Estimated Global Transmural Distribution of Activation Rate and Conduction Block During Porcine and Canine Ventricular Fibrillation

Jonathan C. Newton, William M. Smith, Raymond E. Ideker

Abstract—We quantified ventricular fibrillation (VF) activation rate, conduction block, and organization transmurally in pigs and dogs, whose transmural Purkinje distribution differ. In six pigs and five dogs, 75 to 100 plunge needles, containing four electrodes for the right ventricle (RV) and six electrodes for the left ventricle (LV) and septum, were inserted in vivo. Six VF episodes were electrically initiated and allowed to last for 47 to 180 seconds. From the FFT power spectra, dominant frequency (DF), an estimate of activation rate, and incidence of double peaks (DPI), an estimate of conduction block, were calculated every 8 ms at each electrode. DF was highest at the epicardium and lowest at the endocardium, whereas DPI was highest at the endocardium and lowest at the epicardium for the entire LV and the RV base in both pigs and dogs for the first 70 seconds of VF. This distribution changed little throughout the first 3 minutes of VF in pigs but reversed in dogs by 2 minutes of VF. In conclusion, estimated activation rates and conduction block incidence during VF are not uniformly distributed transmurally. During the first minute of VF, the faster activating LV base epicardium exhibits less estimated block than the slower endocardium, raising the possibility that faster activating epicardium generates wavefronts that drive the endocardium early during VF. Constancy of this pattern in pigs but its reversal by 2 minutes in dogs is consistent with the hypothesis that activation during later VF is driven by Purkinje fibers. (Circ Res. 2004;94:836-842.)

Key Words: cardiac electrophysiology ■ arrhythmia ■ mapping ■ ventricular fibrillation

Little previous research has investigated the global transmural activation rates during ventricular fibrillation (VF), primarily due to the difficulties of making simultaneous recordings from hundreds of sites buried within the myocardium. As a result, the electrophysiological characteristics of VF have primarily been analyzed on the epicardium or occasionally the endocardial surface.

A recent study recorded from the cut surface across the wall, but the new boundary created at the cut surface may have altered VF electrophysiologic characteristics. One of the few studies that utilized transmural recordings during VF in dogs recorded from five plunge electrodes in the free wall of the left ventricle (LV), each with five transmural electrodes. It showed that by 2 minutes of VF a gradient of activation rate developed across the wall with the epicardium slower than the endocardium and that this gradient then increased with time. The distribution of activation rates during early VF in pigs and dogs is unknown. This knowledge is needed to guide and focus future mapping studies to determine the mechanisms for VF maintenance. For example, one hypothesis is that VF is maintained by a “mother rotor” in the fastest activating region of the myocardium, which gives rise to wavelets that propagate throughout the remainder of the myocardium where they block. If the conduction block involves the entire wavelet, it is terminated. If only a portion of the wavelet blocks, the wavelet either fractionates into daughter wavelets or it begins to rotate at the site of the wavelet. To test this hypothesis, the fastest activating myocardial region should be identified so that it can be mapped in detail. In addition, the distribution of Purkinje fibers is markedly different in pigs and dogs, with Purkinje fibers largely confined to the endocardium in dogs but extending almost transmurally in pigs. Thus, if the Purkinje tissue plays a role during the maintenance of VF, the intramural location of the fastest activating region may not be the same in dogs and pigs. Therefore, the goals of this study were to determine estimates of the intramural distribution of activation rate and the incidence of block during VF throughout the free walls of both ventricles in pigs and dogs.

Materials and Methods

Animal Preparation
Preoperative and operative care complied with section 6 of the Animal Welfare Act of 1989, and adhered to NIH publication No.
The experiments were performed in six pigs (39.7±1.7 kg) and five dogs (18.4±1.1 kg). Anesthesia was established in pigs using telazol (4.4 mg/kg IM), xylazine (2.2 mg/kg IM), and atropine (0.04 mg/kg IM), and in dogs with intravenous thiopental (30 mg/kg). Anesthetic maintenance was achieved with isoflurane in 100% O2 by inhalation in both species. Cardiac rhythm was monitored with ECG lead II.

To determine the mean DF and DPI in different ventricular areas at different transmural depths in both pig and dog hearts during VF, the myocardium was divided into five regions: the LV base (LVB) and apex (LVA), the RV base (RVB) and apex (RVA), and the septum (Figure 1). Each region was divided into levels corresponding to the position of the recording electrodes on the needles, resulting in four levels for the RV and six for the LV and septum, yielding 26 zones in total.

Results

DFs

Pig

There existed a spatiotemporal distribution of activation and DFs throughout the fibrillating swine myocardium (Figures 2A and 3A). The fastest activating region from 10 seconds to 3 minutes of VF was the LVB epicardium (P<0.01 by ANOVA). The DFs for the epicardial levels were significantly higher than for the endocardial levels in the LV and

Data Acquisition

Unipolar epicardial potentials from the electrodes were recorded with a 528-channel mapping system as follows: 2-kHz sample rate, 0.5- to 500-Hz bandpass filter, 50× gain, with reference and ground electrodes at the right and left legs, respectively. Signals from broken electrodes were excluded from analysis.

Power Spectra

The first five VF episodes were divided into 5-second epochs beginning after 5, 10, 15, 20, 25, 30, 35, and 40 seconds of VF and the longer sixth VF episodes included three more 5-second epochs beginning after 1, 2, and 3 minutes of VF. An FFT was performed and analyzed for each epoch from each electrode according to the method of Evans et al, which has a specificity of >99% and a sensitivity of >70% for conduction block detection during VF (Figure 1). For each electrode tracing, the signal mean was subtracted to remove any DC offset and the signal was downsampled to 125 samples per second. An FFT was performed on each downsampled recording using a Hamming window spanning 80 downsampled datapoints (640 ms) and zero-padded on either side to 128 points (1.024 seconds and 0.976-Hz resolution). An FFT window centered at every downsampled datapoint in the 5-second epoch was computed, resulting in a dominant frequency (DF) and a binary positive or negative variable for the occurrence of double peaks (DPs) at each time point in the epoch for every electrode recording. The DF, an estimate of activation rate, was the frequency of the peak in the power spectrum with the highest power in the range 4 to 20 Hz. The mean DF during each 5-second epoch was determined by averaging the DF for each time point in the epoch at which a DP was not present. DP, an estimate of conduction block, was identified at a time point if a secondary peak was present in the power spectrum that was within a maximum separation of 80% of the dominant (highest) peak frequency and that had an amplitude of at least 20% of the amplitude of the dominant peak. The fraction of time a DP was present (the DF incidence, DPI) was determined for each 5-second epoch.

To determine the mean DF and DPI in different ventricular areas at different transmural depths in both pig and dog hearts during VF, the myocardium was divided into five regions: the LV base (LVB) and apex (LVA), the RV base (R VB) and apex (RVA), and the septum. Each region was divided into levels corresponding to the position of the recording electrodes on the needles, resulting in four levels for the RV and six for the LV and septum, yielding 26 zones in total.

Statistics

Data are given as mean±SD. A value of P≤0.05 was considered significant. To test for differences in DF or DPI, an ANOVA followed by a Fisher’s PLSD test for significant differences and for independence of samples within the 26 zones for the short episodes of VF (Statview, Abacus Concepts Inc). For the longer episodes, differences between the DFs and DPs at the epicardial and endocardial zones, and the RV, LV and septal zones for 1, 2, and 3 minutes of VF, were analyzed for significance using a paired t test (Excel, Microsoft Corp.) A linear regression analysis was performed correlating the mean DF and the mean DPI of each zone determined during the short VF episodes.
RV base throughout the first minute. In the RV apex, this transmural distribution of activation rates only occurred after 20 seconds. This gradient of DF across the wall became more evident with the duration of VF, so that the difference between the endocardial and epicardial levels was greater at 40 seconds than at 5 seconds (Figure 3). The DFs recorded in the septal midwall were not significantly different than DFs recorded at the septal endocardial surfaces, but the electrodes near the RV chamber had higher DFs than the electrodes near the LV chamber for all epochs. These same distributions were seen after 2 and 3 minutes of VF with the LV continuing to exhibit the greatest gradient from a faster epicardium to a
slower endocardium. By 2 minutes of VF, the septum activated slower than the LVB, but not significantly different from the other regions.

The global mean DF from 5 to 45 seconds after VF induction for all episodes for all animals was 9.3±0.63 Hz. The global mean DF decreased from 5 to 45 seconds after VF induction (Figure 4).

**Dog**

The LV epicardial electrodes exhibited significantly higher DFs than the endocardial electrodes with a gradual decrease across the wall, but the highest DF was found 3 mm below the epicardial surface of the LVB from 15 to 40 seconds of VF (Figure 3B). This significant transmural gradient was also seen in the LVA and the RVB. This gradient increased as VF progressed during the first minute of VF (Figure 2B) and was maximal after 40 seconds of VF in the LVB, LVA, RVB, and RVA. The septum did not exhibit a significant DF gradient until after 20 seconds of VF. The electrode located at the LV endocardial surface of the septum activated faster than its immediate neighbor 25 seconds after VF induction. The distribution of DF across the wall did not significantly change during the first 65 seconds of VF. The distribution did change after 2 minutes of VF with the DF gradient reversing in the LV, RV, and septum. After 3 minutes, the endocardium exhibited a DF that was over 2 Hz faster than the epicardium in the LV and approximately 1 Hz faster in the RV. The septum was the slowest activating region for the first 25 seconds, but by 1 minute was not different in rate than the rest of the heart.

The mean DF from 5 to 45 seconds after VF initiation for all episodes for all animals was 10.1±0.87 Hz. The DFs at each of the regions and depths increased with time until 45 seconds, after which it decreased (Figure 3B).

**DPI**

**Pig**

There existed significant differences in DPI between various ventricular regions and depths throughout the first 3 minutes of VF. DPI decreased from the endocardium to the epicardium across the ventricular walls in the LV and the RV for the first 45 seconds and in the LV for the entire 3 minutes (Figure 5A). The middle of the septum exhibited significantly lower...
DPIs than did septal levels adjacent to the ventricular cavities except during 40 to 60 seconds of VF. The global mean DPI showed a continuous significant increase after 15 seconds of VF (Figure 6). The mean global DPI for the first 45 seconds of VF was 0.21 ± 0.078, ie, the electrodes recorded DPs 21% of the time. After 2 and 3 minutes of VF, the distribution of DPI across the LV continued to exhibit a significant decrease from endocardium to epicardium (Figure 5A). This distribution was not evident for the RV and the septum. The septum experienced a significantly higher DPI than the LVB. The mean DF at each level of each zone was negatively correlated with the corresponding DPI (A) and 5 dogs (B) were correlated with the corresponding DPI values to determine if activation rate correlated with incidence of block. Pigs: R = 0.31, P < 0.0001; dogs: R = 0.27, P < 0.0001.

**Figure 6.** Mean and standard deviation of global DPI at different VF durations for pigs (●) and dogs (○).

**Figure 7.** DF versus DPI for 45 seconds of VF. Average DF at each level for each zone for the epochs beginning 5, 10, 15, 20, 25, 30, 35, and 40 seconds after the onset of VF for the 6 pigs (A) and 5 dogs (B) were correlated with the corresponding DPI values to determine if activation rate correlated with incidence of block. Pigs: R = 0.31, P < 0.0001; dogs: R = 0.27, P < 0.0001.

discussion
The major findings of this study investigating transmural and global VF are as follows. (1) The distribution of activation rates estimated by DFs is not uniform across the ventricular wall in pigs and dogs. (2) A gradient of increasing activation rate from the endocardium to the epicardium of the swine LV exists, and this gradient becomes more prominent with the progression of VF. (3) The gradient of activation rates across the ventricles in dogs reverses from a faster LV epicardium early during VF to a faster LV endocardium after 2 minutes of VF. (4) The canine VF activation rate increases over the first 60 seconds and then decreases; the porcine activation rate decreases for 2 minutes but increases by 3 minutes of VF. (5) The distribution of conduction block estimated by DPs during VF is not uniform across the pig or dog hearts. (6) A gradient of decreasing estimated conduction block from endocardium to epicardium exists in the swine LV for 3 minutes but only for 1 minute in the dog before it reverses by 2 minutes. (7) A significant negative correlation between DF and DPI exists in the pig during the first 45 seconds of VF and a significant positive correlation between DF and DPI exists in the dog during the first 45 seconds of VF.

**DFs**
A significant gradient of activation rate existed across the ventricular walls, with the endocardial zones exhibiting lower frequencies than the epicardial zones during the first minute of VF in both the pig and the dog. Although this gradient persisted throughout the first 3 minutes of VF in the pig, it reversed by 2 minutes in the dog. Worley et al also showed a similar gradient of decreasing activation from endocardium to epicardium across the wall after 2 minutes of VF in dogs. Although not commented on in the text, Figure 2 of that article suggests that a gradient of increasing activation rate from endocardium to epicardium may have existed at 45 seconds, consistent with our results. The plunge needles of Worley et al were anchored on the endocardium so that the most endocardial electrodes were almost in direct contact with the endocardial surface. Our needles were fixed at the epicardium so that the epicardial most electrodes were 1 mm
from the epicardium, but the most endocardial electrodes bore no constant relationship with the endocardium and probably were not directly on the endocardium. Thus, we may have missed a rapid activation rate in dogs that is limited solely to the Purkinje fibers, because the Purkinje fibers are largely confined to the endocardium in this species.6

Cha et al10 previously reported that the endocardium activated faster than the epicardium after 1, 3, 5, and 10 minutes of VF in dogs. The difference was small after 1 minute of VF, but increased markedly after 3 to 10 minutes. Exposing the endocardium to air instead of blood did not alter this difference in activation rate, suggesting that increased \( \text{O}_2 \) in the subendocardium from blood in the ventricular cavity was not the cause of the rate difference. However, in dogs in which the Purkinje system had been ablated with Lugol’s solution, there was no significant difference between activation rates at the endocardium and epicardium.10 This suggests that the Purkinje system plays an integral role in maintaining activation during the later stages of VF in dogs because the faster activating endocardial region containing the Purkinje fibers may be the source of activation fronts that propagate into the slower regions toward the epicardium.11 Although the level of maximal DF had moved intramurally by 1 minute of VF in our study, only by 2 minutes was the endocardium the fastest activating region, which is consistent with the Purkinje fibers driving VF after 1 minute but a separate mechanism, such as reentry in the working myocardium, driving the earlier stages of VF.

The Purkinje fibers of pigs extend from the subendocardium almost to the epicardium,5,6 potentially influencing the effect of the Purkinje fibers on the transmural distribution of the VF activation rate in swine such that it differs from that in dogs. As in the dog, our data in pigs are consistent with the working myocardium maintaining VF for the first minute or so and the Purkinje fibers maintaining VF thereafter, although because the Purkinje fibers extend almost transmurally in swine, it is also possible that the Purkinje fibers are responsible for VF maintenance even during the first minute in this species. Berenfeld and Jalife11 found in a simulation study that the Purkinje fibers play an important role in the development of VF, but once intramural reentry has been established, they are no longer involved. Among other limitations, this simulation did not consider the effects ischemia may have on VF dynamics.

Another possible reason for the distribution of DFs is the heterogeneous distribution of ion channels and cell types across the ventricular wall. Ito has a greater expression in the epicardium than endocardium of humans.12 Ito has also been shown to have a gradient of expression from endocardium to epicardium in rats.13,14 ERG, a component of the delayed rectifier \( I_{\text{Ks}} \), has been shown to vary throughout the ferret ventricular wall with increased levels of expression at the epicardial layers.15 For a more complete review of the distribution of ion channels across the wall, see the review by Schram et al.16 During paced rhythm slower than VF activation rates, the endocardium has been shown to have longer ERPs than the epicardium and this distribution may be present during VF.17 The presence of M cells in the midmyocardial wall may also play a role in the transmural DF gradient,18 but the exact role is unclear as the longer action potential of the M-cell is lost at cycle lengths of less than 1 second.18,19 It is unlikely that differences in energy metabolites during VF influence the transmural DF gradient because Worley et al.20 showed in dogs that no transmural gradient of energy metabolites exists during the first 20 minutes of VF and our data were limited to the first 3 minutes of VF.

The septum of the pig exhibited an increase in DF in the center of the wall and a decrease toward both ventricular chambers. The dog never exhibited this distribution, although there was a tendency for the DF to increase toward the midseptal-RV endocardial regions after 25 seconds of VF. Thus, the activation rate decreases with proximity to the endocardium in both the ventricular free walls as well as the septum.

**DP Incidence**

This study is the first to estimate the distribution of the incidence of conduction block across the ventricular wall during VF. The LV transmural decrease in DPI from endocardium to epicardium is opposite to the transmural increase in DF from endocardium to epicardium, suggesting that conduction block is more likely to occur in the slower activating regions of the LV. This inverse relationship between the activation rate and the incidence of estimated conduction block during fibrillation has been reported in studies of ventricular epicardium20 and the atria.21 Zaitsev et al.22 proposed that as activations propagate from a region of higher activation rate to a region of lower activation rate, some activation fronts periodically block due to the presence of refractory tissue in the slower activating region, so that the borders between these regions of differing rate exhibit more block. They also reintroduced the idea that the fastest activating region contained a stable reentrant circuit, the mother rotor, which gave rise to daughter wavelets that propagated into the slower activating regions where they frequently blocked. The statistically significant inverse correlation we found between DF and DPI in pigs indicates that the degree of estimated conduction block increases in slower activating regions, as predicted by the mother rotor hypothesis. Although significant, the correlation was not strong in pigs and was reversed in dogs (Figure 7). Whereas study of the mother rotor in the guinea pig ventricle revealed relatively large domains of a single DF with the DF of the fastest domain as much as twice that of the slowest domain and with conduction block confined primarily to the boundaries between domains, in pigs and dogs, we found a much narrower range of DFs and a heterogeneous distribution of DFs and DPIs.

If a mother rotor does exist during the first minute of VF in either species, then our data suggest the LV epicardium would be the most likely place to find it because it is the fastest activating region. The fact that the faster activating region early during VF is on the epicardium means that the search for the mother rotor can be undertaken by optical or electrical mapping of the epicardium. However, a previous electrical epicardial mapping study did not find evidence for a mother rotor in this region, but instead found circumstantial evidence pointing to the septum as a possible site of mother rotor activity.22 Yet, our study did not find that the septum
activated faster than the LV, which would be expected if the septum were the site of the mother rotor. However, only three plunge needles were placed in the septum, so a faster activating region in the septum could have been missed. Indeed, another study found that the septum activated faster than the LV or RV free walls. The insertions of papillary muscles and trabeculae into the endocardial surface may also contribute to the increase in estimated conduction block at the endocardium. Reentry and increased wave splitting have been associated with the roots of papillary muscles. The high incidence of wave splitting at the insertions of papillary muscles and trabeculae may manifest as increased block in our experiment.

Limitations
The spacing between each electrode was approximately 1 cm in the RV and LV, so that the resolution was not sufficient to reconstruct activation fronts. Thus, instead of directly observing conduction block by mapping activation sequences, the occurrence of block was estimated by analyzing single electrograms. Although the method used to estimate conduction block has a high specificity, it has a moderate sensitivity; so some instances of conduction block were probably not detected by this method.

The use of plunge needles can damage tissue and disrupt the structure of the heart; however, the needles were thin, which may have limited the damage to the tissue.

The 2-kHz sampling rate was too slow to reliably detect Purkinje spikes. However, the small spikes occasionally seen preceding the activation complexes in the endocardial electrodes of dogs after longer VF duration (Figure 2B) raise the possibility that the Purkinje fibers are driving the working myocardium at this time.

Acknowledgments
This work was supported in part by NIH Research Grants HL-28429, HL-66256, and HL-67961.

References
Estimated Global Transmural Distribution of Activation Rate and Conduction Block During Porcine and Canine Ventricular Fibrillation
Jonathan C. Newton, William M. Smith and Raymond E. Ideker

Circ Res. 2004;94:836-842; originally published online February 5, 2004;
doi: 10.1161/01.RES.0000120860.01645.17
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/94/6/836