The Distribution of Refractory Periods Influences the Dynamics of Ventricular Fibrillation

Bum-Rak Choi, Tong Liu, Guy Salama

Abstract—The spatial and dynamic properties of ventricular fibrillation (VF) may be random or related to cellular electrical properties of the normal heart. Local activation intervals (AIs) in VF may depend on the local refractory period (RP), and sustained VF may require a steep action potential (AP) restitution curve. In guinea pig hearts, AP durations (APDs) and RPs on the epicardium are shorter at the apex and progressively longer toward the base, producing gradients of RPs that may influence the spatial organization of VF. In the present study, the influence of APDs on VF dynamics is investigated in perfused guinea pig hearts stained with a voltage-sensitive dye by comparing APD gradients to the dynamics of VF elicited by burst pacing. In VF, AIs had no clear periodicity, but average AIs were shorter at the apex (57.5±8.1 ms) than the base (76.1±1.5 ms, n=6, P<0.05) and had gradients similar to APD gradients (correlation coefficient 0.71±0.04). Analysis of local velocity vectors showed no preferential directions, and fast Fourier transform (FFT) power spectra were broad (10 to 24 Hz) with multiple peaks (n=6). However, the selective inhibition of delayed K+ rectifying currents, I_{Kr} (E4031; 0.5 μmol/L, n=3), shifted FFT spectra from complex to a lower dominant frequency (10 Hz) and altered repolarization but retained the correlation between mean AIs and RPs. Thus, VF dynamics are consistent with a multiple wave-make and wave-break mechanism, and the local RP influences VF dynamics by limiting the range of VF frequencies and AIs at each site. The full text of this article is available at http://www.circresaha.org. (Circ Res. 2001;88:e49-e58.)

Key Words: action potential ■ refractory period ■ ventricular fibrillation ■ action potential duration ■ fast Fourier transform

Ventricular fibrillation (VF) had been traditionally thought of as a highly disorganized process of random electrical activity, but more recent studies described a substantial degree of structure and organization to seemingly random events.1–5 Optical mapping of electrical activity using a CCD camera in dog and sheep hearts showed that activation waves in VF emanate from a single periodic source.7

In contrast, other studies predicted that fibrillation consisted of wandering wavelets with a high level of disorganization, nonrepeatability, resulting in sporadic short-lived, ever-changing reentry.4,8 Still, activation intervals (AIs) in VF were correlated with the RPs of the normal action potential (AP)4 and agents that alter RPs also changed the number of activation fronts per second, demonstrating a correlation between tissue RPs and VF structure.5 Action potential durations (APDs) and RPs are dynamic parameters that change as a function of diastolic interval.9–11 The adaptation of the APD to a premature impulse or the restitution kinetics curve was proposed as a predictive cellular property that promotes wavebreaks when the slope of the curve is >1.11,12 According to the wave breakup hypothesis, waves are annihilated and new waves are created continu-
ously to maintain the complex behavior of VF. Plots of AIs versus the preceding AI revealed no clear periodicity, consistent with the continuous wave breakup hypothesis. Other studies showed that VF could exhibit multiple wavelet reentry in intact pig hearts, whereas in cryoablated hearts (with a thin layer of surviving epicardium) VF exhibited a single wavelet reentry.14

Both hypotheses regarding the nature of VF implicate spatial heterogeneities of RPs as a major determinant of VF structure and stability. In the continuous wave breakup hypothesis, the correlation between local RP and AI in VF would suggest that AIs in VF were spatially heterogeneous, like RPs. In the dominant spiral wave hypothesis, changes in RPs delineate the boundaries separating large domains containing a single spiral wave. Despite the significance of the spatial distribution of RPs, no studies have compared detailed maps of RPs to AIs and frequencies in VF.

Guinea pig hearts are now used to investigate the spatial distribution of VF dynamics and its correlation with gradients of APDs and RPs because the sequence of repolarization and RPs had been extensively characterized in this animal model. In guinea pig hearts, repolarization of the right and left epicardium was shown to start at the apex and to spread systematically toward the base.15-17 In the absence of anatomical or ischemic injury, RPs showed no abrupt heterogeneities, changed gradually along the epicardium, and were homogeneous along the endocardium.16 The sequence of repolarization and RPs was independent of cycle length (CL) or various sites on the ventricles (apex, base, anterior, or posterior) produced markedly different activation patterns yet similar repolarization patterns.15,16 The findings indicated that heterogeneities of intrinsic cellular properties of ventricular myocytes along the epicardium produce gradients of APDs, but the exact distribution of inward (I\text{Na} and I\text{Ca}) can account for rapid depolarizations in VF, the rapid repolarizations are more difficult to explain. Studies on the distribution of Erg (the channel protein underlying I_{Ks}) and the kinetics of the I_{Ks} current suggest that I_{Ks} could account for the gradients of APDs, rapid repolarizations in VF and VF frequencies, making I_{Ks} a dominant contributor to VF dynamics (see Discussion).

In this study, fluorescence signals from voltage-sensitive dyes were used to map APDs from 252 sites on paced guinea pig hearts. Next, VF was elicited by burst stimulation, and AIs were correlated to the local AP measured in paced hearts. Local conduction velocity vectors were used to test for the occurrence of Wenckebach-like conduction blocks in VF. AIs and frequency distributions of FFT spectra were correlated with APDs, in the absence and presence of a selective blocker (E4031) of the delayed K′ rectifier current (I_{Ks}) to prolong APDs and hence test the link between RPs and VF dynamics. Preliminary reports of these data appeared in abstract form.18

Materials and Methods

Preparation

Guinea pigs (female, ~400 g) were procured from Hill Top Lab Animals, Inc (Scottdale, Pa). Experiments were carried out in accordance with the Animal Welfare Act and the NIH Guide for the Care and Use of Laboratory Animals. Guinea pigs were anesthetized (pentobarbital 35 mg/kg) and injected with heparin (200 U/kg IP). Hearts were perfused in a Langendorff apparatus with (in mmol/L) NaCl 130, NaHCO$_3$ 25, MgSO$_4$ 1.20, KCl 4.7, dextrose 20, and CaCl$_2$ 1.25, at pH 7.4, and gassed with 95% O$_2$ and 5% CO$_2$ at 37.0±0.2°C. Perfusion pressure was adjusted to ~70 mm Hg by controlling the flow rate of perfusate and was continuously monitored. Hearts were placed in a chamber to reduce movement artifacts without using chemical uncouplers to arrest contractions.16,19,20 The voltage-sensitive dye (di-4-ANEPPS, 10 µL of 1 mg/mL dimethyl sulfoxide) was added to the perfusate while continuously recording heart rate, electrocardiograms, and perfusion pressure to ensure that staining did not produce lasting pharmacological effects.14

Optical Apparatus, Data Acquisition, and Analysis

An image of the heart was focused on the array by focusing the image on a graticule (Graticules Ltd), located on a plane paralfoocal to the array (Figure 1A). Outputs from 256 (16×16) diodes were amplified, digitized (12-bit) (Microstar Laboratories, Inc) and stored in computer memory.17,20 Unless stated, all maps were obtained from the anterior surface of the heart (Figure 1E). Figure 1C depicts a map of the array; each box represents a diode with APs detected by that diode. APDs were calculated from the activation ([dF/dt]max of the AP upstroke) and repolarization ([dF/dt]max of the AP downstroke) time points.19 Maps of APDs (Figure 1E) differ from maps of activation (Figure 1D) and demonstrate gradients of APDs from apex to base.16,17,19 Repolarization time points determined from ([dF/dt]max were shown to be equivalent to 97% recovery to baseline and coincident with the RP.19

VF was induced by burst stimulation (2× threshold, 2-ms duration, 30- to 50-ms CL for 1 second). After 2 minutes of VF, data were acquired as a series of scans (4.096 sec/scan) at 2000 frames/sec. During VF, the local ([dF/dt]max was considered an activation event, if >10% of maximum ([dF/dt]max (Figure 1). The time delay between activation events (eg, AIs) was measured from >50 beats to plot AI (AI_{E4031}) versus the previous AI (AI), as previously described.13

Local conduction velocities vectors were calculated for each diode from the differences in activation time points of that diode (determined from [dF/dt]max) and its 8 nearest neighbors, as previously described.20 Local velocity may be underestimated in VF because wavefronts may originate from deeper layers. The maximum local velocity vector was normally ~0.8 m/sec but exceeded 1.1 m/sec in VF in 18±4% of activation events (n=4 hearts). Local velocities ≥0.95 m/sec (maximum velocity +1 SD) could detect transmural propagation and were deleted from the analysis.

FFT analysis used an optimized FFT routine, FFTW (available at http://www.fftw.org) to speed up calculations. Each trace lasting 4.096 seconds (8192 points) was transformed to the power spectrum in the range of 2 to 40 Hz, providing a frequency resolution of 1/4.096=0.244 Hz. FFT analysis >4.096 seconds at the highest frequency resolution of the apparatus provided accurate frequency distributions across the heart. To investigate VF structure in various regions of the ventricles, hearts were rotated around the aortic cannula to record signals from all accessible surfaces (anterior, posterior, and right and left epicardia) (n=4 hearts).

Results

Spatial Distribution of AIs

The initiation of VF is illustrated in Figure 2A where AIs are recorded during 3 basic beats (CL 300 ms) followed by a burst of electrical stimuli (2 ms in duration, 50-ms CL for 1 second), which triggered long-lasting VF. Maps of activation...
and APDs recorded during the basic beat from the same heart before the induction of VF are shown in Figures 1D and 1E. A set of simultaneously recorded signals during VF shows that signals from the edges of the heart had lower signal to noise (S/N) ratio (Figure 2B). Hence, data from the edges were not included in the analysis of VF dynamics to avoid a bias of the results. Figure 2C (top trace) shows an expanded trace of voltage changes during VF and the first derivative of the signal (bottom trace). Movies (not shown) and isochronal maps of activation and during VF revealed no regular patterns or structural organization during the first 30 minutes of VF. An example of random activation patterns is depicted in panels 1 through 6 (Figure 2D), which shows activation maps from 6 sequential VF beats (labeled 1 through 6 in Figure 2C). Each VF beat produced a markedly different activation sequence because the initiation site(s), zones of functional conduction block, densities of isochronal lines, and directions of wavefronts varied from one VF beat to the next.

VF dynamics were examined by plotting $AI_n$ versus $AI_{n-1}$, as previously described by Garfinkel et al.13 (Figure 3A). The open circles represent a series of 74 AIs detected by a diode recording from the apex, and the solid triangles represent a series of 53 AIs from a diode recording from the base. Consistent with electrode studies,13 AI plots produced a random distribution, with no discernible clusters of data points, consistent with a nonperiodic process. However, the average AIs from sites at the apex and base were statistically
significant different with values of 57.5±8.1 and 76.1±1.5 ms (P<0.05, n=6), respectively. The shorter AIs at the apex than the base were found to correlate with the local APD measured from the same hearts, before VF. AIs were averaged over 4 seconds from 220 (of 252) diodes and plotted against APDs, demonstrating a statistically significant correlation (correlation coefficient 0.70) between the local mean AI in VF and the local APD in paced hearts (Figure 3B). A best fit between mean AI and local APD favors a linear relationship between these two parameters with a slope of 0.59±0.08 (n=6). Note that the slope is 1, because APDs in hearts paced every 300 ms are in the range of 160 to 200 ms whereas in VF, AIs fell in the range of 60 to 120 ms. The correlation of AIs in VF with local APDs in paced hearts is equivalent to the correlation of AIs to RPs because APDs measured from the second derivative of the AP downstroke are coincident with the RP. The correlation between APDs and AIs also implies that the ionic currents responsible for APDs in normal conditions influence VF dynamics.

Like the restitution kinetics of the AP amplitude, the rate of depolarization of a VF beat depends on the recovery of inward currents, which might depend on the previous AI. Figure 3C plots (dF/dt)max of VF depolarizations versus the previous AI for a site at the apex and a site at the base of the heart, showing a linear correlation between the rate of depolarization of a VF cycle and the duration of the previous AI. The analysis was repeated for a column of 16 diodes oriented from the base to the apex; all showed the same tight correlation between (dF/dt)max and the previous AI. Best-fit analysis produced lines with similar slopes but statistically significant differences in x-intercepts in going from the apex to the base (not shown). In 6 hearts, the correlation coefficients between (dF/dt)max and previous AI were 0.64±0.09 for sites at the base and 0.63±0.08 for sites at the apex. These findings indicate that determinants of refractoriness and recovery from RPs influence VF, resulting in gradients of AIs and (dF/dt)max in VF.

Local Velocity Vectors in VF

The distribution of the amplitude and orientation of local velocity vectors were also analyzed as an alternative approach to detect organized repetitive wavefronts in VF. The limitation of the approach was that activation wavefronts do not spread strictly along the surface of the heart but may contain transmural components of propagation emerging from deeper layers. Thus, conduction velocities measured from 2D data must be considered as apparent rather than

Figure 3. Refractoriness influences VF. A, Poincaré maps of AIs at the apex and the base. For a diode recording from the apex and the base of the heart, AIs were plotted as a function of the previous AI in a VF elicited by burst pacing. Poincaré plots show no clear periodicity, but AIs were consistently longer at the base than the apex, with a statistically significant difference in their distribution (P<0.01). B, Correlation between AIs and APDs. A plot of mean AIs versus APDs shows a high correlation (r=0.7) between APDs in paced hearts and AIs in VF. C, Effect of AI on the depolarization rate of the next VF beat. The maximum rate of rise of each depolarization in VF, (dF/dt)max, was plotted against the AI of the previous VF cycle. In this preparation, the correlation coefficients between (dF/dt)max and previous AI were 0.65±0.09 for 6 diodes at the base and 0.70±0.05 for 6 diodes at the apex. The dashed and solid lines represent the best fits for (dF/dt)max versus the previous AI using a linear regression algorithm, for sites on the apex and base, respectively.

Figure 4. Distribution of local conduction velocities in VF. Local conduction velocity vectors were measured during VF for 4 seconds (equivalent to 50 to 80 consecutive beats as described in Materials and Methods). Vectors were obtained from a diode recording signals from the base and the apex of the heart in panels A and B, respectively. Average data are depicted as histograms of the number of waves versus the direction of propagation in degrees for 50 consecutive vectors measured from 5 diodes recording from the base (panel C) and 5 diodes recording from the apex (panel D).
exact conduction velocities. Errors produced by transmural activation can be partially overcome by deleting from the analysis data diodes that record zones of synchronous activation, which indicate zones of epicardial breakthrough. The distribution of local conduction velocities during VF was analyzed from sites at the base (Figures 4A and 4C) and the apex (Figures 4B and 4D) of the heart. Local velocity vectors revealed a complex pattern of angles and amplitudes with no preferential amplitude or direction, even for a few consecutive cycles of VF, which is contrary to expectations for repetitive reentry circuits. However, the distribution of local velocity vectors at the base (Figure 4A) showed a slight tendency to cluster near 100 degrees, which might be caused by the neighboring atrioventricular boundary. The directions of local vectors were also calculated for 5 adjacent channels at the base and 5 at the apex and are displayed as histograms of angular distribution (Figures 4C and 4D). Histograms of local velocity vectors showed no preferential orientation (n=4 hearts). Hence, the analysis of local velocity vectors did not support the hypothesis that VF is an organized process.

Spatial Distribution of FFT Power Spectra

Another approach to test for spatial organization in VF is to analyze FFT spectra from various sites on the heart. As shown in Figure 5, FFT spectra recorded from a site on the base (Figure 5A, top trace) and from the apex (Figure 5B, bottom trace) were broad, with multiple peaks and no indication of a single dominant frequency. FFT spectra from the base had lower frequencies (Figure 5A, 12.05 ± 0.6 Hz) compared with the apex (Figure 5B, 16.3 ± 0.5 Hz). The analysis was then extended for all sites on the anterior surface of the hearts (n=4 hearts, 220 diodes per heart) to relate the normal gradient of APDs to the range of FFT frequencies at each site. Figure 6 is a 3D plot, with FFT frequencies plotted along the z-axis as a function of x-y location on the anterior surface of the hearts.

Figure 5. Power spectra of voltage oscillations during VF. FFT spectra from the base (A) and the apex (B) of the heart. Spectra had broad bandwidths, with multiple peaks but with different energy distributions at different sites. Apex regions had higher frequency components than the base consistent with the shorter AIs and APDs at the apex than the base. The FFT algorithm used a Hanning window to prevent smearing or spectral leakage caused by the finite time interval of sampling the signal.

Figure 6. Spatial distribution of FFT power spectra during VF. FFT spectra were analyzed from each diode over a 2-second interval of voltage oscillations during VF, and the frequency distributions (10 to 20 Hz) were plotted along the z-axis versus x-axis and y-axis, the spatial coordinates from base to apex, and from right to left ventricles, respectively. A map of APDs measured from the same hearts during a basic beat (CL 300 ms) was superimposed at the bottom of the 3D plot to visually correlate APDs in the paced heart to frequency distributions in VF. Note that frequency distributions had the inverse relationship to the APD distribution, decreasing systematically from apex to base. Maps of APDs measured, as described in Materials and Methods, are equivalent to maps of RPs.

Figure 7. Power spectra across the perimeter of the heart. FFT energy spectra were recorded around the perimeter of a group of hearts (n=4) to characterize the frequency distributions across the epicardium in VF and search for regions with a single dominant frequency indicative of a mother rotor. Hearts were rotated by 90° around the aortic cannula to record voltage oscillations in VF and measure FFT spectra from the right ventricle (A), anterior region (B), left ventricle (C), and posterior region (D). The most organized region was found to be a small region at the base of the right ventricle as shown in panel A. However, simple rotors were not observed even from the region that shows the most organized FFT spectrum.
surface of the heart. A 2D plot of APDs across the surface of the heart is superimposed below the 3D plot of FFT frequencies. FFT frequencies increased gradually from base to apex whereas APDs decreased progressively from base to apex. Those findings support the hypothesis that ionic currents responsible for gradients of APDs in normal cardiac rhythm influence the dynamics of the heart under VF.

The complex FFT spectra measured at all sites on the anterior surface of the heart imply that VF was not sustained by one or several rotors. Still, the possibility that the mother rotor (or zone with a single dominant frequency) existed in a region beyond the field of view was investigated by repeating the measurement around the perimeter of the hearts (n = 3). Figures 7A through 7D illustrate FFT spectra recorded from the right, left, anterior, and posterior surfaces of a heart, respectively. Although FFT spectra had somewhat different spectral characteristics in the different regions of epicardia, all regions exhibited complex broadband FFT spectra rather than a single dominant frequency in VT. The narrowest FFT spectra (ie, most organized) were found in a small region (~3×3 mm) at the base of the right ventricle, which tends to have the longest APDs (Figure 7A). It is unlikely that the base of the right ventricle (Figure 7A) can be the source of a mother rotor that sustains VF throughout the heart because activation maps showed no periodicity, dimensions were too small, and the maximum FFT frequency was not faster than elsewhere in the heart.

**Figure 8. Effect of APD prolongation by I\textsubscript{Kr} blockade on VF dynamics.** VF dynamics were analyzed; E4031 (0.5 μmol/L) was then added to the perfusate during VF, and measurements were repeated 10 minutes later (n = 3). A and B, APD maps (isochronal lines are 3 ms apart) measured in the absence (control) and presence of E4031 (0.5 μmol/L), respectively. APDs were measured at a CL of 300 ms (n = 3). C and D, Maps of mean AIs measured during VF in the absence and then 10 minutes after perfusion with E4031 from a heart in VF (isochronal lines are 10 ms apart). Patterns of APD correlated to patterns of mean AI, before and after E4031. E and F, FFT of traces before and after E4031 from a diode identified by the open circle on the maps shown in panels C and D. G and H, Poincaré plots of AIs for 2 sites on a heart. Two sites on the hearts were selected on the left ventricle (open circles) and right ventricle (asterisks) because they had similar APDs in a heart paced at a CL of 300 ms. E4031 altered APDs and AIs heterogeneously across the anterior surface of the heart, revealing a spatial heterogeneity of I\textsubscript{Kr}. E4031 prolonged APDs and mean AIs more at the apex of the right ventricle than at the apex of the left ventricle, eg, at sites labeled with an asterisk and an open circle, respectively. Panels G and H compare Poincaré plots from these two regions identified by an open circle and an asterisk in panels C and D, before and after E4031. In control plots (panel G), AIs from these 2 sites were random, forming a single cluster. With E4031 (panel H), APDs (panel B) and AIs (panel D) were prolonged in the right ventricle, eg, at a site labeled with an asterisk, resulting in marked differences in AIs in VF at the right and left ventricles (panel H).

**Ionic Currents Underlying APD Gradients and AIs in VF**

The link between the spatial distribution of APDs and AIs shown in the present study suggests that the heterogeneous current distributions that produce APD gradients likewise influence AIs and frequencies in VF. To investigate the role of I\textsubscript{Kr} as a possible determinant of the spatial distribution of APDs and AIs in VF, hearts were perfused with the selective I\textsubscript{Kr} antagonist E4031. E4031 (0.5 μmol/L) produced the maximum prolongation of APDs from 172±9 to 241±6 ms (n = 3) and marked changes in the distribution of APDs (compare Figures 8A and 8B), which indicated that I\textsubscript{Kr} is heterogeneously distributed on the epicardium. The effects of E4031 on VF dynamics were tested by inducing VF, mapping AIs, and then adding E4031 to the fibrillating hearts.
remapping AIs (n=3 hearts). In the absence of E4031, the mean AI maps were similar to control maps of APDs, with a pattern of short to long AIs from apex to base (Figure 8A versus 8C). With E4031, FFT spectra changed from complex broad energy distributions (Figure 8E) to narrow-bandwidth lower-frequency (Figure 8F) spectra, within 10 minutes. With E4031, maps of mean AIs (Figure 8D) were again similar to maps of APDs in the presence of E4031 (Figure 8B).

To highlight the link between APDs and AIs in VF, we selected 2 sites on the heart, one on the right ventricle and another on the left ventricle, that had similar APDs in control maps but different APDs after treatment with E4031. Figures 8G and 8H show Poincaré plots of AIs recorded from these 2 sites identified on the mean AI maps (Figures 8C and 8D) by an open circle and an asterisk. In this example, Poincaré plots show that in the absence of E4031, AIs from the 2 sites occurred with similar AI values of 45.8±9.7 ms on the left ventricle (open circle) and 38±9.1 ms on the right ventricle (asterisk) (Figure 8G). AI values were consistent with the APD values measured at the same sites and with the correlation of AIs with APDs shown in Figure 3B. With E4031, AIs from these 2 sites separated into 2 clusters with long (asterisks, 137±7.6 ms) and short (open circles, 72.5±22.3 ms) durations (Figure 8H), consistent with marked dispersion of long and short APDs recorded at the same sites in the presence of E4031 (Figure 8B). Maps of mean AIs (Figures 8C and 8D) showed substantial structure and organization much like those obtained in maps of APDs with or without E4031 (Figures 8A and 8B). The data show that the prolongation of APDs decreased VF frequencies and AIs and the inhibition of Is slowly narrows the bandwidth of FFT spectra most likely as a result of the substantial increase in RPs throughout the heart.

Discussion

The properties of VF and the possible relationship to the electrical properties of the normal myocardium are important concepts that could help us to better understand the mechanisms that sustain VF and improve strategies to interrupt VF. The present study investigates the relationship between VF dynamics and RPs by analyzing their spatial distribution. The main findings are that VF is not organized into zones of reentrant waves with a single dominant frequency but has a broad range of frequencies and that AIs are tightly correlated with the local APD and RP, producing gradients of AIs across the surface of the heart. Hence, heterogeneities of repolarizing K+ currents underlie gradients of APDs and RPs seen in the normal heart and influence VF dynamics.

Dispersion of Refractoriness and VF Structure

Heterogeneities of excitability and/or conduction velocities have long been implicated as mechanisms that initiate arrhythmias. Enhanced dispersion of refractoriness caused by tissue damage or pathological condition can produce a functional arc of conduction block. For instance, a premature impulse originating in an area with a short RP can capture, propagate, then encounter a zone with longer RPs, resulting in unidirectional propagation and a reentrant circuit that progresses to fibrillation. However, once VF reaches a steady state, it is not known whether properties of the normal myocardium (ie, gradients of APD and refractoriness) continue to dictate limits on VF dynamics. Once formed, stable spiral waves can be the source of activation wavefronts, and their periodicity may in turn depend on the local excitability and recovery of the tissue where the core is located. Optical recordings of atrial and ventricular signals using a CCD camera suggested that fibrillation is composed of a few domains, each with a single dominant frequency separated by boundaries of slow Wenckebach-like conduction. Chen et al. proposed that a single 3D scroll is sufficient to account for the spatial and temporal features of VF, and the boundaries between these reentrant waves can be attributed to zones with different RPs. In contrast, Moe et al. proposed a tight relationship between RPs and AIs in atrial fibrillation, and Janse reported a high degree of correlation between local APDs and AIs of VF in dog hearts. Previous studies did not directly measure the distribution of RPs and did not have sufficient spatial resolution to correlate AIs and VF frequencies to RPs at multiple sites. Hence, the relationship between RPs and VF dynamics and whether RPs dictate spatial and/or temporal features of VF dynamics were not firmly established and remained a matter of conjecture.

Validity of the Approach and the Animal Model

In our study, a 16×16 photodiode array was used to record transmembrane potentials from multiple locations with good S/N ratio (~100:1) and high temporal resolution (2000 frames/sec). Guinea pig hearts were chosen to study the spatial and temporal dynamics of VF because the electrophysiology and the initiation and termination of VF in these hearts have been well characterized and the gradients of APDs and RPs have been extensively investigated. In guinea pig hearts, AIs are short at the apex and are progressively longer toward the base, resulting in a gradient of APDs on the left, right, and anterior surface of the heart. In previous studies, we showed that in normoxic hearts, the repolarization time point taken as (dF/dt)max of the AP downstroke was shown to be coincident with the RP such that a map of APs can be used to generate an instantaneous map of RPs.

The size of guinea pig hearts was not a limitation because VF can be readily elicited by burst pacing, was maintained for >30 minutes, and could be terminated and re-induced, in the absence of ischemia or any anatomical block. Movement artifacts were reduced effectively by pressing the hearts gently against the glass window of the heart chamber. Numerous precautions were taken to ensure that hearts did not become ischemic when pressed against the glass window. Hearts were perfused at a constant flow rate; the aortic pressure was continuously monitored to check for an increase in coronary resistance, an indication of blocked vessels by the chamber glass. We required that the optical APs had stable plateau phases and long APDs rather than the triangulated shape characteristic of APs in ischemia. These precautions ensured that the heart was not ischemic, particularly within the field of view, and APD recordings were measured several times over 10 minutes before VF to guard against unstable preparations.
Guinea pig ventricular myocytes have been extensively studied, and the ionic channels and currents underlying the generation of the AP are well characterized with notable features such as the lack of $I_{\text{Kr}}$ currents. The present study demonstrates a tight correlation between AIs in VF with local APDs and RPs in paced hearts, indicating that a form of refractoriness persists in VF. Activation waves in VF invade regions of myocardia before the myocytes recover fully from the previous depolarization; yet AIs in VF still influence the next AI, its rate of depolarization, and mimic the distribution of APDs in the normal heart.

**Distribution of $I_{\text{Kr}}$ May Underlie APD Gradients and AIs in VF**

The distribution of APDs along the epicardium of guinea pig hearts has been extensively demonstrated, but the ionic channels that are heterogeneously distributed on the epicardium to produce APD gradients are unknown. Repolarizing $K^+$ channels ($I_{\text{Ca}}, I_{\text{ks}}, I_{\text{Kr}}$) are heterogeneously distributed along the ventricular wall, which could account for the observed dispersions of repolarization. Several studies are consistent with the notion that $I_{\text{Kr}}$ may contribute to the gradients of repolarization and the dispersion of AIs in VF, shown in Figure 8. The delayed rectifying outward current, $I_{\text{ks}}$, was distributed heterogeneously in various species of mammalian hearts. In ferret hearts, immunohistochemistry of Erg, the channel protein responsible for the rapid component of the delayed rectifier current $I_{\text{ks}}$, was shown to be heterogeneously distributed (decreasing from apex to base), consistent with the gradients of APDs reported in the epicardium of guinea pig heart. In rabbit ventricles, the ratio of $I_{\text{Kr}}/I_{\text{ks}}$ currents was found to be greater at the apex than at the base, consistent with the data on ferret hearts. Guinea pig ventricular myocytes do not express the channel protein underlying the transient outward current, $I_{\text{to}}$, leaving the distribution of $I_{\text{ks}}$ as the most likely current to contribute to gradients of APD and its kinetics to account for rapid repolarization during VF beats. In guinea pig myocytes, $I_{\text{ks}}$ was known to activate early in the plateau phase of the AP and was thought to have a negligible contribution to the early phase of repolarization. Gintant reexamined the contribution of $I_{\text{ks}}$ to the early phase of the AP downstroke and proposed that $I_{\text{ks}}$ contributed a considerable component of the total repolarizing $K^+$ current. During an AP, most $I_{\text{ks}}$ channels activate early in the plateau phase, then rapidly shift to an inactivation state while still in the plateau phase. As the plateau potential decreases, a threshold voltage for $I_{\text{ks}}$ channels is reached, resulting in a shift to the open state and a large $K^+$ repolarizing current before channel closure. Thus, $I_{\text{ks}}$ could account for APD gradients and rapid voltage repolarizations that are required to produce voltage oscillations in VF. Indeed, the $I_{\text{ks}}$ blocker E4031 produced marked changes in VF dynamics, changing a complex frequency distribution to a major dominant frequency and considerably longer AIs, particularly at sites with prolonged APDs (Figures 8G and 8H).

**Single or Multiple Wavelets?**

Multiple wavelet reentry has been extensively studied as a possible mechanism for VF, where spiral waves can be subdivided by wavebreaks to produce multiple wavelets. In the present study, FFT spectra had a broad power spectrum with multiple peaks appearing from a 4-second analysis of VF, and broad spectra were observed at all sites on the epicardial surface. The complex power spectra are consistent with the hypothesis that VF is composed of multiple wavelets undergoing wave breakup. Our data are inconsistent with a single spiral or 3D scroll because we failed to observe large areas with a monolithic dominant frequency ($n=7$). Several factors could account for the different findings reported here and previous data from a CCD camera. The photodiode array provided a significant improvement in sampling rate and S/N ratio compared with CCD cameras, hence a superior temporal resolution and accuracy of FFT spectra. On the other hand, CCD cameras have a greater spatial resolution than photodiode arrays (if spatial averaging is not required to improve the S/N ratio) so that each FFT spectrum was derived from smaller regions of tissue. Another difference is that FFT spectra were determined from 4 seconds of continuous data compared to 2 seconds in previous studies that used a CCD camera. The longer time of analysis increases the frequency resolution and improves the details regarding the frequency distributions in VF signal. However, the FFT algorithm represents the time-averaged behavior during the time of analysis and contains no information regarding when each peak occurred within that time interval. Thus, each peak in the frequency domain could appear and disappear and hence more peaks are seen when the time of analysis is increased. To examine the possibility that the FFT spectra will have a markedly altered energy distribution, the FFT analysis window was reduced from 4 seconds to 0.5. Windows of 2 seconds (0.5-Hz resolution) did not produce a single peak in the frequency domain. With a 0.5-second (2-Hz resolution) window, FFT spectra showed a single peak, but different regions of the heart had different peak frequencies, which would remain inconsistent with a highly organized process. Further studies are required to adjust the window of analysis to encompass the lifetime of each peak in the frequency domain, which represents the lifetime of spirals and their breakup.

Species differences may also account for the different findings on the structure of VF. Guinea pig ventricles lack $I_{\text{to}}$ currents, so different ionic currents, with different spatial distributions, are involved in setting limits to VF dynamics. In rabbit heart, $I_{\text{to}}$ currents produce spatial distributions of repolarization that contribute to repolarization in VF, which may alter VF dynamics and VF frequencies. Finally, edema of the ventricular myocardium must be avoided to protect the heart and avert changes of conduction velocities and VF dynamics.

**Analysis of Local Conduction Velocity**

The spatial and temporal distribution of local velocity vectors was used in attempts to quantitatively characterize Wenckebach-like activation waves in VF that might occur at boundaries separating rotors of different phases and/or fre-
quencies. Previous studies had shown that such boundaries were invaded by wavefronts emanating from the two rotors that could alternatively propagate or be blocked on encountering functional conduction blocks due to spatial and temporal changes in refractoriness, resulting in Wenckebach-like conduction. Such periodic wavefronts were identified on the epicardium of hearts in VF using space-time plots, an approach that lacks quantitative information regarding the velocity and orientation of the waves. To quantitatively assess the occurrence of Wenckebach-like conduction on the epicardium during VF, the magnitude and direction of local velocity vectors were analyzed as previously described at multiple sites on the epicardium for 4-second intervals during VF. These data illustrate the findings for sites on the apex (5 diodes) and the base (5 diodes) (Figure 4) and showed a random distribution of angles, which is inconsistent with a periodic process or Wenckebach-like wavefronts.

The restitution kinetics of APDs and conduction velocities are also important to understand the stability of spiral waves. Restitution curves with steep slopes predict an increase in APD oscillations and increased vulnerability to VF. The role of conduction velocity oscillations is less understood, but simulation studies predict that velocity oscillations produce CL oscillations and conduction blocks that may lead to wave breakups and new spiral waves in VF elicited by burst stimulation. The role of conduction velocity oscillations in VF has not been confirmed experimentally because of the technical difficulties of measuring conduction velocities in 3D. Hence, it should be emphasized that local conduction velocities measured in the present study may contain components of transmural propagation even after precautions were taken to eliminate zones of highly synchronous activation. For this reason, the analysis of conduction velocities should be limited to test for the occurrence of Wenckebach-style conduction and should not be overinterpreted to provide evidence for the wave breakup or the mother rotor hypotheses.

Limitations: Ischemia and Effects of 3D Structure of the Heart

The findings correlating VF dynamics to dispersion of refractoriness are limited to VF in normoxic hearts with constant coronary perfusion. In the clinical setting, VF will rapidly trigger a loss of perfusion pressure, ischemia, and acidosis. These conditions would compromise the metabolic state of the heart, depolarize the resting membrane potential, and alter RPs (ie, postpolarization refractoriness) and perhaps the dispersion of refractoriness. Ischemia also alters ionic currents, eg, ATP-sensitive K+ currents modifying the myocardial substrate compared with normoxic hearts, thus changing VF dynamics and the correlation between electrical activity in VF with those of the normoxic paced heart.

Another limitation is that this study does not address the role of 3D structure of the heart and how this modifies VF dynamics. Cryoadiblation and chemical ablation of the specialized conductile system and modeling studies show that the 3D structure of hearts can contribute to complex wavefronts in VF. In hearts with thick walls, the propagation of the activation wave in the deeper layers produces more complex pathways, and the emergence of these waves to the surface results in more complex epicardial wavefronts. In the left ventricle of guinea pig hearts, the apex has a thicker wall than the base and one should consider the possibility that the apparent correlation between AIs and APDs may be due to gradients of tissue thickness across the heart. However, this study examined VF dynamics around the perimeter of the heart and failed to find zones with periodic spiral waves. If wall thickness is the important parameter to correlate VF dynamics, then according to this hypothesis, the middle region between the right and left ventricles should show the shortest AIs. Our data reveal no clear relationship between AIs and anatomical landmarks and support the gradient of RPs as the important factor limiting AIs. The influence of wall thickness was examined by cryoadiblation of the endocardium to produce a thin layer of surviving tissue on the epicardium as described by Allessie et al. However, in agreement with previous reports, VF was very difficult to induce by burst stimulation in cryoadiblated hearts (n=6 hearts), possibly as a result of the reduction of total tissue mass and/or the ablation of the Purkinje system, which may also play a pivotal role in sustaining VF. An elucidation of the role of Purkinje fibers to promote persistent VF and as a determinant of VF dynamics will hopefully be achieved with the development of 3D mapping techniques.

Acknowledgments

This work was supported by grant awards from the National Institutes of Health, R01 HL57929 and HL59614 (to G.S.), and a predoctoral fellowship from the Western Pennsylvania Affiliate of the American Heart Association (to B.-R.C.). Thanks are due to the staff of our Departmental Machine Shop: Scott J. McPherson and William B. Hughes for the construction of optical components and heart chamber.

References


The Distribution of Refractory Periods Influences the Dynamics of Ventricular Fibrillation
Bum-Rak Choi, Tong Liu and Guy Salama

Circ Res. 2001;88:e49-e58
doi: 10.1161/01.RES.88.5.e49

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/88/5/e49

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/