Organization of Myocardial Activation During Ventricular Fibrillation After Myocardial Infarction
Evidence for Sustained High-Frequency Sources

Stuart P. Thomas, BMEd, PhD, FRACP; Aravinda Thiagalingam, MB, ChB, FRACP; Elisabeth Wallace, BSc; Pramesh Kovoor, MD, PhD, FRACP; David L. Ross, MBBS, FRACP

Background—Studies of ventricular fibrillation (VF) in small mammals have revealed localized sustained stationary reentry. However, studies in large mammals with surface mapping techniques have demonstrated only relatively short-lived rotors. The purpose of this study was to identify whether sustained high-frequency activation with low beat-to-beat variability was present at intramural sites in a postinfarct ovine model of VF.

Methods and Results—VF was induced in 12 sheep 77±40 days after anterior myocardial infarction. Electrical activation was recorded with 20 multielectrode transmural plunge needles. Unipolar electrogram frequency content and local cycle duration variability were studied in 30-second recordings beginning 5 seconds after the onset of VF. Higher mean beat frequency was associated with lower SD of the cycle duration intervals (r = −0.91, P < 0.001). The mean beat frequency and the SD of cycle duration intervals of the highest-frequency electrode were 8.8±2.0 Hz and 17±11 ms. In 3 cases, a region with regular activation throughout the recording was identified (SD of the cycle duration interval, 6.0±0.7 ms). Two of these sites and 67% of all sites with low local cycle duration variability were intramural. They occurred within regions with a high dominant frequency as determined by fast Fourier transform of the unipolar electrogram.

Conclusions—Regions with the highest frequency of activation during VF were always associated with a low local cycle duration variability and usually intramural in this chronic infarct model. In a minority of cases, a region of stable, rapid, and very regular activation could be identified. These findings support the hypothesis that relatively stable periodic sources form a component of the mechanism of VF in this model. (Circulation. 2005;112:157-163.)

Key Words: arrhythmia ■ death, sudden ■ fibrillation ■ ventricles

Ventricular fibrillation is characterized by wave breaks and the formation of wavelets.1–4 This process results in irregular activation of the myocardium. This pattern results from several mechanisms. Myocardial activation and the corresponding characteristic surface ECG may be the result of multiple fleeting scroll waves,5,6 a single scroll wave, the core of which moves through the myocardium,7 or a fixed scroll wave activating the bulk of the myocardium via a series of wavelets formed by wave breaks.8,9 Technical limitations in the duration of recordings, spatial resolution of recorded data, the difficulty of recording intramural activity, and the difficulty of interpreting the large quantity of data have prevented investigators from determining which of these mechanisms is dominant in clinical ventricular fibrillation.

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An important characteristic of ventricular fibrillation is the considerable variability in the intervals between local myocardial activation times (cycle duration) on a beat-to-beat basis. Despite this variability (local cycle duration variability [LCDV]), a clear regional dominant frequency (DF) of activation has been identified in experimental ventricular fibrillation. Regions characterized by a dominant high frequency of activation correspond to rotors identified by activation mapping.10 Several studies have suggested that such rotors are transient and may not represent sustained sources responsible for the perpetuation of the arrhythmia. However, the distribution of phase singularities and therefore rotors appears to be anatomically determined.11 Failure to identify more sustained fixed rotor activity may be due to the techniques used to examine myocardial activation. More sustained rotors or scroll waves may exist deep in the myocardium in regions not examined by surface mapping of ventricular fibrillation.7

In the present study, we describe characteristics and distribution of LCDV during ventricular fibrillation in a myocardial infarction model of ventricular fibrillation. The incidence of ventricular fibrillation is increased by previous myocardial infarction.12 The mechanism of ventricular fibrillation onset in ischemic cardiomyopathy has been the subject of recent studies.13,14 We hypothesized that regions of reduced LCDV and high frequency of activation (beat rate) representing relatively fixed activation sources are present in...
an animal model of ventricular fibrillation after myocardial infarction. We further hypothesized that these regions of high-frequency, low-variability activation would be large enough to detect with a relatively small number of transmural electrodes. The purpose of this study was to describe the extent and characteristics of high-frequency, low-LCDV regions during experimental ventricular fibrillation.

**Methods**

**Infarct Induction**

Myocardial infarction was induced in 12 Wether sheep (45±6 kg) with the percutaneous technique described by Reek et al.15 Briefly, anesthesia was induced with intravenous thiopental (15 mg/kg) after premedication with intramuscular xylazine (0.1 mg/kg). The sheep were intubated, and anesthesia was maintained with isoflurane (1% to 3%) in 100% oxygen. A 6F guiding catheter was inserted into the right femoral artery and used to introduce an angioplasty wire and a 3.0-mm angioplasty balloon into the left anterior descending equivalent artery. Intravenous procainamide (1 g), metoprolol (5 mg), and verapamil (5 mg) were administered to reduce the incidence of ventricular fibrillation. The balloon was inflated in the mid left anterior descending artery for 180 minutes. The surface ECG demonstrated anterior ST-segment elevation. After deflation of the balloon, the sheep recovered and were administered sotalol 40 mg QID until 7 days before arrhythmia induction.

**Signal Recording and Processing**

After 77±40 days, the sheep were again anesthetized, and a left lateral thoracotomy was performed. The heart was suspended in a pericardial cradle, and an array of quadrupolar recording needles was inserted into the ventricular myocardium. The recording needles have previously been described.16 Briefly, each needle had a diameter of 0.8 mm and had 4 cylindrical electrodes 1.5 mm long and spaced at 1.5-mm intervals. The needles (20 per study) were inserted perpendicularly from the epicardial surface with a needle spacing of ~10 mm.

Unipolar electrograms were obtained with the chest clamp as the indifferent electrode. The electrograms were amplified and digitized with an acquisition frequency of 1 kHz (Pruka, Marquette). The high- and low-pass filters were set at 0.2 and 300 Hz, respectively. The data were subsequently exported to a custom-built signal analysis application (CEPAS, Madry Technologies). Activations were detected with a semiautomated technique. Activations were defined as the time of the nadir of the first derivative of the unipolar electrogram where the nadir fell below a preset threshold.17,18 The threshold was initially set at −0.1 V/s. The threshold was decreased to eliminate activations occurring within 50 ms to a minimum of −0.5 V/s. The activations were viewed manually with adjoining electrodes and calculated bipoles to eliminate activations resulting from artifacts. The resulting activation time data were used to study temporal changes in cycle duration (Figure 1). This representation of the data was referred to as the cycle duration series. The cycle duration series was analyzed with the indexes defined in Table 1. Some of the abbreviations defined are deliberately similar to those used for analysis of surface electrogram heart rate variability for ease of comprehension. A fast Fourier transform (FFT) was performed on the unipolar signal to determine the DF (DFFF). These data were compared with the DF of a cycle duration histogram constructed from the cycle duration series (DFhist; Figure 1).

**TABLE 1. Abbreviations and Indexes Used for Analysis of the Cycle Duration Series**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Cycle duration</td>
<td>Time between consecutive local myocardial electrical activation</td>
</tr>
<tr>
<td>LCDV</td>
<td>Variability in local cycle duration</td>
</tr>
<tr>
<td>n</td>
<td>No. of beats during the recording (30 s)</td>
</tr>
<tr>
<td>Beat rate</td>
<td>Beats per second over entire recording (30 s); n/30</td>
</tr>
<tr>
<td>DFhist</td>
<td>Frequency value (defined by central value) with highest frequency (occurrence) on cycle duration frequency histogram</td>
</tr>
<tr>
<td>DFFF</td>
<td>Frequency in the power spectrum of the unipolar signal with highest peak level</td>
</tr>
<tr>
<td>FF</td>
<td>Interval between 2 consecutive local ventricular activations measured from the same electrode (ms)</td>
</tr>
<tr>
<td>SDFF</td>
<td>SD of FF intervals from a single recording from a single electrode</td>
</tr>
<tr>
<td>Cycle duration series</td>
<td>Linear interpolation of measured tachycardia cycle durations charted against time to estimate temporal changes in instantaneous cycle duration</td>
</tr>
</tbody>
</table>

![Figure 1. Analysis of data from a single electrode. A, Unipolar signal. B, Marked activation times. C, Cycle duration series. D, FFT of unipolar signal (A). E, Frequency histogram of cycle durations from B. F, FFT of cycle duration series (C) representing frequency content of cycle duration variation over the duration of the recording. Example illustrates rapid series with DFFF of 10.2 Hz corresponding to mean cycle duration of 98 ms as seen in corresponding histogram.](image-url)
Induction of Ventricular Fibrillation
Ventricular fibrillation was induced by insertion of a transvenous electrode catheter into the right ventricular apex and programmed stimulation. Rapid pacing was performed with a 100- to 150-ms cycle length, 20-mA pulses, and 20-ms pulse duration. Recordings were started 5 seconds after the onset of ventricular fibrillation and continued for 30 seconds (Wiggers stage II). After completion of the study, the heart was removed, and the relative positions of the electrodes were identified and mapped. The hearts were examined macroscopically and microscopically to define the extent of myocardial infarction. The position of needles relative to the infarct scar was determined. Needles within 10 mm of the scar were considered to be at the periphery (peri-infarct zone) of the infarct.

Statistical Analysis
The Student t test was used for paired comparisons. The rank correlation coefficient was used to describe the relationship between beat rate and measures of beat-to-beat variability because those data were not normally distributed. A Bland-Altman analysis was used to assess the accuracy of activation detection. Continuous variables were expressed as mean±SD.

Results

Accuracy of Activation Detection
The accuracy of activation detection was checked by comparing DFFT and DHist. There was good correlation between them. The mean difference, SD of the difference, and mean error were 0.1 Hz, 1.6 Hz, and 12%, respectively. The minor residual error may be due to inherent errors associated with either technique and systematic errors associated with binning of the histogram data.

Identification and Characterization of Regions of High Activation Frequency and Low LCDV
There was an inverse relationship between beat rate and the SD of activation intervals (SDFF; Figure 2; r=-0.91, P<0.001; see Table 1 for a description of variables). Regions with a high frequency of activation had a lower beat-to-beat variability as determined by the SDFF. The typical patterns of distribution are illustrated in Figure 2. The SDFF may be influenced by the cycle duration, so this relationship was reassessed after correction of the SDFF for this variable (SDFF per cycle duration).

The SDFF was still inversely related to beat rate after this correction (r=-0.77, P<0.001).

In each sheep, a region of high frequency activation and low LCDV was identified. Characteristics of these regions are detailed in Table 2. They were widely distributed. However, in 9 of the 12 sheep, they were close to the interventricular septum. Eight sites were intramural, 3 were epicardial, and only 1 was endocardial. The mean frequency of activation or beat rate was 8.9±2.0 Hz (corresponding to a cycle duration of 120±32 ms) at the site of highest-frequency activation. The baseline ventricular effective refractory period was 232±35 ms.

In most recordings, there were pauses in otherwise relatively regular sequences of activation. These pauses represented failure to detect activations at the recording site that were likely to be due to failure of impulse propagation into the region adjacent to the recording electrode. In a small proportion of electrodes (1.3%), activation persisted at a regular rate throughout the recording. In these examples, none of the intervals varied from their predecessors by >50 ms, and the SDFF was <10 ms. Such regions were identified in 3 of the 12 sheep. The SDFF at these sites was 6±1 ms. These sites also corresponded to regions of high DFFT (Figure 3). In sheep 2, 4, and 5, we found that 1, 6, and 7 electrodes, respectively, had a very regular activation pattern, with no interval differing from its predecessor by >50 ms. In the groups of electrodes from sheep 4 and 5, there was 1:1 activation throughout the recording, corresponding to activation of the electrode with the lowest SDFF. In these 2 sheep, the groups of electrodes sharing 1:1 activation throughout the recording were adjacent. In sheep 4, they included an entire transmural needle and the endocardial pair of an adjacent needle (Figure 4). In sheep 5, they consisted of the most epicardial electrodes of 3 closely spaced plunge needles. The numbers of recorded beats in these examples were identical, suggesting 1:1 conduction within these small muscle volumes (~1 cm³). The unipolar recording with the lowest SDFF in this group is illustrated in Figure 5.

In the first 8 sheep, the frequency content of the cycle duration series was examined in the range of 0.15 to 2.5 Hz (Figure 1F). This is another method of describing the LCDV.
TABLE 2. Description of the Site of Lowest LCDV and Highest Beat Rate for Each VF Episode

<table>
<thead>
<tr>
<th>Site</th>
<th>Frequency, Hz</th>
<th>SDFF, ms</th>
</tr>
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<tbody>
<tr>
<td>1 Posterior basal septum/intramural/nonscar</td>
<td>10.2</td>
<td>24</td>
</tr>
<tr>
<td>2 Margin left ventricle/intramural/nonscar</td>
<td>10.2</td>
<td>6</td>
</tr>
<tr>
<td>3 Inferoapical/intramural/per-infarct</td>
<td>9.5</td>
<td>20</td>
</tr>
<tr>
<td>4 Posterior mid septum/intramural/nonscar</td>
<td>7.7</td>
<td>7</td>
</tr>
<tr>
<td>5 Apical/endocardial/per-infarct</td>
<td>5.9</td>
<td>5</td>
</tr>
<tr>
<td>6 Mid anterior septum/intramural/per-infarct</td>
<td>10.6</td>
<td>12</td>
</tr>
<tr>
<td>7 Mid acute margin/epicardium/nonscar</td>
<td>10.0</td>
<td>13</td>
</tr>
<tr>
<td>8 Mid posterolateral/epicardium/nonscar</td>
<td>12.2</td>
<td>11</td>
</tr>
<tr>
<td>9 Anterior septum/epicardium/nonscar</td>
<td>9.0</td>
<td>11</td>
</tr>
<tr>
<td>10 Posterior apical septum/intramural/nonscar</td>
<td>8.0</td>
<td>36</td>
</tr>
<tr>
<td>11 Anterior septum/intramural/scar</td>
<td>5.4</td>
<td>39</td>
</tr>
<tr>
<td>12 Anterior septum/intramural/per-infarct</td>
<td>6.9</td>
<td>17</td>
</tr>
</tbody>
</table>

It enabled us to determine the frequencies of cycle duration oscillation. As expected, the sites with the lowest SDFF had the lowest variability as assessed by the total spectral power (569 versus 54,220 U²Hz; P=0.001). Closer analysis of the distribution of spectral power demonstrated a change in frequency content between the sites of low SDFF and other sites. Sites with lowest SDFF were characterized by relatively larger high-frequency variations in the cycle duration series (range, 0.4 to 2.5 Hz). The proportion of power in the high-frequency range was 0.67±0.15 compared with 0.31±0.15 for other sites (P<0.001).

**Activation Sequence Mapping**

In the 2 examples with >1 electrode sharing high-frequency 1:1 activation, we examined the activation sequence throughout the recording. The activation times were charted relative to the electrode with the lowest SDFF. The results are illustrated in Figure 6. The activation sequence remained stable throughout most of the recording, but there was variability in the relative timing of activation. In the example shown in Figure 6A, there is a change in the earliest electrode 32 activations into the recording. This lasts for only a single beat. We examined the signals at this perturbation; they are shown in Figure 6B and 6C. The change in activation sequence is associated with an oscillation in the cycle duration series and an altered morphology of the extracellular potential from the reference electrode and electrode B1. The recording from electrode B1 gradually develops a lower-frequency downsloping segment, resulting in a relatively small reduction in cycle duration for a few beats. This suddenly corrects, with a steep downsloping segment and a corresponding prolongation in the cycle duration for a single beat. The second example (not illustrated) was more complex. Again, there was consistency of the activation sequence throughout most of the recording. However, at 1 point, there was a brief period of turbulence in the cycle duration series, resulting in dramatic changes in the relative activation sequence. After the period of turbulence, the pattern of activation resumes in an orderly sequence, with remarkably small variability in relative activation times but a new activation sequence.

**Discussion**

The main findings of this study were that regions characterized by the highest frequency of local activation during ventricular fibrillation were regions with the lowest beat-to-beat variability in the local activation time. This pattern of activation was stable at these high-frequency sites for the duration of the recordings. Thus, regions of stable, nearly regular activation exist within the myocardium during sustained ventricular fibrillation. This pattern of activation may represent activity in or in close proximity to rotors responsible for the maintenance of ventricular fibrillation. Other sites were characterized by relatively lower beat rates and higher LCDV. These findings lend support to the hypothesis that a relatively stable periodic source with fibrillatory conduction to the remainder of the ventricle is the mechanism for ventricular fibrillation in this model. Identification of regions of high frequency and low LCDV may be helpful for identifying critical rotors responsible for maintenance of ventricular fibrillation.

**Measures of LCDV**

The measures of LCDV in the present study are each attempts to describe the curve of the cycle duration series. This series is an interpolated estimation of the cycle duration at any point in time within the recording. Perhaps the most obvious measure of LCDV is the SDFF. The very sensitive SDFF is able to detect minor differences in LCDV between apparently very regular series. It is also very sensitive to gaps in the series created by failure of propagation into the recording site or failure to detect an underlying activation. Other indexes...
that may be useful for detecting high-frequency activity include the DF$_{hist}$ and DF$_{FFT}$. These measures are less likely to be influenced by occasional errors in detecting a local activation. However, intermittent failure of propagation to an otherwise regular site suggests that the recording site is not driving the arrhythmia. Frequent errors in the detection of beats in an otherwise regular series or intermittent entrance block to a recording site would produce a harmonic in the cycle duration histogram. The frequency of the cycle duration series (Figure 1F), representing the pattern of cycle duration variation over time, was also useful in identifying regions of low LCDV. Cycle durations at regions with high regularity tended to oscillate at higher frequencies compared with other sites. Thus, a combination of measures rather than a single index may be appropriate for examining LCDV.

**Spatial and Temporal Stability of DFs During Ventricular Fibrillation**

Previous studies of activation frequency detected by analysis of the transmembrane action potential have indicated clear spatial gradients in the frequency of activation during ventricular fibrillation. These studies concentrated on short periods of arrhythmia. Newton et al., using a different methodology and longer recordings, also demonstrated spatial gradients in DFs from epicardium to endocardium. The regularity of high-frequency activation at the sites of high DF has not been examined in detail. Several studies have demonstrated that organized patterns of activation may be nonstationary and relatively fleeting. Choi et al demonstrated that the DF at any particular site may change rapidly with time. They emphasize the importance of considering variations in the frequency of activation over time. Although Zaitzev et al found examples with clear spatial gradients of DF, they also found that the regions of similar DF were relatively small (1.1 cm$^2$) and may be separated from each another. This degree of DF heterogeneity may appear relatively organized in a small isolated tissue fragment but translates to a more complex picture in the whole heart. Our finding of complex spatial gradients of dominant activation frequency supports the findings of Zaitzev et al and emphasizes the importance of structural complexity and functional variations in conduction over time. In the present study, most recording sites were characterized by marked variation in cycle durations. However, we were able to demonstrate that the degree of cycle duration variability is also spatially distributed and that regions of comparative stability can often be detected.

**Significance of Sites With High Frequency of Activation and Low LCDV**

Regions of high DF may play an important role in the maintenance of ventricular fibrillation. Chen et al and Samie et al from the same laboratory found that high-frequency periodic sources underlie ventricular fibrillation in the iso-
Defining Local Activation

An important component of this study was obtaining intramural activation data. Unipolar electrograms were used to minimize the influence of directionality on electrogram characteristics. It is well established that the minimum slope of the extracellular unipolar electrogram corresponds to the local activation time when the activating wave front is planar.\(^{18,26,27}\) Determining the time of local activation during ventricular fibrillation with unipolar electrograms may be problematic because electrotonic interactions between the recording area and adjacent myocardium may confound the usual relationship between transmembrane and extracellular voltage changes.\(^{28,29}\) Furthermore, the relationship between these electrotonic influences and local activation is complex because of the nonconcordance of activations in regions located very close. As a result, the signals were more likely to be influenced by activation of neighboring myocardium. To partially overcome this problem, the electrograms were viewed with a neighboring unipolar electrogram and a calculated bipolar electrogram. The presence of simultaneous deflections in adjacent unipolar signals with an absence of a bipolar signal suggests that the deflection may not represent local activity. Errors may occur when the 2 electrodes are activated simultaneously by a planar wave front. Such errors were less likely when activation was predictably periodic. Therefore, this problem of inaccuracy in determining the activation time is less likely to occur at the site of regular periodic activation.

Transmural Differences in LCDV

Mapping of ventricular fibrillation with multielectrode arrays is difficult even when large numbers of electrodes are used. To overcome this problem, researchers have used either larger numbers of electrodes or optical mapping techniques that allow a very high density of recording sites. These techniques are limited by the need to study surfaces. intact or cut. Detailed mapping of activation patterns would not be possible with the number of plunge electrodes used in the present study. However, we have demonstrated that a relatively small number of electrodes may be useful for identifying regions of high-frequency activation.

Most studies of ventricular activation during ventricular fibrillation have recorded either endocardial or epicardial activity. Little is known of the intramural activation pattern during this arrhythmia.\(^{24}\) Zaitsev et al\(^{22}\) demonstrated there is no or poor correlation between epicardial and endocardial dominant frequencies of activation. More recently, Newton et al\(^{23}\) demonstrated gradients in activation rates from the faster epicardium to the slower endocardium.\(^{23}\) This finding suggested that study of endocardial or epicardial activation patterns may not be sufficient for identifying high-frequency sources of ventricular fibrillation if indeed they are present. Study of cut slices may also be misleading because of the boundary effects created by interrupting the tissue. In many of the examples from the present study, the region with the lowest-LCDV and highest-frequency activation was an intramural site. Therefore, more detailed intramural mapping techniques are required to confirm that the patterns of regular periodic activation detected in the present study are due to stationary or nearly stationary intramural scroll waves.
References

CLINICAL PERSPECTIVE

Ventricular fibrillation is the most common cause of sudden cardiac death, but the actual mechanisms that maintain the characteristically rapid irregular ventricular activation have not been fully defined. High-frequency depolarization of the ventricles may originate from multiple fleeting wavelets or rotors that form scroll waves in 3 dimensions or by a single scroll wave that meanders through the myocardium. Alternatively, wave breaks from a single stable source may generate daughter wavelets that activate the bulk of the ventricles. Knowledge of the underlying mechanism may permit modification of the substrate to prevent the arrhythmia. In large animals and humans, studies of electrical activation at the heart surface may fail to show sources of rapid activation that are concealed within the ventricular wall. In the present study, needle electrodes were used to survey the ventricular myocardium during ventricular fibrillation induced in sheep with chronic infarction. Sites with the highest frequency of activation during ventricular fibrillation also had relatively regular activation, consistent with a source-driven fibrillation. Most of these sites were intramural and present intermittently during the recording period. Regions of stable, rapid, and very regular activation throughout the recording period that suggested a stable driving source were observed in only 3 of 12 animals. These findings support the hypothesis that sources of relatively regular, rapid activation contribute to the maintenance of ventricular fibrillation. In this model, these sources are often intramural in location, where they would escape detection by most mapping methods.
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