Three distinct phases of VF during global ischemia in the isolated blood-perfused pig heart

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Huizar JF, Warren MD, Shvedko AG, Kalifa J, Moreno J, Mironov S, Jalife J, Zaitsev AV. Three distinct phases of VF during global ischemia in the isolated blood-perfused pig heart. Am J Physiol Heart Circ Physiol 293: H1617–H1628, 2007. First published June 1, 2007; doi:10.1152/ajpheart.00130.2007.—Changes in ventricular fibrillation (VF) organization occurring after the onset of global ischemia are relevant to defibrillation and survival but remain poorly understood. We hypothesized that ischemia-specific dynamic instability of the action potential (AP) causes a loss of spatiotemporal periodicity of propagation and broadening of the electrocardiogram (ECG) frequency spectrum during VF in the ischemic myocardium. We recorded voltage-sensitive fluorescence of di-4-ANEPPS (anterior left ventricle, 35 × 35 mm, 64 × 64 pixels) and the volume-conducted ECG in six blood-perfused hearts during 10 min of VF and global ischemia. We used coefficient of variation (CV) to estimate variability of AP amplitude, AP duration, and diastolic interval (CV-APA, CV-APD, and CV-DI, respectively). We computed excitation median frequency (Median_F), spectral width of the AP and ECG (SpW-AP and SpW-ECG, respectively), wavebreak incidence (WBI), and recurrence of propagation direction (RDP). We found three distinct phases of local VF dynamics: “relatively periodic” (≤1 min, high Median_F, moderate AP variability, high WBI, low RPD), “highly periodic” (1–2 min, reduced Median_F, low AP variability, low WBI, high RPD), and “aperiodic” (3–10 min, low Median_F, high AP variability, high WBI, low RPD). In one experiment, spontaneous conversion from the aperiodic to the highly periodic phase occurred after 5 min of ischemia. The SpW-ECG was correlated with SpW-AP, CV-APD, and CV-APA. We conclude that 1) at least three distinct phases of VF dynamics are present in our model, and 2) the newly described aperiodic phase is related to ischemia-specific dynamic instability of the AP shape, which underlies broadening of the ECG spectrum during VF evolution.

ventricular fibrillation; action potential; electrocardiogram

ONE IMPORTANT DECISION that an emergency care professional faces at the scene of cardiac arrest is whether to initiate cardiopulmonary resuscitation (CPR) before the application of a shock, or to defibrillate first. Population studies indicate that the “CPR first” strategy improves survival if the ventricular fibrillation (VF) duration exceeds 3–4 min (“circuitual phase”) but not at the earlier “electrical” phase of VF (18). This observation motivated recent studies aimed at estimating VF duration based on the structure and spectral content of the electrocardiogram (ECG) waveform (6, 27, 34). Furthermore, various quantitative measures of “order” in the ECG waveform can predict the likelihood of rescue shock success, restoration of circulation, and survival to hospital discharge (4, 6). However, the relationship between the structure of the ECG waveform and the spatiotemporal dynamics of the fibrillatory waves in the myocardium remains unknown.

VF organization evolves naturally after its onset as a result of global myocardial ischemia. Yet, the effects of ischemia on VF dynamics are complex and poorly understood. Reduced excitability resulting from hyperkalemia increases spatiotemporal periodicity and stabilizes sources of VF in isolated heart preparations perfused with physiological solutions (23, 25, 43). However, in the open-chest dog, Huang et al. (19) have recently found a biphasic type of evolution of activation patterns during VF, with a transient phase of increased spatiotemporal organization between 1 and 1.5 min after VF onset followed by a decrease in the organization in later phases of VF. While the transient phase of increased organization may be attributed to the effect of hyperkalemia as found in isolated heart preparations (23, 25, 43), the mechanism of decreased organization during later phases of VF remains unknown.

Dynamic instability in the form of discordant alternans of action potential (AP) duration (APD) was observed in blood-perfused rabbit hearts after 2–3 min of global ischemia (32). We hypothesized that similar instabilities could occur during ischemic VF and explain the loss of spatiotemporal periodicity of propagation (19) and broadening of the ECG frequency spectrum (34) observed at later stages of VF. Therefore, we performed a systematic analysis of the dynamical properties of the optical AP, wave propagation, and ECG during the first 10 min of global ischemia in isolated blood-perfused porcine hearts. We describe a new type/phase of fibrillatory dynamics characterized by a highly unstable AP shape occurring 3–10 min after the onset of global ischemia. This phase, which we termed “aperiodic,” is recognizable in the ECG and may be relevant to the “circulatory” phase of clinical VF (18).

METHODS

This investigation conformed to the National Research Council’s Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 85-23, revised 1996) and was approved by the Institutional Animal Care and Use Committee of the University of Utah (protocol no. 05-04009).

Isolated heart preparation. Young pigs (18 ± 3 kg) of either sex (n = 6) were premedicated with ketamine (350 mg), azaperone (80

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mg), and atropine (0.5 mg, im) and anesthetized with pentobarbital (20 mg/kg, iv). Pigs were intubated and ventilated with room air. Heparin (1,000 IU) was administered intravenously. The chest was opened through a midsternal incision. The heart was excised and Heparin (1,000 IU) was administered intravenously. The chest was maintained at 37 ± 0.5°C during both normal coronary perfusion and ischemia. The gradient of temperature across the left ventricular wall never exceeded 1°C.

Optical recordings. The optical mapping system has been described previously (38, 47). We recorded fluorescence of the voltage-sensitive dye di-4-ANEPPS using a digital charge-coupled device (CCD) camera. The diameter of the field of view was ~35 mm (64 × 64 pixels, 300 fps). The field of view covered the epicardial area of the anterior left ventricle with the upper left corner just viewing the left anterior descending coronary artery. To minimize motion artifacts, the heart was gently pressed against the glass chamber wall while acquiring a 5-s movie. No electromechanical uncouplers were used.

Volume-conducted ECG. Volume-conducted ECG (VCECG) was recorded using two active electrodes on the right and the left sides of the heart and the ground electrode at the bottom of the superfusion chamber. All three electrodes were located at a distance of ~2.5 cm from the heart. The VCECG signal was recorded continuously at a sampling rate of 500 Hz throughout the entire experiment (high-pass and low-pass filters, 0.05 and 100 Hz, respectively) and was resampled in software to match the video frame rate. We extracted 1,024 sample-long segments of the VCECG synchronous with the optical recordings to perform frequency analysis. The CCD camera set-up provided a TTL output signal that was set to high throughout the entire duration of video recording. This signal was continuously recorded in parallel with the VCECG, which allowed for time alignment of optical recordings and VCECG with the accuracy of the VCECG sampling interval, 2 ms.

Experimental protocol. VF was induced using a 9-V DC battery and allowed to reach steady state during 5 min before induction of global ischemia by interruption of aortic perfusion. Optical recordings of VF were acquired between 0 and 10 min after the onset of ischemia at 1-min intervals.

Data analysis. We computed the fast Fourier transform on 1,024 data points to obtain the power spectrum for both optical APs and VCECG and analyzed the frequencies in the range of 2–30 Hz. We define dominant frequency (DF) as the frequency corresponding to the highest peak in the power spectrum (Fig. 1A). Median frequency (Median_F) is taken at the frequency value that divides the spectrum into two subranges with equal total power. Spectral width (SpW) is defined as the range of frequencies centered at Median_F that contained N% of the total power in the range of interest. In this study, N = 67%. See METHODS for more detail.

Optical AP analysis is illustrated in Fig. 1B. Custom software (AZaitsev) identified individual APs in each single pixel recording as follows. The algorithm first determined the local minimum and maximum in the initial n number of points of the time series. If the point of local maximum was ahead of the point of local minimum, then the segment was identified as a descending segment. Conversely, if the point of local maximum was behind the point of local minimum, then the segment was identified as an ascending segment. Starting with either an ascending or a descending segment, the algorithm recursively appended consecutive n-point segments of the same modality until it encountered the first segment of the opposite modality. The point of transition between an ascending and a descending phase defined a robust maximum. The point of transition between a descending and an ascending phase defined a robust minimum. A robust maximum flanked by two robust minima defined an AP (see Fig. 1B). Note that constant-level regions of the signal, if present, were randomly included either in an ascending or a descending phase. This did not have any noticeable effect on the parameters derived from the optical AP analysis (below). Note also that the segment length n effectively imposed the lower limit for the cycle length of detected APs, because at most one robust maximum or minimum could be identified within n points. We set n equal to 10 frames (33 ms), which was much shorter than the shortest cycle length we found by extensive visual inspection of single pixel recordings (18 frames, ~60 ms).

Fig. 1. A: parameters derived from spectral analysis. Note that, in general, dominant frequency (DF) and median frequency (Median_F) do not coincide. B: action potential (AP) analysis. The algorithm first identified robust maxima (●) and robust minima (○) in individual pixel recordings. These resulted in 9 candidates for AP (impulses a–i in the selected time window). The line connecting robust minima identified the “floor of AP” (bottom dashed line in each AP), which provided a reference level for measurement of the AP amplitude (APA), AP duration (APD), and diastolic interval (DI). APD was measured at 80% of repolarization (the top dashed line in each AP) as explained in METHODS. Note that impulse h, detected by the algorithm, was rejected because its APA fell below the amplitude threshold (15% of maximum APA (APAmax)), and therefore it was considered to be a part of DI. SpWN, spectral width defined as the range of frequencies centered at Median_F that contained N% of the total power in the range of interest. In this study, N = 67%. See METHODS for more detail.
To define the 100% repolarization level, two consecutive minima defining a single AP cycle were connected by a straight line (“the floor of the AP”). Since during VF the end-repolarization level varies from cycle to cycle, this line generally was not horizontal (see Fig. 1B), which introduced uncertainty in the definition and measurement of the APD and AP amplitude (APA). This is a common problem of AP analysis during VF. Nevertheless, our approach was that we calculated APA as the height of the vertical line connecting the peak of AP and the “floor of AP” (see Fig. 1B). We calculated APD at the level of 80% repolarization by drawing a virtual line parallel to the floor of AP that crossed the upstroke and the downstroke of the AP at 80% of the distance from the peak of AP (see Fig. 1B). In each pixel, the maximum APA was calculated (APAmax). APs with amplitude <15% of APAmax were rejected and considered a part of the diastolic interval (DI) (see Fig. 1B). We tested thresholds of 5, 10, 15, and 20% and found that the results were fairly constant between 10 and 20%, whereas at 5% threshold, we started picking up noise. On the other hand, the 20% level was close to the minimal amplitude of propagating waves after prolonged periods of ischemia (see Fig. 3F). Therefore, we concluded that the amplitude threshold equal to 15% of APAmax was optimal.

The moment of activation in each AP cycle was defined as the time point where the upstroke of the AP reached 50% of the APA. The interval between the two subsequent activations defined the cycle length (CL). The DI was then calculated as DI = CL – APD. In each pixel, we calculated the coefficient of variation (CV) (SD normalized to the mean value) for APA, APD, and DI (CV-APA, CV-APD, and CV-DI, respectively). To obtain restitution density plots, two-dimensional histograms were created such that bin \( i,j \) contained the number of DI,APD pairs where DI was equal to \( i \) frames and APD was equal to \( j \) frames (12). The bins were sorted in the descending order of their count (i.e., the no. of DI,APD pairs residing in a given bin). The first \( n \) largest bins with a cumulative count equal or exceeding 50 or 90% of the total count constituted the 50 and 90% density areas, respectively.

For assessment of spatiotemporal periodicity, we estimated recurrence of propagation direction (RPD) following a previously described method (2). Briefly, for each pixel, direction of propagation was obtained from activation times in a 5 × 5-pixel neighborhood. Histograms of all the activation directions yielded the predominant direction (PD) of propagation. The recurrence of the predominant direction (RPD) in a pixel was measured from such histograms as the ratio of the number of activations in the PD to the total number of activations. For statistical analysis, all pixel-wise measurements were averaged among all pixels of a given movie.

For detection of wavebreaks (WBs), singularity point (SP) trajectories were determined in phase movies (16, 38) using SCROLL software (S. Mironov). A starting point of an SP trajectory was considered as the site of a new WB. The total number of WBs per 1,024 frames was determined for each movie of VF. To obtain WB incidence (WBI), this number was normalized to the size of mapped area and the average number of excitation cycles estimated as DF × T, where \( T \) is the duration of the movie. Time-space plots is a plot of instantaneous intensity of fluorescence along a selected line of pixels vs. time (37, 46). Time-space plots presented here were constructed for a vertical line across the center of the field of view.

Statistics. Data are given as means ± SD. One-way repeated-measures ANOVA (RMANOVA) was applied to test whether VF descriptors changed significantly over the course of ischemia. Post hoc Bonferroni test was used for selected pairwise comparisons between 0 and 1 min and between 0 and 3 min of ischemia. Post hoc linear trend test was applied to SpW-AP and SpW-ECG. Friedman test (nonparametric RMANOVA) with Dunn’s multiple comparison test was applied for analysis of RPD. All tests were performed using the InStat program (GraphPad software). A difference at \( P < 0.05 \) was considered statistically significant.

RESULTS

Overview of the spatiotemporal organization of VF at different stages of ischemia. In each experiment, we observed nonmonotonic changes in the spatiotemporal organization of VF during the first 10 min of global ischemia. Figure 2 illustrates the relationship between spatial and temporal dynamics of VF at 0, 2, and 5 min of ischemia in a typical experiment. Specifically, at 0 min of ischemia (Fig. 2, top), a snapshot of a phase movie shows a complex activation pattern

![Fig. 2. Relationship between temporal and spatial dynamics of ventricular fibrillation (VF) at different stages of global ischemia. Top to bottom: results obtained at 0 (top), 2 (middle), and 5 min (bottom) of ischemia. For each time point, we show the following data representations. Left: a snapshot of the phase movie. White circles indicate location of singularity points (SPs). Center-top: a time-space plot constructed for the vertical line across the center of the mapped area (vertical dashed line). Center-bottom: the signal recorded from the central pixel (dashed line in time-space plot). Right: the power spectrum for the signal recorded from the central pixel. SpW-AP, SpW of optical AP.](http://ajpheart.physiology.org/)
with four WBs in the field of view. However, the temporal order is rather high, as evidenced from the time-space plot and the recording taken from the center pixel. Indeed, the cycle length is relatively stable, and although the amplitude and shape of the optical AP vary, the white bands in the time-space plot corresponding to consecutive activations clearly form a rather periodic grid over time. This is in agreement with the single peak in the power spectrum of the center pixel (at DF = 12.8 Hz) and the relatively narrow SpW (SpW-AP = 1.76 Hz). At 2 min of ischemia (Fig. 2, middle), activation is slower (DF = 8.5 Hz), and a strikingly high degree of organization is present both in space and in time. The snapshot of the phase movie shows no WBs in the field of view. In each impulse, single propagating wavefronts (yellow band) consistently entered the upper right quadrant of the mapped area, yielding an RPD of 77%. The time-space plot and single pixel recordings clearly indicate a very high degree of periodicity reminiscent of monomorphic ventricular tachycardia. This high degree of periodicity translates into a very narrow peak in the power spectrum, such that SpW-AP is only 0.88 Hz. Finally, at 5 min of ischemia (Fig. 2, bottom), there is a decrease in organization that cannot, however, be classified as a return to the original pattern (i.e., at 0 min of ischemia). Although the phase movie shows an increase in the WBI, the temporal organization is strikingly different from that at 0 min of ischemia. The data were obtained at 0, 2, and 5 min of ischemia (from top to bottom). Figure 3, A, C, and E, shows time-space plots of the DI (black) and APD (white). Figure 3, B, D, and F, shows the time-space plot of the AP wavefronts (upstrokes) color coded according to the APA normalized to the APA_{max} in the same pixel during the respective movie. Similar to Fig. 2, the experiment in Fig. 3 clearly demonstrates qualitatively distinct types of dynamics at the selected time points of ischemia. At 0 min (Fig. 3A), the excitation frequency is the highest; however, the variability of APD and DI is moderate with the exception of the WB sites (zigzag patterns) where neither parameter is well defined. Similarly, the variability of the APA (Fig. 3B) is also moderate outside the WB sites where APA is usually in the range of 60–80% of the APA_{max}. Near the WB sites, the APA is always low (<50% of maximal APA). At 2 min (Fig. 3, C and D), the excitation frequency is slower, and the degree of spatiotemporal periodicity is the largest among all phases. Both APD and DI are longer and show little variation (Fig. 3C). WBs are rare and so are waves with low amplitude (Fig. 3D). In stark contrast, at 5 min of ischemia (Fig. 3, E and F), APD, DI, and APA all fluctuate markedly both in space and time. Note also that at 5 min of ischemia, low-amplitude waves (<50% of APA_{max}) spanning the entire time-space plot are commonly seen (Fig. 3F). Some of the low-amplitude waves, especially those following critically short DI, failed to propagate. For example, wave a in Fig. 3, E and F, was blocked in the downward direction after a DI approaching zero and reentered that area later. However, many of the low-amplitude waves were able to propagate without interruption and could recover to a higher amplitude as they propagated (e.g., Fig. 3F, cycle d). Note that at 0 min of ischemia, low-amplitude waves were seen only in the vicinity of WBs (see Fig. 3B). It should be noted that beat-to-beat fluctuations of APA, APD, and DI often showed a considerable degree of spatial discordance during later stages of VF/ischemia (see Fig. 3, E and F, cycles b–e). Specifically, in cycle b, APD was relatively uniform along the y-coordinate; however, the APA was larger in the center than in the periphery, following a relatively long DI. In cycle c, both APA and APD were larger in the periphery than in the center, although the DI preceding beat c was relatively uniform along the y-coordinate. The DI following beat c was the longest in the center (following an AP with short APD and low APA) but became shorter toward the periphery. In cycle d, both APA and APD were large in the center (following the longest DI) and small in the periphery (follow-
ing short DI). The DI following beat d was the shortest in the center (following an AP with long APD and large APA). Finally, in cycle e, both APA and APD were larger in the periphery than in the center (similar to beat c). Note that in beat e, the preceding DI was clearly not a sole determinant of the APA and APD. The lowest values of APA and APD (center) and the largest values of APA and APD (the uppermost y-coordinate) correspond to similar lengths of the preceding DI. In general, after 3 min of ischemia, long DIs were consistently followed by APs with large APA and long APD, but short DIs were less predictive of the parameters of the following AP.

Figure 4 shows restitution plots of APD and normalized APA in the same experiment as shown in Fig. 3. At 0 min of ischemia, the relationship between APD and DI of all AP cycles (Fig. 4A) forms a tight cluster of points where variation of APD is larger than variation of the DI. Such a distribution is compatible with a steep restitution slope, but no functional relationship can be quantified because of the very narrow range of the independent variable, the DI. Similar to APD, variation in APA is large (Fig. 4D), but no functional relationship with DI can be determined. Note that at 0 min, a substantial proportion of APs have amplitudes <50% of APA_{max}. As was shown in Fig. 3, these low-amplitude APs are usually associated with the sites of WBs. At 2 min of ischemia (Fig. 4, B and E), the range of DIs is shifted to longer values but remains rather narrow. Additionally, both APD and APA variability are less than at 0 min with only few APs having APA <50% of APA_{max}. As was shown in Fig. 3, this is associated with a low incidence of WBs in this phase. At 5 min of ischemia (Fig. 4, C and F), the most striking difference with previous phases is a wide range of DIs. Furthermore, there is also some dependence of APD and APA on the preceding DI. In the case of APD (see Fig. 4C), this dependence is complex, such that long DIs are associated with a narrow range of intermediate APD values, but at shorter DIs, the range of possible APD values progressively broadens. The relationship between APA and DI at 5 min of ischemia (Fig. 4F), however, shows a weak but detectable linear correlation ($R = 0.63$, $P < 0.0001$). In all experiments, the slope of the APD restitution (APDR) curve was uncertain at all phases of ischemic VF because of the lack of clear functional relationship between APD and the preceding DI. This is consistent with our previous microelectrode data (47). However, after 3 min of ischemia, there was a measurable “restitution” of APA that resulted in low-amplitude waves following short DIs (as seen in Fig. 3F).

Changes in AP shape variability with time of ischemia. Whereas the restitution relationships (see Fig. 4) resisted robust quantification, the variability of the AP shape seemed to show consistent changes with time of ischemia. Figure 5 shows plots of CV-APA, CV-APD, and CV-DI vs. ischemia duration in six experiments. In most experiments, each of the AP variability metrics reached a minimum at 1 or 2 min of ischemia, followed by a sharp increase between minutes 2 and 4, a maximum around 4 min, and a plateau or a slow decline thereafter. The notable exception was experiment 6 in which AP variability returned to low levels after 5 min of ischemia. Nevertheless, for all the metrics presented in Fig. 5, the changes with time of ischemia were highly significant ($P < 0.0001$, ANOVA). The results of selected pairwise comparisons are presented in Table 1. Unlike CV-APA and CV-DI, CV-APD was significantly different between 0 and 1 min of ischemia. However, all AP variability parameters were significantly different between 0 and 3 min of ischemia. We interpret these results as indicative of the presence of a phase in ischemic VF that starts at 3–4 min of ischemia and is characterized by the highest variability of the AP shape.

Changes in wave propagation with time of ischemia. An important advantage of high-resolution optical mapping is the ability to compare local dynamics of the AP with those of propagating wavefronts. Figure 6A demonstrates that in each experiment, WBI reached an absolute minimum at 1 or 2 min of ischemia followed by a sharp increase within a minute from the time at which that minimum was reached. Additionally, in each experiment, WBI reached a maximum at 3–5 min of ischemia, exceeding the respective value at 0 min. WBI slowly decreased between 4 and 10 min of ischemia. In experiment 6 (green curve in Fig. 6), the time

![Fig. 4. Density scatter plots of APD and APA restitution at 0, 2, and 5 min of VF/ischemia. Each plot represents data collected from 40,000–80,000 individual AP cycles from the same experiment as shown in Fig. 3. In each plot, black color indicates the area with the highest density of points (50% of all points); the union of black and gray colors indicates the area containing 90% of all points. APD_{50}, APD at 80% repolarization.](http://ajpheart.physiology.org/)
course of WBI declined faster than in other experiments and approached zero at 9–10 min of ischemia. Statistical analysis indicated that the variation of WBI with time of ischemia was highly significant. WBI was significantly different between 0 and 1 min of ischemia and between 0 and 3 min of ischemia (Table 1).

It was proposed that type II VF, which is characterized by increased spatiotemporal periodicity of activation, occurs during long-duration VF (8, 24, 43). Figure 6B shows that average RPD, an estimator of spatiotemporal periodicity, had a time course with three distinct phases. At 0 min of ischemia, RPD was relatively low in five of six experiments, which reflects the fact that everywhere in the field of view, the activation waves frequently changed the direction of propagation. Then, in each experiment, there was a transient increase in RPD at either 1 or 2 min of ischemia, followed by a return to the preischemic values. A gradual increase in RPD was observed between 4 and 10 min of ischemia. The largest increases were observed in experiment 6, where RPD exceeded 50% at 9 min of ischemia. There was a statistically significant difference between RPD values at 0 and 1 min but not between 0 min and 3 min of ischemia (see Table 1). Interestingly, experiment 6 is a clear outlier in terms of VF evolution. Indeed, unlike the rest of the experiments, experiment 6 exhibited a “reversed” evolution between 5 and 10 min of VF in terms of AP variability (Fig. 5), WBI (Fig. 6A), and, finally, RPD (Fig. 6B).

Frequency analysis of optical APs during ischemic VF. Figure 7A shows that Median_F of optical APs decreased gradually and reached minimum at 3–4 min of ischemia, with a small increase between minutes 5 and 10. Median_F was highly correlated with the inverse of average cycle length of optical APs ($R = 0.96$, not shown). Note that despite multiphasic time courses of the Median_F, in most experiments the average SpW-AP (Fig. 7B) progressively increased over time of ischemia, the only exception being experiment 6 (green curve) in which SpW-AP returned to low values after 5 min of ischemia. The average SpW-AP showed a significant linear trend over time of ischemia ($P < 0.0001$, $R^2 = 0.37$).

Interpretation of ECG during ischemic VF. The temporal organization of the ECG decreases with time after the onset of VF (4). It remains unknown how the changes in the ECG during VF are related to the dynamics of electrical waves in the heart. On ECG, changes in the organization may be due to changes in both temporal and spatial dynamics of fibrillatory waves (see DISCUSSION). However, our results indicate that the evolution of the ECG spectrum predominantly reflects changes in local dynamics. Figure 8A demonstrates parallel changes in periodicity of local optical APs and VCECG. Note that despite the expected differences between the waveforms of optical AP and VCECG, both signals share a high degree of periodicity at 2 min and a low degree of periodicity at 5 min of ischemia. Figure 8B highlights the coincidence between an abrupt broad-

Table 1. Statistical analysis of VF descriptors

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<td>CV-APA</td>
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<td>CV-DI</td>
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<td>WBI</td>
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<td>RPD</td>
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VF, ventricular fibrillation; CV, coefficient of variation; APD, action potential (AP) duration; APA, AP amplitude; DI, diastolic interval; WBI, wavebreak (WB) incidence; RPD, recurrence of propagation direction; NS, not significant.
ening of the ECG spectrum and an increase in the measures of AP variability in a typical experiment. The broad ECG spectrum and high degree of AP variability persist between 3 and 10 min of ischemia. In contrast, in the atypical experiment (experiment 6) shown in Fig. 8C, the broadening of the ECG spectrum is transient but so is the increase in the AP variability. Thus the ECG spectrum closely tracks the degree of AP variability during both typical and atypical VF evolution.

In Fig. 9, we compared the strength of correlation between the spectral content of the ECG and various metrics of local VF dynamics. Data from experiment 5 are not presented because the VCECG recording from this experiment was not available. It can be seen that Median_F of VCECG directly correlates with Median_F and with the inverse of the cycle length (1/APCL) of local optical recordings (Fig. 9, A and B, respectively). Likewise, SpW-ECG directly correlates with the SpW-AP (Fig. 9C) and with the metrics of AP variability (CV-APA and CV-APA in Fig. 9, D and E, respectively). Thus the spectral content of ECG can be interpreted in terms of local excitation rate and regularity of the AP even without knowledge of spatial organization of excitation waves.

**DISCUSSION**

The data presented here establish a link between the AP dynamics and the volume-conducted ECG during the first 10 min of VF/global ischemia, a time window that is relevant to cardiac resuscitation (18). At the level of the optical AP, which is a surrogate of cellular activity, we describe for the first time the aperiodic phase/type of VF characterized by a large dynamic instability. This AP instability underlies the broadening of the power spectrum and loss of periodicity that were previously observed in the ECG waveform during VF evolution in anesthetized pigs and in humans (5, 26).

Natural evolution of VF. In the open-chest dog, Wiggers (41) described four different stages of VF: 1) undulatory or tachysystolic (1–2 s); 2) convulsive incoordination (15–40 s); 3) tremulous incoordination (2–3 min); and 4) atomic fibrillation, which usually develops 2–5 min after the onset of VF. In a similar model, Huang et al. (19) recently distinguished five phases (phases i–v) during the first 10 min of VF, based on a quantitative analysis of spatiotemporal dynamics of wavefronts extracted from multielectrode epicardial maps. Most notably, they found a nonmonotonic evolution of activation patterns during long-duration VF, with a transient phase of increased periodicity and organization between 63 and 86 s after VF onset and a rapid decrease in organization in later phases of VF (19).

Similar to Huang et al. (19), we found a nonmonotonic VF evolution in the isolated blood-perfused pig heart. Furthermore, we found the dynamic instability of AP shape to be a defining factor of VF dynamics at later stages of VF/ischemia. Taking into account the dynamics of cellular electrical activity, we defined three qualitatively different phases/types of VF occurring during the first 10 min of ischemia progression (Table 2). Specifically, the relatively periodic phase of VF is defined by a high excitation frequency, a complex propagation pattern (high WBI and low RPD), and yet a considerable degree of local temporal periodicity (moderate AP variability). The highly periodic phase is characterized by intermediate values of DF, a very low degree of AP variability, a low WBI, and a high RPD. Finally, the aperiodic phase is characterized by a low/intermediate DF, a high AP variability, a high WBI,
Finally, our aperiodic phase presumably corresponds to stabilization of both the AP shape and reentrant sources. This is the transient phase of an increased organization and periodic phase clearly corresponds to Huang’s (19) established VF not yet affected by ischemia. Then, our highly corresponds to Huang’s (19) and a low RPD. Our relatively periodic phase most likely corresponds to Huang’s (19) phase ii, which is the phase of established VF not yet affected by ischemia. Then, our highly periodic phase clearly corresponds to Huang’s (19) phase iii. This is the transient phase of an increased organization and stabilization of both the AP shape and reentrant sources. Finally, our aperiodic phase presumably corresponds to Huang’s (19) phase iv and the initial part of phase v.

In phase i (1–11 s after VF induction) of Huang et al. (19), there is an increase of the excitation frequency and the number of wavefronts per second amid a relatively constant incidence of conduction block. This initial acceleration of VF probably reflects a gradual accommodation of the AP due to so-called cardiac memory (20, 36, 39). Analysis of microelectrode recordings during VF in open-chest pigs showed that the role of cardiac memory in VF dynamics is the largest within the first 30 s of VF (20). We observed a monotonic increase in Median_F and a relatively constant WBI within the first 20–30 s of VF regardless of whether the aortic perfusion was maintained or stopped at the moment of VF induction. However, at VF durations exceeding 30–40 s, Median_F and WBI measured during perfused and nonperfused VF started to diverge (see Supplemental Figure; supplemental data are available at the online version of this article).

From the discussion above, we can conclude that the intrinsic evolution of VF, which is independent of ischemic changes, is limited to a gradual increase in the excitation frequency during the first 10–30 s of VF. Despite changes in excitation frequency, WBI is fairly constant during this time (19). The further evolution of VF organization occurs only in the presence of ischemia and is probably dominated by cellular ischemic changes, although the modulatory roles of mechanical stress and autonomic influences cannot be excluded.

It is important to note that the timing of different phases of VF varied among experiments in our study. This effect probably reflected the individual differences in the underlying time course of ischemic changes. Interestingly, in the atypical experiment (experiment 6) a reverse transition from aperiodic VF to highly periodic VF occurred after 5 min of ischemia. In other experiments, there was also a trend toward increased organization at −10 min of ischemia. Thus it is possible that the aperiodic phase of VF is also a transient. However, this phase is prominent during the time window when gains in survival are still possible (18). In any case, given individual differences in the course of VF/ischemia, in the clinical setting it may be more useful to recognize specific phases of VF or underlying stages of ischemia rather than to estimate the absolute amount of time elapsed since the onset of cardiac arrest (4).

**Phases vs. types of VF dynamics.** Recently, Wu et al. (42) and Chen et al. (8) proposed a synthetic theory postulating two distinct types of VF. According to this theory, type I (fast) VF is associated with a steep APDR, a flat conduction velocity restitution, and a multiple wavelet mechanism. Type II (slow) VF is associated with flat APDR, broad conduction velocity restitution, decreased excitability, and spatiotemporal periodicity in activation maps (8, 42). Type I and type II VF were identified in the normoxic and ischemic rabbit ventricle, respectively (24, 42). The question arises as to how the three distinct phases observed in our study can be related to the type I and type II VF. Formally, our relatively periodic phase and type I VF refer to the same physiological conditions, namely, the stage when VF is already established as a highly complex mode of propagation (with multiple WBs) but is not yet modified by ischemia. However, the definition of “type I VF” postulates a single mechanism of WB, divergent fluctuations of APD due to a steep slope of the APDR curve (8). We could not detect any functional relationship between the APD and the preceding DI during our relatively periodic phase (see Fig. 4), which corroborates previously published microelectrode data obtained during VF in the whole pig heart (20, 47). The preceding DI accounts for <30% of the variation in APD

**Fig. 8.** Relationship between volume-conductor ECG and optical AP during VF evolution. A: fragments of a single pixel AP recording (left) and the ECG (right) at 2 and 5 min of ischemia. B: a temporal relationship between the evolution of the ECG spectrum and metrics of AP variability in a typical experiment. The time courses of CV-APA (solid line) and CV-APD (dashed line) are superimposed on the spectrogram of the ECG (white on black). In the spectrogram, each vertical line represents a power spectrum for a 5.12-s segment of the ECG. Gray-scale levels represent the power at respective frequencies. C: same as in B, but in an atypical experiment (experiment 6).
during the first minute of VF in the porcine heart in situ (20). The restitution relationship during VF may be distorted by spatial effects, such as cycle-to-cycle changes in activation sequence (3) and memory effects (20). In any case, given the low predictive power of the DI with respect to the following APD, it is difficult to argue that the APDR mechanism is the main determinant of recurrent WB during early, largely nonischemic VF (relatively periodic phase). Several mechanisms of spiral wave breakup that are independent of APDR slope have been proposed (for review, see Ref. 12). We think that the presently available experimental information including the results of the present study do not allow the exclusion of any of the proposed spiral breakup mechanism or the mother rotor mechanism with fibrillatory conduction. However, our observations of relatively stable DI and APD and narrow power spectra of local activations during the relatively periodic phase seem to differ substantially from the results of computer models of APDR-related spiral wave breakup (=type I VF), which usually demonstrate wide fluctuations of the APD and DI and a low degree of temporal periodicity (e.g., Fig. 4D in Ref. 13, Fig. 6 in Ref. 37, Fig. 5D in Ref. 45, and Fig. 7C, tracing b, in Ref. 1). Thus whether the mechanism of the relatively periodic phase of VF is the same as or different from type I VF remains to be established.

During the highly periodic phase, we frequently observed a clear candidate for a mother rotor in the form of either a single wavefront (e.g., see Fig. 2) or a single stable spiral wave (e.g., see Supplemental Movie “2 min_ischemia.avi”) that lasted throughout the entire movie (~30–40 cycles). Coupled with high RPD and narrow spectra of optical APs (see Fig. 2) and the ECG (see Fig. 8), this observation in our opinion leaves little doubt that a “mother rotor” mechanism underlies the highly periodic phase.

Of all three phases, the aperiodic phase exhibits the slowest frequency (see Fig. 2). In addition, it is characterized by large spatiotemporal fluctuations of the APD and DI, WBs associated with critically short DIs (see Fig. 3F), and a grossly aperiodic appearance of the ECG. It is interesting that this set of features is compatible with those produced by computer models of spiral wave breakup, or type I VF (see, for example, Fig. 6 in Ref. 37 and Figs. 5 and 7 in Ref. 45). However, the

### Table 2. Three phases/types of VF in globally ischemic isolated porcine heart

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<td>Intermediate</td>
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<td>AP variability</td>
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<td>Low</td>
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aperiodic phase is different from type I VF for at least two reasons: 1) slow frequency of excitations and 2) clear presence of ischemic changes. Our aperiodic phase is probably equivalent to phase iv (2–5 min of VF) in the study of Huang et al. (19). The following phase v (5–10 min) in that study exhibits a rapid decay in all VF descriptors, which may indicate a rapid deterioration of the mapped epicardial tissue because of ischemia. In our study, most of the VF descriptors remained much more stable between 5 and 10 min of ischemia, which may indicate that in the pig heart, ventricular epicardium is more resistant to ischemic injury than in the dog heart.

**Possible mechanisms of VF evolution in the ischemic pig heart.** Extracellular K$^+$ accumulation is a prominent factor of myocardial ischemia (7). Reduced excitability resulting from hyperkalemia increases spatiotemporal periodicity and stabilizes sources of VF in isolated heart preparations (23, 25, 43). These effects have been explained in terms of the increase in the core size of the spiral wave (25) or flattening APD (23). Thus hyperkalemia may be a sufficient mechanism for the increased organization of VF observed at least transiently in the dog, (19) rabbit (25, 43), and pig (this study).

What causes an abrupt transition from a highly periodic to an aperiodic phase? A dynamic instability in the form of discordant alternans of APD (32) and Ca transient (31) was observed in paced blood-perfused rabbit hearts after 2–3 min of global ischemia. This coincides in time with the onset of our aperiodic phase characterized by large, spatially discordant fluctuations of APA, APD, and DI (see Fig. 3, E and F). Interestingly, Ca transient alternans during ischemia were greatly potentiated in the presence of blood compared with saline perfusion (44). Thus the prominence of the aperiodic phase of VF might depend on blood perfusion before the onset of ischemia. It is difficult to speculate at this point how blood cells and/or various humoral factors present in blood might promote the dynamic instability of the AP. However, the ultimate dynamic mechanism may involve the observed dependence of APA on the DI (“broad” APA restitution, see Fig. 4F). During ischemia, the recovery of sodium channels from inactivation is slow, sodium current (I\text{Na}) is diminished, and L-type calcium current (I\text{Ca,L}) contributes significantly to the later portion of the upstroke (7). Under these conditions, an instability may arise if, after a critically short DI, the I\text{Na}-driven portion of the upstroke reaches a level close to the activation threshold of I\text{Ca,L} (−40 to −20 mV). In that case, a small variation in the APA can produce an “all-or-none” activation of I\text{Ca,L}, which in turn will produce the all-or-none plateau resulting in large fluctuations of APD. These fluctuations of APD, in a simple case of a constant cycle length, will in turn produce large fluctuations of the DI, leading to fluctuations of APA, and so on. Elevation of extracellular potassium concentration ([K$^+$]) may be a necessary, but not sufficient, factor in this hypothetical mechanism. Ischemia inhibits both release and reuptake of Ca into the sarcoplasmic reticulum (SR) (14, 28), which potentially can cause alternans of intracellular calcium (Ca$_i$) cycling (10, 35) and, consequently, augment beat-to-beat fluctuations of I\text{Ca,L} via a feedback involving Ca-induced inactivation of I\text{Ca,L} (40). In that regard, it is worth mentioning that blockade of SR Ca release by ryanodine selectively affects the aperiodic phase of VF (21). Thus a combination of hyperkalemia and altered SR Ca cycling may underlie the aperiodic phase of VF in a globally ischemic heart. Future work can test such assumptions.

**Clinical relevance.** The ECG is commonly the only source of information about VF available to a health care professional at the scene of an acute cardiac event. The organization of the ECG waveform correlates with the outcome of defibrillation and survival (6, 27, 34). The body surface ECG is a result of the summation of local electrical dipoles in the heart, where the contribution of each dipole depends on the magnitude of the dipole, its direction, and its distance from the ECG leads (33). The magnitude and direction of a microscopic dipole are determined by the local spatial gradient of the transmembrane potential. At each point of the heart, the largest changes in the dipole amplitude and direction occur during the passage of the depolarizing front and the repolarizing tail of the cardiac wave. If all waves in the heart have consistent spatial profile and propagation direction in each cycle, then the variation of the dipole magnitude and direction would be periodic in time, which should lead to a periodic pattern of ECG. Conversely, beat-to-beat changes in either AP shape or propagation direction or both, if they occur in the majority of ventricular sites, should lead to aperiodic pattern of ECG.

Our results suggest that the spectral content of the ECG during VF reflects the average spectral content of local APs, even without knowledge of spatial pattern of propagation (see Figs. 8 and 9). In particular, broadening of the ECG spectrum indicates destabilization of the AP. This observation offers a physiologically meaningful interpretation of VF on the ECG, which so far has remained in the domain of abstract mathematical analysis (4). Continuous ECG recordings during long-duration VF in patients are expectedly scarce, but the one example we found (26) shows an evolution of the ECG spectrum qualitatively similar to the one recorded in this study. Namely, the ECG spectrum was relatively narrow up to 2–3 min of VF, at which point it suddenly became very broad. Thus the onset of the aperiodic phase, the broadening of the ECG spectrum, and the onset of the clinically defined circulatory phase of VF (18) occur approximately at the same time and may have a common cellular/metabolic mechanism relevant to worsening of resuscitation outcome after prolonged periods of VF.

The link between changes in VF organization over the course of ischemia and the outcome of defibrillation is yet to be determined. A recent modeling study (17) demonstrated that multiple co-existing spirals waves required larger shock energies for their termination than a single spiral wave, implying that an increase in WBI per se may be a factor of defibrillation outcome. The dynamic instabilities during ischemic VF may also affect defibrillation outcome. For one thing, spatiotemporal variation of AP shape may increase heterogeneity of the responses to virtual electrode polarization (9, 11, 15), thus increasing a chance of forming new reentrant waves postshock. In addition, the ischemia-specific AP instability may be an indicator of destabilized Ca$_i$ dynamics (21), which in itself may be a factor of defibrillation success or failure (22). Further studies are required to test these possible mechanisms.

**Limitations.** The optically recorded APs do not reproduce true cellular activity with full fidelity because 1) each pixel recording represents hundreds of cells, and 2) some residual motion artifacts cannot be excluded even when using mechanical restraint. Nonetheless, the pixel resolution (−0.5 mm)
should be sufficient for assessment of spatiotemporal AP dynamics because it is comparable with the space constant (30), which sets the lower bound for the scale of AP heterogeneity. Whereas motion artifacts may have caused some overestimation of AP variability at early stages of VF, contractility completely ceases by 4–5 min of ischemia, and therefore the largest AP variability observed during the aperiodic phase cannot be attributed to motion artifacts. Optical recordings were limited to the epicardial surface of the anterior left ventricle. It cannot be excluded that other parts of the ventricles had a different time course of VF evolution. However, the correlation observed between the spectral content of optically recorded APs and that of global ECG suggests that the AP dynamics found in the mapped area represent at least a majority of the ventricular tissue. Another limitation of the study is that the hearts were normal, and no chronically administered drugs were present. Cardiac disease as well as drugs could have altered the findings and their clinical implications.

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