrated within the PV in the baseline state, suggesting that at least in normal dogs, electrotonic inhibition resulting from intercellular coupling may suppress automatic activity in individual pulmonary cardiomyocytes. We did demonstrate focal activity in the presence of isoproterenol, which in several cases responded to pacing in a manner suggestive of triggered activity, similar to that suggested by the single-cell studies of Chen et al. However, in these normal dogs, the rate of discharge was relatively slow.

In other studies, discrete sites of high-frequency periodic activity have been demonstrated during AF in the isolated sheep heart.\(^9\) The sites with the highest dominant frequency were localized to the posterior left atrium, near or at the PV ostium. Stable microreentrant sources were thought to be the most likely underlying mechanism of AF in this model. More recently, Hocini et al\(^10\) described regions of slow and heterogeneous conduction in canine PVs, especially in regions that demonstrate changes in muscle fiber orientation, and implied that this may provide a substrate for reentry in the veins. Slow and fractionated activation electrograms were characteristic of these regions of slow conduction. However, because the area of anisotropic conduction in each vein was very small (longest zone of conduction delay in any vein was <15 mm), reentrant circuits, if present, would be very difficult to demonstrate with standard extracellular recording techniques. Moreover, interactions of regions of slow conduction and prolonged refractoriness could not be evaluated with extracellular recordings.

Using high-resolution optical mapping, we have demonstrated that PVs possess both anisotropic conduction and repolarization heterogeneity, with evidence of reentry in response to extrastimulus testing. We and others have demonstrated that the proximal vein contains abrupt changes in fiber orientation, which may account for this conduction slowing.\(^5,10\) Although conduction slowing was most marked in the proximal vein, the reentrant loops that were noted also seemed to require participation of regions of prolonged refractoriness, usually more distally in the vein. The reentry was therefore consistent with the classic “leading-circle” model described by Allessie et al\(^11\) in that it seemed to occur in the absence of anatomic obstacle (ie, was functional) and seemed to require only recovery of a very small region of depolarized vein to persist. This is further supported by the finding that isoproterenol allowed the sustenance of reentry with no effect on conduction velocity, only on APD_{80}. The rates of reentry, which were frequently faster than 300 ms, were compatible with those found in patients with focal drivers.

We also noted slower, focal activity more proximally in the PV that was enhanced by burst atrial pacing, suggesting triggered activity as a possible mechanism. Nonetheless, focal activity also seemed to be suppressible by faster atrial rates, suggesting focal automaticity as a potential mechanism. Regardless of the true underlying mechanism, it is possible
that the slow proximal focal activity may constitute the focal triggers that are encountered clinically. In the setting of diseased atrial substrate, these triggers may allow the initiation of AF.

It is conceivable, therefore, that the PV may provide a substrate for both focal (or triggered) automaticity and reentry. A slow, automatic (or triggered) focus originating from close to the PV ostium may then be maintained as a rapid reentrant circuit because of the functional barriers provided by the slow conduction in the rest of the PV. This may explain the unusually rapid rates of these foci that have been observed clinically (focal drivers).

**Study Limitations**

Our study demonstrates the potential substrate for arrhythmias in normal canine PVs and therefore may not represent that of patients with AF. However, our study does seem to confirm some of the substrate characteristics that have been postulated on the basis of clinical studies in the PV. Moreover, it is possible that our observations may be a 1-dimensional snapshot of more complex spiral/scroll wave activity in a potentially very heterogenous medium (ie, the PV). Additional work is needed to better clarify these mechanisms.

Although our main finding demonstrated reentry within the PV as one mechanism, we were unable to demonstrate the exact mechanism of focal activity observed in the proximal endocardium, although there was some evidence supporting both triggered and focal mechanisms. Moreover, focal discharge was seen only on the endocardial aspect and not on the epicardial aspect of the vein. A potential explanation is that the focus may be deeper than the surface field of view of the optical mapping technique and may lie closer to the endocardial surface of the PV. Alternatively, the observed focus may also be the exit site of reentry that may be occurring deeper within the vein (and may therefore not be observed on optical mapping). However, the presence of reentry is the unique finding of this study.

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**References**

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