Activation Patterns of Purkinje Fibers During Long-Duration Ventricular Fibrillation in an Isolated Canine Heart Model

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Background—The roles of Purkinje fibers (PFs) and focal wave fronts, if any, in the maintenance of ventricular fibrillation (VF) are unknown. If PFs are involved in VF maintenance, it should be possible to map wave fronts propagating from PFs into the working ventricular myocardium during VF. If wave fronts ever arise focally during VF, it should be possible to map them appearing de novo.

Methods and Results—Six canine hearts were isolated, and the left main coronary artery was cannulated and perfused. The left ventricular cavity was exposed, which allowed direct endocardial mapping of the anterior papillary muscle insertion. Nonperfused VF was induced, and 6 segments of data, each 5 seconds long, were analyzed during 10 minutes of VF. During 36 segments of data that were analyzed, 1018 PF or focal wave fronts of activation were identified. In 534 wave fronts, activation was mapped propagating from working ventricular myocardium to PF. In 142 wave fronts, activation was mapped propagating from PF to working ventricular myocardium. In 342 wave fronts, activation was mapped arising focally. More than 1 of these 3 patterns could occur in the same wave front.

Conclusions—PFs are highly active throughout the first 10 minutes of VF. In addition to retrograde propagation from the working ventricular myocardium to PFs, antegrade propagation occurs from PFs to working ventricular myocardium, which suggests PFs are important in VF maintenance. Prior plunge needle recordings in dogs indicate activation propagates from the endocardium toward the epicardium after 1 minute of VF, which suggests that focal sites on the endocardium may represent foci and not breakthrough. If so, in addition to reentry, abnormal automaticity or triggered activity may also occur during VF. (Circulation. 2007;116:1113-1119.)

Key Words: action potentials ▪ mapping ▪ fibrillation ▪ electrophysiology ▪ endocardium

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PFs in the dog are found in the subendocardial layer and rarely course transmurally, as in porcine models.8,9 Chemical ablation of this area of endocardium in dogs slows the activation rate near the endocardium so that it is no longer significantly faster than near the epicardium during VF.10 Even after extensive infarction of myocardial tissue, subendocardial PFs in canine models remain structurally and electrophysiologically intact and capable of rapid activation.11–13 Using 3-dimensional modeling, Berenfeld and Jalife14 have shown that focal activity may arise at the Purkinje muscle junctions, leading to initiation of VF. This model predicted that the PFs become irrelevant after intramyocardial reentry is established during the early stages of VF.14 However, global ischemia rapidly develops as VF progresses, and because PFs are more resistant to ischemia than is the working ventricular myocardium (WVM), PFs may play an important role during late VF.11–13

The objectives of this study were to (1) record PF signals reliably from the endocardium of an isolated canine heart model, (2) quantitatively evaluate the activation patterns of PFs during VF, and (3) make inferences about the role of PFs...
in the maintenance of VF. We hypothesized that PFs are intimately involved in the maintenance of VF, either by acting as a substrate for reentry or as a spontaneously activating source of wave fronts.

**Methods**

All of the animals were managed in accordance with the guidelines established by the American Heart Association on research animal use,¹¹ and the protocol was approved by the University of Alabama at Birmingham’s Institutional Animal Care and Use Committee.

**Animal Preparation**

Six 22- to 28-kg (24±3 kg, mean±SD) mongrel dogs (Marshall Bioresources, North Rose, NY) were fasted overnight and anesthetized with sodium thiopental 25 mg/kg IV, intubated, and mechanically ventilated with 2% to 3% isoflurane in 100% oxygen. Core body temperature, arterial blood pressure, arterial blood gases, and the anterior descending arteries to expose the endocardial surface. A perfusion catheter was inserted and ligated within the left main coronary artery, and a 504-electrode array (24 columns by 21 rows) with 1-mm spacing was attached to the aortic root.

**Heart Isolation**

The heart was exposed via a right lateral thoracotomy approach. The left main coronary artery was identified and clearly exposed with left coronary artery was excised for isolation.

**VF Induction and Mapping Ex Vivo**

A 504-electrode array (24 columns×21 rows) with 1-mm spacing acquisition was seated over the endocardial insertion of the anterior papillary muscle. Unipolar electrograms were recorded with respect to a reference electrode located on the aortic root. The recordings were band-pass filtered with a high-pass filter of 0.5 Hz and a low-pass filter of 500 Hz. Data were sampled at 2 kHz and recorded with 14-bit resolution. A pacing wire on a hook electrode was placed at the superior border of the array. Initially, the heart was defibrillated with a Lifepak 12 biphasic defibrillator (Medtronic Physio-Control, Redmond, Wash) at 30 to 60 J. Pacing was briefly induced to ensure that the ventricular myocardium could be captured and to confirm the orientation of the array. To allow the heart to rewarmin from the isolation procedure, the postisolation VF episode was not induced until after ∼5 minutes of reperfusion. VF was then induced with a 9V battery applied to the right ventricle, and recording was performed for 10 consecutive minutes. Perfusion was continued for the first 30 seconds of VF and then discontinued for the remaining time. Six segments of data, each 5 seconds long, were analyzed 0, 1, 3, 5, 7, and 10 minutes after the start of VF.

**Quantification of Activation Patterns**

Quantitative analysis of VF activation patterns was performed with a computer-generated 2D visual display. A single temporal sample at a recording site was deemed to represent an activation in the underlying tissue when dV/dt was less than −0.20 V/s. Activations that occurred within 0.5 ms of each other at neighboring electrodes, ie, electrograms that were horizontally, vertically, or diagonally adjacent to one another, were identified as forming a wave front. PF activations were quantified and distinguished from WVM activations by evaluation of recorded potential and their temporal derivatives for a more rapid propagation across the array and were correlated with a sharp Purkinje potential of 1 to 2 ms in duration, as previously described in canine studies by others.¹⁷–²¹ The manner in which PF activations arose was quantified as either (1) depolarization propagating from WVM to PF, presumably through retrograde conduction at a PF–WVM junction site; (2) PF arising from the leading edge of a WVM activation, presumably through retrograde conduction at a PF–WVM junction site; (3) propagation of PF from the border of the mapped region, activating WVM in the mapped region; or (4) PF or WVM arising de novo in the mapped region.

The overall difference between the PF-to-WVM and WVM-to-PF patterns of Purkinje activation occurred in >84% of activation wave fronts throughout the 10 minutes of VF. Three primary patterns of PF or focal activations occurred: (1) a WVM wave front propagating into PFs (WVM to PF), (2) a PF wave front propagating into a WVM (PF to WVM), and (3) wave fronts that arose focally in either the PF or WVM. More than 1 of these 3 patterns could occur during the same PF activation. The Table describes the summed numbers for each activation pattern was given in parentheses.
they were less closely coupled during VF. Figure 2 illustrates that despite a longer coupling interval, there remained a 1:1 relationship between the PF and ventricular activation.

A total of 1018 wave fronts were identified over the 36 segments of data during which PF activation occurred. In 534 PF wave fronts (52%), activation was mapped propagating from WVM to PF. Two different WVM-to-PF types of activations were identified. Type 1 WVM-to-PF activation involved a wave front of myocardial activation with a PF activation emanating from it as a leading edge, likely from a retrograde conduction. Figure 3 illustrates the type 1 WVM-to-PF pattern in which the PF arises from and then leads the WVM activation because of the faster PF conduction velocity to the top of the array. The wave front labeled A represents propagation of WVM without evidence of PF interaction as is seen in the wave fronts labeled B through F, during which retrograde penetration of the Purkinje system has occurred. Of the 534 wave fronts with a WVM-to-PF pattern, 300 (73%) were type 1.

Type 2 WVM-to-PF activation was characterized by a myocardial activation that appeared to stimulate the PF network retrogradely from a PF–WVM junction site, which led to an expanding “starburst” appearance of PF propagation. Figure 4 illustrates the type 2 pattern of WVM to PF, in which a WVM activation courses from the top of the array to the bottom-left portion, at which time a PF–WVM junction site is stimulated that leads to activation of the PF network in an expanding starburst pattern. The WVM activation continues off the mapped region without change in direction or fractionation. The temporal derivative tracings show the PF...
activation conducting more rapidly than the WVM activation. Of the 534 wave fronts with a WVM-to-PF pattern, 234 (27%) were type 2.

In 142 wave fronts (14%), activation was mapped propagating from the PFs to the WVM. Figure 5 illustrates an example of this pattern in which a rapidly activating PF wave front enters the array from the top, moves leftward, and stimulates a PF–WVM junction site, after which an expanding PF ring of activation appears, followed by a more slowly activating ring of ventricular myocardium. The PF to WVM pattern of activation existed throughout VF, even in the first minute of VF (Table).

A total of 342 wave fronts (34%) were mapped that arose focally, with no preceding activations recorded in the vicinity.
for at least 50 ms. Figure 6 demonstrates a focal activation in which the PF wave front arises de novo from the middle of the array, with propagation to the top and bottom-right aspect of the array, followed by a WVM activation directed to the left of the array. The temporal derivative tracings in Figure 6 show propagation that begins at electrode A in the center of the array and propagates upward toward electrode E and downward toward electrode D.

Focal activations could arise in either PFs or WVMs. Of the 342 focal wave fronts, 144 (42%) arose in the PFs. In 26 (8%) of these wave fronts, activation remained confined to the PFs, whereas in 118 (34%), the PF activation appeared to propagate into and initiate a WVM activation. Of the 342 focal wave fronts, 198 (58%) appeared to arise in the WVM. In only 7 (2%) of these wave fronts did retrograde PF activation occur after the focal WVM activation, leaving 191 (56%) of focal WVM wave fronts that remained confined to WVM activation.

**Discussion**

The present study provides the first description of the mapping of PF activation patterns on the endocardium in an ex vivo isolated heart preparation during long-duration VF. Our findings show that the PF network is highly active during all stages of VF and raise the possibility that abnormal automaticity or triggered activity may play a role in the maintenance of VF. In the present study, PFs could be activated by retrograde stimulation from the myocardium and could directly initiate new WVM wave fronts by antegrade stimulation. These findings suggest that PFs are important in VF maintenance. In addition, wave fronts frequently appeared focally on the endocardium from either PF or WVM activation, which raises the possibility that abnormal automaticity or triggered activity is present throughout the first 10 minutes of VF.

Although several studies implicate the active role of PFs in ventricular arrhythmias, the exact mechanism for initiation and maintenance of VF is a mystery that is slowly being unraveled. Previous evidence to support the PF role in VF comes from canine heart studies in which the subendocardium was ablated by painting the endocardium with either Lugol’s solution or phenol. Those studies revealed that chemical ablation of the PF-rich subendocardial layer in canine hearts dramatically elevated the VF threshold, which made inducibility difficult. In addition, 2 groups have shown that rapid polymorphic ventricular tachycardia/VF could be initiated by PF activation via burst atrial pacing in humans with idiopathic VF and that radiofrequency ablation of the PF potentials resulted in noninducibility in these patients. In contrast, in a study by Chen et al that included observations of VF after subendocardial ablation by the flushing of Lugol’s solution in situ canine hearts, VF thresholds remained relatively unchanged despite ablation of the endocardium with Lugol’s solution. However, some PFs may have not been ablated by this procedure, because PF potentials can be recorded at a depth of 2 mm in the left ventricular free wall, whereas application of Lugol’s solution produces necrosis at a level of only 0.5 mm. Further studies are needed to determine whether VF is inducible after complete PF ablation and, if so, how VF is altered by the absence of PF activation.
ischemia is more likely antegrade than retrograde through PF–WVM junctions owing to the increased load in the antegrade direction of propagation.\textsuperscript{11–13}

An intriguing finding in maps in the present study was the frequent occurrence of focal activation patterns. This pattern may have been caused by intramural wave fronts propagating toward the endocardium and breaking through to it. However, after the first few minutes of VF in the dog, plunge needle recordings indicate that most wave fronts propagate from the endocardium toward the epicardium,\textsuperscript{12,33} so that frequent endocardial breakthrough would not be expected. If the focal activation pattern arises from true spontaneous activation that occurs in the PF or WVM tissue, then abnormal automaticity or triggered activity may be present during VF. This would be a startling finding, because the literature about VF maintenance has overwhelmingly dealt exclusively with reentry.

**Study Limitations**

We used a perfused isolated heart model to allow for direct visualization of mapping on the anterior papillary muscle. Isolated heart models lack innervation and therefore lack influence by autonomic control, which alters findings in intact hearts and clinical VF.

With the 2-dimensional map, it is not possible to unequivocally determine whether the focal endocardial activations occurred spontaneously from near the surface of the endocardium or whether they represent intramural activation with breakthrough at the surface of the endocardium. Further studies with plunge needles will be needed to answer this question.

Only a small, flat segment of the left ventricle over the papillary muscle was able to be studied with our array because of the undulating nature of the ventricular endocardium. This limitation prevents our ability to make wider observations about the spread of VF wave fronts in the other regions.

**Acknowledgments**

We thank Frank Vance and Reuben Collins for assistance with the experimental preparation of the animals.

**Sources of Funding**

This work was supported by National Heart, Lung, and Blood Institute grants HL28429, HL66256, HL64184, and HL85370.

**Disclosures**

None.

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**CLINICAL PERSPECTIVE**

Of the hundreds of thousands of sudden cardiac deaths every year, many are caused by ventricular fibrillation (VF). For well over 50 years, it has been thought that VF is maintained by reentry within the working myocardium. Although the Purkinje fibers of the specialized conduction system have been shown to initiate VF in a small number of patients, their role in the maintenance of VF, if any, has not been known. This study mapped the activation sequences of the Purkinje fibers and of the working myocardium on the endocardium of the dog heart during VF that lasted for 10 minutes and found that the Purkinje fibers are active during this period. In addition to the working myocardium, which activates the Purkinje fibers in a retrograde fashion, the Purkinje fibers were observed to activate the working myocardium via an antegrade mechanism, which indicates that the Purkinje fibers are responsible at least in part for the maintenance of VF. In addition, activation appeared to arise focally on the endocardium in some cases, which raises the possibility that abnormal automaticity and/or triggered activity is present during VF. If confirmed, these findings suggest that therapy focused on the Purkinje fibers, on abnormal automaticity, and/or on triggered activity, in addition to therapy focused on reentry in the working myocardium, may cause VF to be easier to halt or to be more likely to stop spontaneously.
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Circulation. 2007;116:1113-1119; originally published online August 13, 2007; doi: 10.1161/CIRCULATIONAHA.107.699264

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