Wavebreak Formation During Ventricular Fibrillation in the Isolated, Regionally Ischemic Pig Heart

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Abstract—Both fixed and dynamic heterogeneities were implicated in the mechanism of wavebreak (WB) generation during ventricular fibrillation (VF). However, their relative roles remain unclear. We hypothesized that during ischemic VF, the WBs are produced primarily because of a fixed heterogeneity; namely, the gradient of refractoriness across the ischemic border zone (BZ). Ischemia was induced in 15 isolated blood-perfused hearts by occluding the left anterior descending coronary artery. Simultaneous video imaging (≈32×32 mm²) of Di-4-ANEPPS fluorescence in the ischemic zone (IZ), the BZ, and the nonischemic zone (NIZ) was performed. Dominant-frequency maps were constructed to assess gradients of refractoriness during VF. We used singularity points analysis to quantify the incidence of WBs per square centimeter per second. During preischemic VF, the distribution of WBs was relatively uniform. Ischemia caused an increase of WBs in the BZ (from 6.2±2.8 to 10.8±4.0) and a decrease of WBs in the IZ (from 5.8±2.8 to 2.8±1.4), without a significant change in NIZ (from 6.4±2.3 to 4.1±1.7). This finding is fully consistent with the dominant-frequency distribution during ischemic VF: the average dominant frequency was significantly slower in IZ than in NIZ (7.8±0.7 versus 10.1±1.0 Hz), suggesting a large gradient in refractory periods across the BZ. We concluded that acute regional ischemia plays a dual role in the maintenance of VF, decreasing the incidence of WB in the IZ while increasing it in the BZ. This suggests a predominant role of fixed heterogeneities in the formation of WB during VF in acute regional ischemia. (Circ Res. 2003;92:546-553.)

Key Words: ventricular fibrillation • myocardial ischemia • optical mapping • wavebreak

Acute myocardial ischemia is a major cause of VF in humans. Although metabolic, electrophysiological, and structural changes related to ischemia have been studied extensively, the role these alterations play in the development and maintenance of ventricular fibrillation (VF) remains unclear. Janse et al showed that a transition from ventricular tachycardia to VF was related to fragmentation of waves in the ischemic zone (IZ). In contrast, Rankovic et al argued that the ischemic region acted as a bystander in the maintenance of VF, because it did not affect activation rate or wave organization in the peri-ischemic region. Furthermore, global ischemia seems to impede the fibrillatory activity.

The hallmark in the mechanism of maintenance of VF is wavebreak leading to wavelet formation. Recent studies in normally perfused ventricular tissues emphasized the role of dynamic changes in action potential duration (APD) as a function of the preceding diastolic interval (APD restitution) in the mechanism of VF. According to the so-called “restitution hypothesis,” a steep (>1) slope of the APD restitution relation would produce divergent fluctuations of APD, and wavebreaks would occur as short wavefronts collide with the tails of preceding longer waves. However, the steepness of APD restitution is decreased rather than increased during acute myocardial ischemia. Hence, to reconcile the restitution hypothesis with the pathophysiology of ischemia, Xie et al proposed that acute ischemia promotes wave breakup in the normal rather than in the ischemic tissue because of acceleration of the reentrant activity in or around the ischemic region. However, such predictions conflict with available experimental results, which show a deceleration of the fibrillatory activity after ischemia.

Here, we analyzed the incidence and spatial distribution of wavebreaks during VF in a regionally ischemic heart. We sought to address two major questions: first, whether acute regional ischemia promotes or impedes the fibrillatory process, ie, formation of wavebreaks, and second, whether the restitution hypothesis is operative in the setting of VF associated with acute myocardial ischemia.

Materials and Methods

Isolated Heart Preparation

Pigs of either sex (n=15) were anesthetized with pentobarbital 20 mg/kg IV. After isolation via midline sternotomy, the heart was perfused in a Langendorff apparatus with a mixture of oxygenated (37±0.5°C) blood and Tyrode’s solution collected from the same animal. All the outflow of perfused blood was collected for...
recirculation (Figure 1A). The heart was superfused with warm oxygenated Tyrode’s solution.\textsuperscript{16}

The left anterior descending coronary artery (LAD) was occluded to induce regional ischemia. The recording camera’s field of view was adjusted so as to have equal areas on both sides of the ischemic margin (Figure 1B). The border zone (BZ) was defined as an 8-mm-wide strip of myocardium along the ischemic border.\textsuperscript{13} The IZ and NIZ were defined, respectively, as the portions of ischemic and nonischemic tissue in the field of view exclusive of the BZ (Figure 1B).

**Optical and Microelectrode Recordings**

We recorded the fluorescence of di-4-ANEPPS using a CCD camera as described elsewhere.\textsuperscript{17} The diameter of the field of view was \( \approx 32 \) mm (64\( \times \)64 pixels) and included approximately equal portions of the IZ, BZ, and NIZ (Figure 1B). Transmembrane potentials (TMPs) were recorded from the IZ by use of floating glass microelectrodes filled with 3 mol/L KCl.

**Experimental Protocols**

In 7 animals (animals 1 to 7), VF was induced electrically (with a 9-V DC battery) and recorded optically 5 minutes before and 15 minutes after LAD occlusion. Each episode of VF lasted <3 minutes and was terminated by a noncontact defibrillation shock. If spontaneous VF occurred during 15 minutes of ischemia, it was also recorded optically and then terminated by a defibrillation shock. In one experiment (experiment 8), VF was started 5 minutes before LAD occlusion and maintained for 15 minutes thereafter. Optical recordings were taken at 2-minute intervals. In a separate series of 5 experiments (experiments 9 to 13), TMPs were recorded from cells in the IZ during control and ischemic VF.

Two additional experiments were performed to ensure the stability and reproducibility of excitation frequency during VF in a normally perfused heart. In experiment 14, VF was induced electrically, recorded optically, and terminated by an electric shock at \( \approx 15 \)-minute intervals over a period of 60 minutes. In experiment 15, VF was induced electrically and maintained for 60 minutes, whereas optical recordings were taken every 5 minutes.

**Data Analysis**

Our previous publications describe DF mapping,\textsuperscript{18} time-space plots (TSP),\textsuperscript{19} and singularity point (SP) analysis\textsuperscript{20} in detail. Local DF was used as an estimator of VF cycle length (VFCL). The latter was shown to correlate with local refractory period duration.\textsuperscript{21} Trajectories of individual SPs were tracked over 500 consecutive frames (1.65 seconds). A starting point of an SP trajectory was considered to be the site of a new wavebreak. The average number of wavebreaks per square centimeter per second (wavebreak density) was determined for each optical recording of VF.

TMPs were analyzed in 12.288-second segments of VF recordings. \( \text{APD}_{90} \) was calculated as the temporal difference between the point of maximum slope (\( \text{dV/dt}_{\text{max}} \)) and the point of 90% repolarization.\textsuperscript{22} Diastolic interval (\( \text{DI}_{90} \)) was defined as VFCL − \( \text{APD}_{90} \).\textsuperscript{23} For each recording, the average values of CL, \( \text{APD}_{90} \), \( \text{DI}_{90} \), action potential amplitude, and \( \text{dV/dt}_{\text{max}} \) were calculated. In addition, the SD of CL (SD\text{CL}), \( \text{APD}_{90} \) (SD\text{APD}_{90}), and \( \text{DI}_{90} \) (SD\text{DI}_{90}) were computed. The ratio SD\text{APD}_{90}/SD\text{CL} was used as an estimate of the dynamic restitution relationship during VF.\textsuperscript{8} The dynamic restitution plots were fitted by a single exponential function.

**Statistical Analysis**

Data are given as mean ± SD. Paired \( t \) test and two-way ANOVA with a Bonferroni post hoc test were used as appropriate; a value of \( P \leq 0.05 \) was considered statistically significant.

An expanded Materials and Methods section can be found in the online data supplement available at http://www.circresaha.org.

**Results**

In normally perfused hearts, the DF of excitation during VF was stable and relatively uniform over the mapped area, as can be seen from Figures 1C and 1D. In Figure 1C (experiment 14), VF was continuous for 1 hour. In Figure 1D (experiment 15), VF was induced repeatedly and terminated by a defibrillation shock over the same period of time. In either case, both the temporal variation and spatial dispersion of the DF over the mapped were within 13% of their average values.

**Frequency Analysis of VF in Regionally Ischemic Heart**

Acute regional ischemia dramatically affected the distribution of DF during VF. Figure 2A shows that during the control (preischemic) VF episode, the spatial distribution of the DF

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\( \text{SD}_{\text{APD}_{90}}/\text{SD}_{\text{CL}} \)

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\( \text{DI}_{90} \)

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\( \text{dV/dt}_{\text{max}} \)

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\( \text{SD}_{\text{CL}} \)

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\( \text{SD}_{\text{APD}_{90}} \)

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\( \text{SD}_{\text{DI}_{90}} \)

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\( \text{VFCL} \)

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\( \text{VFCL} \)
was relatively uniform (see also power spectra of points a and b in Figure 2C) and random. Most of the mapped area exhibited DFs between 9.4 and 10.7 Hz. In contrast, as shown in Figure 2B, during ischemic VF there was an abrupt transition in DF exactly at the BZ, leading to a bimodal distribution of DFs (see also Figure 2D). Although the DFs were virtually unchanged in the NIZ, in the ischemic tissue, the DFs were reduced by 20% to 25%.

As in the episode shown in Figures 2A through 2D, in all induced ischemic VF episodes (n=6), there was an abrupt change in DF of excitation at the BZ, and the average DF was always lower in the IZ than in the NIZ. The statistical analysis of average DF and DF dispersion in the IZ, BZ, and NIZ during control and ischemic VF is illustrated in Figures 2E and 2F. Collectively, these data indicate a consistent presence of a unidirectional gradient of excitation frequency during ischemic VF, with the largest change occurring in the BZ.

Spatial Distribution of Wavebreak Density

The results presented above led to the hypothesis that the observed distribution of refractoriness revealed by the excitation DF gradients should translate into spatially nonuniform incidence of wavebreak. From our previous study,18 we expected an increased wave fragmentation in the area of the largest gradient of refractoriness, which seemed to coincide with the BZ. Therefore, we used phase analysis20 to reveal the SPs and quantify the incidence of broken waves.20 Figure 3 illustrates an example of a relationship between distribution of DFs and wavebreaks. Figure 3A shows a typical DF map obtained during electrically induced ischemic VF, with a sharp gradient of DF across the BZ. Figure 3B shows a TSP that allows visualization of the propagation pattern for the activation along the line x-x' crossing the BZ during 1 second of activity. The TSP reveals an intermittent phase discontinuity across the BZ. Some of the wavefronts moving from the NIZ to the IZ pass the border uninterrupted (eg, NIZ wave 1); others form a Christmas tree–like pattern characteristic of a spiral wave19 (eg, NIZ waves 2 to 4); still others are completely blocked (eg, NIZ waves 9 and 10). The overall complexity of the pattern at the BZ prevents us from labeling it a Wenckebach-like pattern, but the net ratio of the number of waves approaching the BZ (from nonischemic tissue) and those making it inside the IZ is 12/9, or 4/3. This explains the gradient of the excitation frequency reflected in the DF map (Figure 3A).

Phase discontinuities observed in TSPs imply predominant breaking of waves at the BZ. In Figure 3C, selected snapshots of phase movies from the same VF episode show that this is actually the case. White circles indicate the positions of SPs that correspond to the tips of broken waves. The white lines encompass the BZ as defined in Materials and Methods. Wavebreaks were found more often within or close to the BZ than the IZ or NIZ. In Figure 3D, we have superimposed the starting points (red) and trajectories (thin black lines) of all SPs during 1.65 seconds of VF. The vast majority of starting points are clustered in the BZ. Interestingly, the trajectories of SPs that originated within the BZ were also confined predominantly to the BZ.
The relation between wavebreak formation and DF gradient is shown in Figure 4. Here, the starting points of SP trajectories are superimposed on the same DF map as shown in Figure 2. Figure 4A shows a control (preischemic) VF episode. The spatial distributions of both the DF and wavebreaks were relatively uniform and random. In contrast, as shown in Figure 4B, during ischemic VF, an increased formation of wavebreaks was observed at the BZ, the area of the largest DF gradient. Under these conditions, the IZ was depleted of wavebreaks. In Figure 4C, analysis of 6 experiments demonstrated that ischemia caused a significant increase in wavebreak density in the BZ but a significant decrease in wavebreak density in the IZ.

**Spontaneous Ischemic VF and the Evolution of DF Gradient**

In 7 experiments, there were two spontaneous VF episodes that occurred in two different hearts between 3 and 6 minutes after LAD occlusion. The incidence and timing of these VF episodes were similar to those observed by Janse et al\(^4\) in a similar experimental model. In each case, there was almost no difference in average DF between IZ and NIZ during control (preischemic) VF; there was a large difference (IZ DF > NIZ DF) during ischemic VF (Table).

Interestingly, the magnitude of the DF gradient across the BZ was larger in spontaneous ischemic VF episodes than in induced ischemic VF episodes (see Figure 2E). To determine whether this difference was a result of the mode of VF initiation or the time elapsed after LAD occlusion, in a separate experiment (experiment 8) we maintained VF throughout 15 minutes of ischemia while recording movies at 2-minute intervals. The evolution of the normalized DF gradient across the BZ is shown in Figure 5. It is seen that the magnitude of the DF gradient reached its maximum at minute 5 of ischemia and then decreased gradually for up to 15 minutes. In two experiments (12 and 13), we maintained a stable TMP impalement over the first few minutes of ischemia in a cell at the center of the IZ (circles and triangles in Figure 5, respectively). The time course of the excitation frequency in these cells, measured as 1/CL of the TMP recordings, almost exactly matched the time course of the average DF in the optical IZ data from experiment 8.

Together, the data presented above suggest a biphasic time course of excitation frequency inside the IZ during VF, with a maximum slowing at 3 to 5 minutes and partial recovery between 5 and 15 minutes of ischemia. The magnitude of DF gradient is most likely independent of the mode of VF initiation. At no time after LAD occlusion did we observe any acceleration of VF rate either inside or outside the IZ.

**Cellular Activity in the Ischemic Region During Ischemic VF**

The purpose of the microelectrode study was 2-fold: (1) to validate the frequency analysis of the optical data and (2) to assess the applicability of the dynamic restitution relationship\(^5\) to preischemic as well as ischemic VF. Because maximum DF reduction occurred at 3 to 5 minutes of ischemia (see Figure 5), we selected for analysis the TMP recordings obtained from the center of IZ during this time interval.

Figures 6A and 6B show representative TMP recordings during preischemic and ischemic VF, respectively. The most striking difference between the two is the rate of activity (ischemic 1/CL \(\ll\) preischemic 1/CL) and the appearance of diastolic intervals, which were virtually absent during preischemic VF. In Figure 6C, the respective power spectra are superimposed. In this and all other experiments, the DF during ischemic VF was consistently lower than during preischemic VF. The power spectrum during ischemic VF was usually broader than during control VF, which correlated with an increase in variability of the CL. Accordingly, the correlation between the DF and 1/CL during control VF was better than that during ischemic VF ($r^2=0.98$ versus $0.82$, respectively). However, a clear separation between the main frequency content of the control and the ischemic spectra shows unequivocally a deceleration of VFCL in the IZ.

Figures 6D and 6E show the plots of the dynamic APD restitution relationship (APDR) during control and ischemic VF, respectively. One can see that the control APDR plot is a tight cluster and the ischemic APDR plot is a scattered
cluster of points, rather than a functional relationship. In neither case were we able to obtain a reasonably good fit of these distributions with exponential functions. Analysis of all 5 control and 5 ischemic TMP recordings showed $R^2 < 0.25$. Therefore, the maximum slope of APDR during VF cannot be measured reliably. Nonetheless, it is clear that the distributions of APD and DI are markedly different during control versus ischemic VF. First, the average DI90 is much longer during ischemia than in control. Second, the average APD90 is also longer in ischemic tissue. Finally, the ratio of dispersions, $SD_{APD}/SD_{DI}$, is much larger in control than ischemia.

The statistical analysis of TMP data from the IZ during control and ischemic VF in 5 experiments is presented in Figure 7. The VFCL was significantly longer during ischemia than in control, which was a result of slight (but significant) prolongation of APD and a very significant prolongation of DI. There was a tendency for an increase in CL dispersion ($SD_{CL}$) during ischemia, but it was not significant ($P=0.062$). Not unexpectedly, action potential amplitude and $dV/dt_{max}$ were also reduced during ischemia. Finally, the ratio $SD_{APD}/SD_{DI}$ was significantly lower during ischemia than control. Please note that this ratio was >1 in control and <1 during ischemia.

**Discussion**

The main findings of this study are first, that regional ischemia has both profibrillatory and antifibrillatory effects in the isolated, blood-perfused pig heart; that is, ischemia increases wavebreak incidence at the BZ while at the same time decreasing it inside the IZ. Second, at the cellular level, the main electrophysiological effect of ischemia in VF is widening of the diastolic intervals, which points to an important role of postrepolarization refractoriness in the mechanism of ischemic VF.

**Role of Acute Regional Ischemia in the Maintenance of VF**

In agreement with the report by Rankovic et al,5 we found that acute regional ischemia slows VF frequency within the IZ without significant change in the NIZ. Using linking analysis,5 these authors did not find any significant change in wavefront organization during VF after ischemia and conjectured that the IZ does not play a major role in VF maintenance. Using a different quantitative approach, namely, the analysis of instantaneous phase singularities,20 we did find a significant local increase in a wavebreak incidence at the BZ while at the same time decreasing it inside the IZ. Second, at the cellular level, the main electrophysiological effect of ischemia in VF is widening of the diastolic intervals, which points to an important role of postrepolarization refractoriness in the mechanism of ischemic VF.

<table>
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<tr>
<th>Average DF of Excitation in IZ and NIZ During Control VF Versus Spontaneous Ischemic VF</th>
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<tr>
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collateral circulation, which may reduce the steepness of the electrophysiological gradients across the BZ.

The DF gradient observed during ischemic VF is most likely determined by a large [K⁺]o gradient across the BZ. The center of the IZ, the level of [K⁺]o, usually reaches a plateau at 5 to 10 minutes of ischemia. In the BZ, however, a noticeable recovery of [K⁺]o may occur as a result of diffusion and washout of potassium ions across the BZ. Remarkably, the time course of VF gradient across the BZ in our experiments matches the biphasic dynamics of [K⁺]o in the BZ very well. This implies that the steepness of the [K⁺]o gradient across the BZ may be more important for the frequency transformations and wave fragmentation than the absolute difference in [K⁺]o between the IZ and NIZ.

Although we did not study the mechanisms of VF onset, we hypothesize that in the presence of regional ischemia, VF starts as the result of the establishment of a steep gradient of refractoriness, which sets the stage for recurrent wavebreak and promotes fragmentation of wavefronts that move into the BZ. In fact, the data presented by Janse et al in their classic article provided evidence for that. During the first few beats of an episode of spontaneous VF, additional reentrant circuits form inside but very close to the border of the IZ (see their Figure 10). As VF progresses, however, the reentrant source(s) presumably move into the NIZ, because the wavefronts tend to propagate predominantly from the NIZ toward the IZ (see Figure 3C). Although we did not see stable reentrant sources in the mapped area, our results are consistent with the source(s) located elsewhere in the nonischemic tissue. However, even the most stable sources are not perpetual; therefore, we conjecture that the BZ contributes to maintenance of ischemic VF, providing a ready replacement in case the dominant source(s) cease to exist.

Role of Heterogeneities in VF

The relative contribution of fixed versus dynamic heterogeneities in the mechanism of VF remains a matter of debate. Regional myocardial ischemia is a clinically relevant condition in which very large macroscopic heterogeneities occur naturally. We believe that the size and the organization (ie, microscopic versus macroscopic) of the preexisting heterogeneities do matter. Although random microscopic heterogeneities may be overridden by dynamic changes during VF, large and macroscopically organized heterogeneities (eg, across ischemic BZ) between left and right...
right ventricle in the guinea pig heart, between the left and the right atrium in different species may determine the global organization of fibrillation in terms of predominant localization of the source(s) and the wavefront traffic. This assumption can be substantiated further in experiments in which the position and magnitude of the gradient are under the experimenter’s control. It should also be noted that steep gradients in refractoriness may enhance shock-induced arrhythmogenesis during myocardial ischemia, which may contribute to an increased rate of defibrillation failure in ischemic hearts.

Restitution Hypothesis and Ischemic VF
Although acute ischemia promotes VF, it flattens the restitution curve. So, how does the restitution hypothesis apply to ischemic VF? Xie et al proposed that acceleration of spiral-wave frequency in the IZ drives the normal tissue into a “dangerous” range of short DIs associated with a steep slope of APD restitution and increased wave breakup. Alternatively, they proposed that “an appropriately sized” ischemic region that has become completely unexcitable can promote VF by anchoring a spiral wave in the normal tissue and accelerating its rate through electrotonic interactions. Because the size of the ischemic area in our experiments was too large to anchor a spiral wave, the second mechanism is not applicable. For the first mechanism, shortening of the effective refractory period after APD abbreviation is a critical prerequisite. In general, elevation of [K+]tive potential (postrepolarization refractoriness). In our model, acceleration of VF was not observed either inside or outside the ischemic region during the first 15 minutes of ischemia. This implies that the postrepolarization refractoriness outweighs the APD abbreviation from the very onset of ischemia.

Another prediction by Xie et al is that regional ischemia would lead to break-up in the normal tissue well away from the zone of maximum gradient. In our study, however, the maximum density of wavebreak during ischemic VF was observed at the BZ but not in the normal tissue (see Figure 5).

From our microelectrode data, it is not possible to draw any definitive conclusions about the role of the APDR slope in wave dynamics during ischemic VF. We were unable to obtain good fits for APDR curves (see Figure 6). This implies that, in addition to the preceding DI, other factors influence APD during VF. The ratio SDAPD/SDDI may be a more robust quantitative parameter for APDR during VF. The observed decrease in the ratio SDAPD/SDDI in the ischemic region (see Figures 6 and 7) is compatible with the flattening of the restitution relationship reported by others. It implies that small fluctuations of DI lead to large fluctuations of APD during control VF, whereas large fluctuations of DI lead to small fluctuations of APD during ischemic VF. Only in this sense, the decrease in the incidence of wavebreaks in the IZ is compatible with the restitution hypothesis. It, however, does not explain the observed increased wavebreak incidence at the BZ, nor does it explain the increased propensity to VF associated with acute ischemia.

As Xie et al pointed out, for spontaneous breakup to occur, the DI must be below a critical value, ie, within the range in which the APDR slope becomes >1. Conversely, if a certain condition prevents the system from getting into that range, the breakup would not occur. It follows that the postrepolarization refractoriness that prolongs DIs in the IZ (see Figure 7) may be a major factor in preventing (or attenuating) wavebreak. This is in agreement with the results by Koller et al showing that conversion of VF into a periodic rhythm by hyperkalemia was associated with prolongation of DIs. More generally, one can argue that any intervention that increases DI during VF will effectively attenuate wavefront–wavetail interactions, thereby preventing waves from breaking. This is consistent with recent data indicating that the effectiveness of various drugs against atrial fibrillation is directly correlated with their ability to increase the excitability gap. Hence, imposing a lower limit to the DI may be a sufficient condition to prevent wavebreak. Therefore, a hypothetical intervention that induces postrepolarization refractoriness (ideally without any adverse effects) may be as good as (or better than) an anti-VF paradigm such as flattening the restitution curve.

Frequency Analysis of VF
Fast Fourier transform analysis provides an objective and fully automated means of estimating frequency content of a complex signal. Although some groups adopted the use of the DF as an estimator of rate during VF, other groups questioned its reliability. In the present study, we obtained power spectra for microelectrode recordings during control and ischemic VF. Because the microelectrode is the best-known way to record myocardial TMP, our data suggest that the DF is an accurate and robust estimator of CL, or rate of activation during VF in normally perfused porcine ventricle. During ischemic VF, the power spectra become broader and the correlation between the CL and the DF gets worse. This may be secondary to an increased variation of the CL in the ischemic tissue. This is different from the data obtained during global ischemia in the rabbit heart, in which the ischemic signals were more periodic and organized. Thus, the increased temporal variation of VFCL in the IZ of a regionally ischemic heart might be caused by intermittent conduction block across the BZ (see Figure 3), rather than local ischemic conditions.

Acknowledgments
This work was supported in part by the following grants: PO1-HL-39707 from the National Heart, Lung, and Blood Institute, NIH; Scientist Development Grants 0230281N and 0230311N from the American Heart Association (A.V.Z. and O.B., respectively); and grant 2000D020 from the Netherlands Heart Foundation (J.R.d.G.). We thank Jiang Jiang for technical assistance.

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*Circ Res.* 2003;92:546-553; originally published online February 13, 2003; doi: 10.1161/01.RES.0000061917.23107.F7
*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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Methods (online supplement)

This investigation conformed to the current Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85-23, revised 1996).

Isolated heart preparation.

Pigs of either sex (n=15) were premedicated with ketamine 350 mg, azaperone 80 mg and atropine 0.5 mg (i.m.), and anaesthetized with pentobarbital 20 mg/kg (i.v.). Pigs were intubated and ventilated with room air. Heparin (1000 IU) was administered i.v..

Approximately 1.5 liters of modified Tyrode’s solution (37°C, composition in mmol/L: Na+, 157; K+, 4.7; Ca2+, 1.5; Mg2+, 0.7, H2PO4– 0.5, Cl– 137.6, HCO3– 28.0, glucose 11.0, dextran 4% and insulin 10U) was infused in an external jugular vein, and blood-Tyrode’s mixture was collected at equal rate from a carotid artery. After midsternal thoracotomy, the heart was rapidly excised and immersed in ice-cold (4°C) Tyrode’s solution. The aorta was cannulated and the heart was perfused with blood-Tyrode mixture in a Langendorff apparatus, as described previously. Our preliminary experiments showed that perfusion with blood prevents edema which affects the wave dynamics during VF (not shown). The blood-Tyrode mixture was oxygenated (CO2 5%/O2 95%), heated (37°C) and filtered using a Micro HF capillary oxygenator (COBE, Arvada, Co). A collector tube was inserted into the right ventricle through the outflow tract. Another tube was inserted into the left ventricle through a cut made in the left atrial appendage, to collect the small amount of blood coming out of the Thebesian veins. Thereafter, the great veins
were tied with sutures. All blood leaks were meticulously closed to ensure that all the outflow of perfused blood was collected for recirculation (Figure 1A). The heart was then submerged in warm, oxygenated Tyrode’s solution, which was continuously pumped, without recirculation, at a rate equal to the perfusion rate (120-150 ml/min). The temperatures of both perfusate and superfusate were maintained at 37±0.5°C. A volume-conducted ECG and aortic pressure were recorded throughout the entire experiment using a data acquisition system (BIOPAC Systems, Goleta, CA).

The left anterior descending artery (LAD) was occluded with an atraumatic clamp to induce regional ischemia. An initial occlusion of 30-seconds allowed identification of the visible border between cyanotic and normal myocardium. The recording camera’s field of view (see below) was adjusted so as to have equal areas of both sides of the ischemic margin (Figure 1B). At the end of each experiment, green food colorant was introduced into the perfusion system to confirm the exact position of the ischemic margin. Afterwards, the green dye was washed out and the heart was sliced and immersed in tetrazolium chloride for 30 min to visualize the extent of ischemia throughout the ventricular wall. The border zone (BZ) was defined as an 8 mm wide strip of myocardium along the ischemic border. IZ and NIZ were defined as the portions of ischemic and nonischemic zones, respectively, exclusive of BZ (Figure 1B).

Optical and microelectrode recordings

We used the fluorescent voltage sensitive dye di-4-ANEPPS as described elsewhere. To record fluorescence, we used a fast digital 12-bit CCD camera (DALSA) connected via a
frame grabber (RoadRunner 24, Bitflow, Inc.) to a PC. The diameter of the field of view was ~32 mm (64x64 pixels) and included approximately equal portions of IZ, BZ and NIZ (Figure 1B). To reveal the signal from the raw data, the background fluorescence was subtracted from each frame. Spatial and temporal filtering was performed as described elsewhere. Briefly, a moving median filter (box size, 3 points) and a low-pass spatial filter (weighted average of 3-5 neighboring pixels) were applied to improve the signals. To minimize motion artifacts, the heart was gently pressed to the glass chamber wall for just enough time to acquire the five-second movie (at 300 or 400 frames/s). No electromechanical uncouplers were used. In a separate series of experiments, transmembrane potentials (TMP) were recorded from the IZ using floating glass microelectrodes filled with 3M KCl. Two intramural bipolar electrograms (BEGs) were recorded from IZ and NIZ at the distance of approximately 1 cm from the ischemic border. A TMP together with two BEGs, volume conductor ECG and perfusion pressure were sampled at 2,000 Hz with 16-bit resolution and digitized using a data acquisition system MP-100 (BIOPAC Systems, Goleta, CA).

Experimental Protocols

We used a total of 15 animals. Unless stated otherwise, VF was induced using a 9V battery applied to the outflow tract, far from the area where either optical or electrical recordings were taken.

Protocol 1 (exp.# 1-7): VF was induced and optically recorded 5 min before and 15 min after LAD occlusion. Each episode of VF lasted < 3 min and was terminated by a non-
contact defibrillation shock. If spontaneous VF occurred during 15 min of ischemia, it was also optically recorded and terminated by a defibrillation shock.

Protocol 2 (exp. #8): VF was started 5 min before LAD occlusion and maintained for 15 min thereafter. Optical recordings were taken at 2-min intervals. Data from this experiment were used to assess temporal dynamics of dominant frequency (DF) distribution during ischemic VF (see Figure 5).

Protocol 3 (exp. #9-13): VF was induced and a microelectrode was impaled in a cell located in the central part of the ischemic zone. Once a stable impalement was obtained, the LAD was occluded and the TMP was recorded continuously as long as the microelectrode remained stable inside the cell. The goal was to obtain at least 12 seconds of continuous recording for 3 to 5 min after LAD occlusion from the same cell from which a 12-sec control (pre-ischemic) recording was obtained. In those experiments in which an unstable impalement precluded control and ischemic recordings from the same cell, a new cell was impaled ≤ 1mm apart from the original cell. In two experiments (#12 and #13), we were able to maintain stable microelectrode recordings from the same cell throughout the first 3.5 (exp. #12) or 6 min (exp. #13) of ischemia. The data from these experiments were used to assess temporal dynamics of cellular excitation frequency during ischemic VF (see Figure 5).

The LAD clamp was released ≤ 10 min after occlusion to ensure that the ischemic changes were fully reversible. The heart was then normally perfused for 20 min and the
protocol was repeated. Thus, there were two LAD occlusions per each microelectrode experiment. In each experiment, the data from the first occlusion that met the objective were used for analysis. Since in each experiment at least one occlusion yielded the required data, we analyzed a total of 5 pairs of control vs. ischemic recordings using pairwise comparisons.

Protocol 4 (exp. #14): To ensure reproducibility of excitation frequency duringVF in a normally perfused heart, VF was electrically induced, optically recorded and terminated by an electric shock at approx. 15 min intervals during 60 min.

Protocol 5 (exp. #15): To ensure stability of excitation frequency during continuous VF in a normally perfused heart, VF was electrically induced and maintained for 60 min. Optical recordings were taken every 5 minutes during this period.

Data analysis

The power spectra of 3.4 sec segments of optical signals (1024 points at 300 fr/sec) were obtained using the fast Fourier transform (FFT) with Hanning window function. DF maps were constructed as described elsewhere. Spatial heterogeneity of DF over an area of interest was assessed using standard deviation. Local DF was used as an estimator of VF cycle length (VFCL). The latter was shown to correlate with local refractory period duration. Time-space plots (TSP) and singularity point (SP) analysis are described elsewhere. Individual singularity points were tracked over 500 consecutive frames (1.65 sec) in order to determine their trajectories and lifespan. Singularity points with the
lifespan < 10 ms were excluded from analysis. The starting point of a SP trajectory was considered as the site of a new wavebreak. The average number of wavebreaks per 1 cm² per second (wavebreak density) was determined for each optical recording of VF.

TMPs were analyzed according to the algorithm described by Kim et al. Briefly, in each cycle a point of the maximum slope (dV/dt_{max}) of the action potential upstroke was determined first. The program then searched backward for the nearest robust minimum (V_{takeoff}) and forward for the nearest robust maximum (V_{max}). Action potential amplitude (APA) was defined as \( V_{max} - V_{takeoff} \). Starting at the point of \( V_{max} \), the program searched forward for the point where the TMP curve crossed the 90% level of repolarization (\( V = V_{max} - APA \times 0.9 \)). APD_{90} was calculated as the temporal difference between the point of maximum slope and the point of 90% repolarization. Cycle length (CL) was defined as the temporal difference between the two consecutive points of maximum slope. Diastolic interval (DI_{90}) was defined as CL - APD_{90}. Action potential with dV/dt_{max} < 5 V/s were discarded from analysis.

TMPs were analyzed in 12.288-second segments of VF recordings, which yielded, depending upon the VFCL, 80 – 130 cycles of activation. For each recording, the average values of CL, APD_{90}, DI_{90}, APA, and dV/dt_{max} were calculated. In addition, standard deviation of CL (SD_{CL}), APD_{90} (SD_{APD}) and DI_{90} (SD_{DI}) were computed. The ratio SD_{APD}/SD_{DI} was used as an estimate of the dynamic restitution relationship during VF. We also attempted to fit the dynamic restitution curve by a single exponential function using non-linear fitting utility of Origin 6.0 (Microcal, Northampton, MA).
The power spectra of TMP recordings were obtained by the Welch method: each recording (12.288 s) was divided into two segments (8.192 s each, 50% overlap). The power spectrum of each segment was obtained via FFT with Hanning window function and then averaged and normalized to the total power. The DF of the power spectrum was compared to the inverse of the average CL (1/CL).

Statistics

Data are given as mean ± standard deviation. Single pairwise comparisons were made using two-sided paired Student t-test. Multiple pairwise comparisons were made using two-way ANOVA with a Bonferroni post-hoc test. A p value < 0.05 was considered statistically significant.

Reference List


