Shock-Induced Figure-of-Eight Reentry in the Isolated Rabbit Heart

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Abstract—The patterns of transmembrane potential on the whole heart during and immediately after fibrillation-inducing shocks are unknown. To study arrhythmia induction, we recorded transmembrane activity from the anterior and posterior epicardial surface of the isolated rabbit heart simultaneously using 2 charge-coupled device cameras (32,512 pixels, 480 frames/second). Isolated hearts were paced from the apex at a cycle length of 250 ms. Two shock coils positioned inside the right ventricle (−) and atop the left atrium (+) delivered shocks at 3 strengths (0.75, 1.5, and 2.25 A) and 6 coupling intervals (130 to 230 ms). The patterns of depolarization and repolarization were similar, as is evident in the uniformity of action potential duration at 75% repolarization (131.4±8.3 ms). At short coupling intervals (<180 ms), shocks hyperpolarized a large portion of the ventricles and produced a pair of counterrotating waves, one on each side of the heart. The first beat after the shock was reentrant in 90% of short coupling interval episodes. At long coupling intervals (>180 ms), increasingly stronger shocks depolarized an increasingly larger portion of the heart. The first beat after the shock was reentrant in 18% of long coupling interval episodes. Arrhythmias were most often induced at short coupling intervals (98%) than at long coupling intervals (35%). The effect and outcome of the shock were related to the refractory state of the heart at the time of the shock. Hyperpolarization occurred at short coupling intervals, whereas depolarization occurred at long coupling intervals. Consistent with the “critical point” hypothesis, increasing shock strength and coupling interval moved the location where reentry formed (away from the shock electrode and pacing electrode, respectively). (Circ Res. 1999;85:742-752.)

Key Words: action potential  electrical stimulation  arrhythmia  fibrillation  optical mapping

Understanding the underlying mechanisms of ventricular fibrillation (VF) is crucial in a society in which sudden cardiac death is the leading cause of mortality.1 VF can be induced in healthy hearts when an electrical stimulus occurs during ventricular repolarization (ie, the “vulnerable period”).2–4 Only certain shock strengths induce fibrillation during the vulnerable period5–8; the highest shock strength to induce fibrillation is called the upper limit of vulnerability (ULV).9 It has been shown that the ULV correlates with the defibrillation threshold, which suggests a mechanistic link between fibrillation induction and defibrillation.8,10 The earliest proposed mechanisms of defibrillation and VF induction suggested that a strong electric shock results in the depolarization of cardiac tissue by direct activation followed by propagating activation fronts,11–13 but recent data suggest that shock-induced hyperpolarization also plays a role.14–18

Among the first proposed mechanisms for the induction of fibrillation was the dispersion of refractoriness hypothesis,4,19 which suggests that reentry forms when a wave propagates into a region of cardiac tissue with a nonuniform dispersion of refractoriness. Conduction block occurs in the more refractory area while propagation continues in the less refractory area, giving rise to unidirectional block. Rapid pacing or shock-induced accelerating beats have been shown to increase the dispersion of refractoriness and cause wavefronts to break down into reentry.4 Similarly, Dillon and Kwaku20 suggest that a progressively stronger shock will increasingly depolarize recovered tissue and also progressively increase the synchronization of repolarization. During the vulnerable period, if a shock is not strong enough to synchronize the repolarization of the refractory tissue, the shock-induced wavefront may encounter a dispersion of refractoriness leading to reentry.20

Another proposed hypothesis of fibrillation induction is the “critical point” (CP) mechanism. Introduced by Wiener and Rosenbleuth21 and later extended by Winfree,22 this hypothesis states that a CP will form where a critical degree of refractoriness intersects a critical strength stimulus.12 A shock-induced wavefront will propagate into recovering tissue but block in regions exhibiting residual refractoriness or stimulus-induced prolongation of refractoriness. The location of the CPs on the heart depends on the state of refractoriness at the time of the shock. Therefore, applying a stimulus at different coupling intervals within the vulnerable period will

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affect the location of the CPs. Similarly, varying the shock strength will also modify the extracellular potential distribution and the portion of the heart subjected to the critical stimulus strength, therefore moving the location of the CPs. According to the CP mechanism, ULV is the shock strength that will create a potential gradient greater than the critical value throughout the ventricular myocardium at all coupling intervals.9 If no CPs are formed in the heart, there will be no induction of fibrillation.

Recent experiments using optical mapping18,23,24 and simulations incorporating anisotropic bidomain models25,26 have demonstrated that electrical stimulation simultaneously depolarizes and hyperpolarizes cardiac tissue. The spatial patterns of the change in transmembrane potential (V_m) in anisotropic cardiac tissue during stimulation are usually composed of a “dog bone” shape of one polarity under the electrode and 2 regions of the opposite polarity, one on each side of the electrode along the fiber direction.17 New mechanisms of fibrillation induction27,28 and defibrillation29 have been proposed on the basis of the spatial distribution of V_m. One hypothesis suggests that the location where reentry occurs is at the intersection of shock-induced areas of positive, negative, and no polarization.27,28

The characterization of V_m from the whole heart during and after fibrillation-inducing shocks has not been accomplished. The purpose of this study is to investigate how coupling interval and shock strength influence the outcome of a shock during pacing. By recording V_m before, during, and after shocks, we seek to improve our understanding of the mechanisms responsible for fibrillation induction.

Materials and Methods

Isolated Rabbit Heart

The excised heart was connected to the perfusion system and continuously perfused via the aorta with oxygenated warm (36°C to 38°C) Tyrode solution at a constant flow (50 mL/min). The excitation-contraction decoupler 2,3-butanedione monoxime (diacetyl monoxime; DAM) was added to the perfusate (500 mg/L).30 Subsequently, the heart was perfused via the aorta with oxygenated warm (36°C to 38°C) Tyrode solution. The heart was paced using a bipolar (Guidant) catheter placed at the apical portion of the posterior wall of the left ventricle. The pacing protocol was as follows: 100 ms pacing interval (10-ms duration), 20-s pacing period, 20-s interval between pacing sequence. The pacing sequence was followed by 10-s period of ventricular pacing at the same rate. The pacing and ventricular activation sequence observed were classified as undetermined.

Definitions of Terms

“Activation time” refers to the time at which the action potential (AP) upstroke reaches 50% of the maximal amplitude33 (Figure 1C). “Repolarization time” is the time at which the AP is 75% recovered. “AP duration” (APD_50) is the time difference between the repolarization time and the activation time. “Hyperpolarization/depolarization” refers to a decrease/increase of V_m at the end of an S2 shock compared with the V_m of the ninth S1 at the same time relative to the onset of the (ninth and tenth) pacing stimulus. “Coupling interval” refers to the time interval from the onset of the tenth S1 to the onset of the shock. Values are presented as mean±SD, unless otherwise indicated.

An expanded Materials and Methods section is available online at http://www.circresaha.org.

Results

Overview

Hearts from 7 rabbits (3.8±0.5 kg) were studied. The diastolic threshold was 0.5±0.36 mA. All hearts were mapped from both the posterior and the anterior surfaces. For all 7 hearts, the resolution for both the anterior and the posterior surfaces was 304±10 μm/pixel (size of the field of view, 3.9×3.9 cm). The portion of the ventricles projected to the field of view was 81.5±2.7% (n=4) by using fiduciary markers every 5 mm. The total number of shocks (including defibrillation shocks) given to each heart was 23±2. The energy delivered for 0.75-, 1.5-, and 2.25-A shocks was 0.25±0.02, 1.01±0.07, and 2.28±0.18 J, respectively. The shock impedance calculated from the mean of all shocks measured in 6 hearts was 59.7±3.6 Ω.

The signal-to-noise ratio (SNR) of the raw data was 8.7±1.0 (n=7) at the beginning of the experiment and decreased by ≈50% by the last recording. After the 5-point median filter, the SNR increased to 9.2±1.3. The spatiotemporal conical filter increased the SNR to 24.6±4.6. Fluorescence magnitude (ΔF/F) for all hearts was 8.5±0.7%.

Depolarization and Repolarization During Pacing

The spatial distribution of depolarization and repolarization was studied using the ensemble average pacing episodes. The pacing activation sequence followed an apex-to-base pattern. All rabbit hearts had a very similar spatial pattern of activation and repolarization during pacing. An example is shown in Figure 1; the earliest activity appeared on the anterior apical portion of the epicardium 29.2 ms after the onset of the S1 pulse (panel A, left). The earliest activity on the posterior side occurred 33.3 ms after the onset of S1 (panel A, right).
The earliest recovery time on the heart surface was 150 ms after the onset of the pacing stimulus on the posterior side (panel B). For all hearts, the mean APD₉⁵ for all sites was 131.4±8.3 ms with a mean spatial SD of 9.0±2.5 ms.

The dispersion of V₉⁵, quantified as the spatial SD of V₉⁵ on the epicardium for all ensemble average episodes, is plotted in Figure 1D along with the percentages of induced arrhythmias for each coupling interval (circles). The first peak occurred 50 ms after the onset of the pacing stimulus when the heart is depolarizing. The second peak occurred 170 ms after the onset of the pacing stimulus and coincides with the repolarization of the heart. The dispersion is greatest during depolarization. It is the dispersion of repolarization that is normally associated with arrhythmia induction. The tracing in Figure 1D can be compared with an ECG, with the second peak representing the T wave. In this protocol, short coupling intervals were associated with a high probability of arrhythmia induction, whereas episodes with long coupling intervals mostly resulted in a shock-induced wave that propagated throughout the heart (single beat). At late coupling intervals, inducibility decreased to 0.

**Effect of the Shock on V₉⁵**

Figure 2 shows the effect of coupling interval and shock strength on V₉⁵ at one site, located on the anterior RV near the apex and close to the cathode. The effect of the shock on V₉⁵ varied nonlinearly with coupling interval. For strong shocks and short coupling intervals, shock-induced hyperpolarization occurred, immediately followed by depolarization. The hyperpolarization magnitude occurring during the shock was greatest at the shortest coupling intervals examined. The AP for 1.5 A/150 ms is typical of a pixel located near the line of block of a reentrant circuit (see below). The effect of the shock on V₉⁵ also varied nonlinearly with shock strength. At coupling intervals >190 ms, no hyperpolarization was observed during the shock; however, depolarization occurred during or shortly after the shock. Variations in shock strength did not produce a proportional variation in the magnitude of V₉⁵ changes. A 3-fold increase in shock strength (from 0.75 to 2.25 A) did not produce a 3-fold change in V₉⁵ during the shock at any site. (For all coupling intervals, shocks depolarized the entire atrial epicardium in the field of view.)

**V₉⁵ at the End of the Shock**

V₉⁵ at the end of the shock was dependent on the refractory state of the heart at the time of the shock (ie, coupling interval). At the shortest coupling interval studied (130 ms), the ventricles were mostly depolarized at the time of the shock, with the basal region at high V₉⁵ and the apical region at lower V₉⁵ (Figure 3A, top). At the longest coupling...
interval (230 ms), the entire ventricle surface was recovered (Figure 3A, bottom). To allow a comparison between \( V_m \) and activation patterns, Figures 3, 4, and 5 were generated from the same heart, which we considered representative of the population studied. This heart was selected because it presented good signals throughout the 18 episodes. Figure 3B is a map of \( V_m \) at different times for different shock applications. To facilitate the comparison between the state of the heart without a shock (Figure 3A) and the state of the heart after a shock (Figure 3B), each row of Figure 3A displays \( V_m \) 10 ms after the coupling interval indicated (the equivalent of a 0-A-strength S2 shock) for an ensemble average episode.

By comparing panel B with panel A, one can identify regions of shock-induced hyperpolarization and depolarization. At short coupling intervals, the region near the RV cathode and the apical region of the ventricles were hyperpolarized by the shock. For coupling intervals <190 ms, the size of the region hyperpolarized by the shock decreased with decreasing shock strength and increasing coupling interval (Figure 3). Figure 3B (from 130 to 170 ms) shows an increase in the size of the recovered region (blue and purple colors), yet the size of the hyperpolarized region decreased because of the increased recovery (panel A) as a result of the later application of the shock (ie, increasing coupling interval). At coupling intervals >190 ms, the shock depolarized the heart near the RV cathode. The size of the region depolarized by the cathodal shock increased with increasing shock strength and increasing coupling interval.

First Beat After the Shock

Figure 3A shows isochrones of the beat starting at the onset of the shock. In Panel A, 3 episodes are presented in detail for

Figure 3. Transmembrane potential at the end of the shock. All potential maps are grouped in pairs (anterior view left and posterior view right). A, Potential maps obtained from the ensemble average beat, 10 ms after the time labeled on the left (coupling intervals). At short coupling intervals, most of the ventricles were depolarized (yellow-red), whereas at long coupling intervals, most of the ventricles were recovered (purple). As with the activation pattern, the repolarization followed an apex-to-base sequence (see Figure 2). B, Potential maps of the heart at the end of the shock (strength noted at the top of each column) for all coupling intervals and shock strengths. For 2.25 A at 130-ms coupling interval, much of the basal portion of the ventricles was at high potentials (red), whereas the apical region was mostly hyperpolarized (blue-purple). For 0.75 A at 230-ms coupling interval, most of the ventricles were at low potentials (purple), whereas some depolarization (green/yellow) appeared in the region near the RV cathode. The atria have been removed from the picture for clarity.

Figure 4. Shock-induced activation sequence. Isochrones starting at the time of the S2 shock. A, For 2.25-A shocks applied at 150-, 190-, and 230-ms coupling interval, isochrones are presented for every frame (or 2.08 ms between lines). Hatched region remained depolarized during and after the shock until the next wavefront (200 ms later). Gray regions were activated during the shock. At coupling interval of 150 ms, the first activation occurred 12.5 ms after the onset of S2. B, For all shock strengths at all coupling intervals, isochrones are presented for every fifth frame (or 10.4 ms). × in panels indicates areas that were activated during the shock. Darker lines appear at the boundaries of the ventricles and where many isochrone lines meet (ie, in areas of block and at frame lines). Arrows indicate direction of wavefront propagation.
allowing propagation toward the base. Propagation on the RV through the LV reached the basal RV at the same time, the part of the wavefront propagating refractory than the apical, causing slow propagation on the half of the heart. The basal portion of the heart was more shock initiated a wavefront that rapidly activated the apical heart had repolarized before the shock was applied. The ms (Figure 4A, middle), most sites in the apical half of the sites repolarized and the wavefront moved from the LV apex forming a closed reentrant loop. At a coupling interval of 190 ms (Figure 4A, bottom). The first sites to activate were in the region most hyperpolarized by the shock (near the RV cathode). Because the sites basal to the area hyperpolarized were refractory, no activation larized by the shock (near the RV cathode). Because the sites

the region near the electrode. At a coupling interval of 150 ms (Figure 4A, top), the shock produced hyperpolarization in the apical RV region, whereas basal regions were not affected. The first sites to activate were in the region most hyperpolarized by the shock (near the RV cathode). Because the sites basal to the area hyperpolarized were refractory, no activation propagated toward the base on the RV. The activation propagated through recovered sites from the apical RV to the LV. While the sites at the apex were being activated, the basal sites repolarized and the wavefront moved from the LV apex toward the base and eventually returned where it started, forming a closed reentrant loop. At a coupling interval of 190 ms (Figure 4A, middle), most sites in the apical half of the heart had repolarized before the shock was applied. The shock initiated a wavefront that rapidly activated the apical half of the heart. The basal portion of the heart was more refractory than the apical, causing slow propagation on the RV toward the base. The part of the wavefront propagating through the LV reached the basal RV at the same time, allowing propagation toward the base. Propagation on the RV was not slow enough to qualify as block,12 and reentry was not initiated, resulting in a nonsustained arrhythmia. At a coupling interval of 230 ms, the shock depolarized a large portion of the heart very rapidly, producing a single beat (Figure 4A, bottom).

For coupling intervals of 130 to 170 ms, all strengths (Figure 4B, top 9 panels), the earliest region to activate in the field of view was near the RV cathode. The wavefront moved from the RV cathode first toward the apex and then up toward the base through the left ventricle and finally came back down the RV to complete the reentry loop. The reentrant circuits on the anterior and posterior views had opposite rotation direction when viewed from the epicardium, counterclockwise and clockwise, respectively. For shocks of coupling intervals <190 ms, increasing the shock strength from 0.75 to 2.25 A increased the extent of the line of block away from the RV toward the LV because of faster propagation at the apex after stronger shocks. The wavefront started to curve in the basal direction ≈30 ms after the end of the shock. At 2.25 A (coupling interval<190 ms), both ends of the line of blocks were located on the left ventricle (LV), whereas at 0.75 A, they were both on the RV. At a coupling interval of 190 ms, slow propagation was observed near the RV cathode at the same location where block occurred at shorter coupling intervals; however, reentry did not occur. For coupling intervals >190 ms, the shock depolarized a large portion of the ventricles during the shock, and the entire heart was depolarized within 60 ms after the onset of the shock.

**Second Beat After the Shock**

Figure 5 shows isochrones for the second beat of activation after the S2 shock. Activation times are continuous with the previous beat (Figure 4), and plus signs and asterisks mark the first isochrone of beat 2. Isochrones of sinus rhythm for the 230-ms coupling intervals were omitted. For coupling intervals of 130 to 170 ms at all shock strengths, propagation followed a pattern similar to that of the first beat. For coupling intervals of 190 and 210 ms, focal beats were characterized by breakthrough patterns on the ventricular epicardium (concentric isochronal lines).

**Sustained Versus Nonsustained Arrhythmias**

The numbers of both sustained and nonsustained arrhythmia episodes for each coupling interval at all strengths combined are shown in the Table. The Table presents the number of episodes for each outcome by coupling interval. The first 2 beats of sustained and nonsustained arrhythmia episodes were separated into 4 categories: reentrant, focal, global depolarization, and undetermined. Patterns that we could not classify presumably because of a missing portion of reentrant circuit (wavefronts arising from the lateral boundary of the heart within the field of view) were labeled undetermined. Relatively short coupling intervals were associated with the induction of arrhythmias, whereas episodes with long coupling intervals mostly resulted in a shock-induced wave that propagated throughout the heart (single beat). Many arrhythmia induction episodes were associated with reentrant patterns immediately after the shock (Table, first beat) and later (Table, second beat). At short coupling intervals, reentrant patterns formed immediately after the shock. At a coupling interval of 230 ms, all shocks directly activated a portion of the ventricles resulting in single beats followed by sinus rhythm (no reentry was observed).

Reentrant patterns were observed during the first beat after the shock in 90% of episodes for sustained arrhythmias compared with 48% for nonsustained arrhythmias (P<0.01). During the second beat after the shock, 91.7% of sustained episodes were reentrant compared with 48% of nonsustained
episodes ($P<0.01$). The probability of an episode being sustained given that the second beat was reentrant was 73.3\% (33/45). Similarly, the probability of an episode being non-sustained given that the second beat was focal was 83.3\% (15/18). The undetermined episodes were excluded from the above percentages.

Reproducibility of Shocks

Three episodes of the same coupling interval/strength (190/1.5, 150/0.75, and 210/0.75) were repeated on the same hearts at the end of the protocol. The first 2 resulted in reentrant and focal nonsustained arrhythmias, and the last in a single beat. For all duplicated episodes ($n=3$), the beat sequence was nearly identical. In one case, all duplicate episodes of the nonsustained arrhythmias lasted 6 beats. The similarity of pattern for all 6 beats suggests that activation patterns are deterministic and repeatable up to 500 ms after the shock.

Uniformity Index and Phase Singularities

To quantify the sign of the polarization resulting from the shock, we have measured the uniformity index for all animals at all shock strengths and coupling intervals (Figure 7A). At short coupling intervals (130 to 150 ms), the major effect of the shock was to hyperpolarize $V_m$, whereas at longer coupling intervals (190 to 230 ms), the effect was depolarization.

Phase singularities were selected for every episode in which the isochrone maps demonstrated a reentrant pattern immediately after the shock. Figure 6 displays the spatial distribution of phase for the examples shown in Figure 4A. Phase singularities are defined as sites around which all phase values ($-\pi$ to $\pi$) converge. For a coupling interval of 150 ms, 2 phase singularities formed immediately after the shock (one on each side of the heart, Figure 6A, top) and 33 ms later (Figure 6B, top) were established and moved toward the tip of the line of block (shown on the isochrones of Figure 4B; 2.25 A, 150 ms). For a coupling interval of 190 ms, the phase pattern at the end of the shock did not demonstrate the formation of phase singularities (Figure 6A, middle), and 33 ms later (Figure 6B, middle) the phase gradient had almost disappeared, demonstrating a global pattern of depolarization. For a coupling interval of 230 ms, the shock did not produce much phase variation throughout the ventricles (Figure 6A, bottom), and 33 ms after the shock, the phase was synchronized over the whole heart (Figure 6B, bottom).

The location of the phase singularity was manually selected from phase maps at 33 ms after the onset of the shock. The distances ($d_y$) in mm between the apex and the phase singularities for all 3 strengths were fitted to a linear regression ($d_y=-3.9\times$coupling interval +0.1, $P<0.0001$, Figure 7B). The distance ($d_y$) in mm between the edge of the RV and the phase singularities for all coupling intervals was fitted to a linear regression ($d_y=1.4\times$S +8.1, $P<0.0001$, Figure 7C), where S is the shock strength. Overall means and SEs of phase singularity location are plotted in panels B and C of Figure 7. These equations suggest that by applying the shock at later coupling intervals, the location of phase singularities moved further away from the apex. Similarly, by applying a stronger shock, the location of phase singularities moved further away from the RV edge. The distribution of $V_m$ before the shock for all sites identified as phase singularities is plotted in Figure 7D. $V_m$ before the shock, for sites identified as phase singularities, was $-23.5\pm13.8$, whereas $V_m$ at all other sites was $-36.7\pm26.0$. Both the mean and variance were significantly different ($P<0.0001$).
Discussion

Spatial Distribution of $V_m$

For a long time, investigators have related the outcome of an electrical stimulus or the vulnerability of the heart to the dispersion of repolarization.19 Traditionally, dispersion has been measured as the maximum difference between repolarization times at several sites. Such measurements have provided evidence that a critical dispersion of repolarization times is required to induce fibrillation for localized stimulus.37,38 Kirchof et al.39 have shown that the dispersion of repolarization times is a better predictor of the vulnerable period than T-wave parameters obtained from the ECG. Laurita et al.40 have recently reported that the dispersion of repolarization can be influenced by a premature stimulus and that a decrease in dispersion results in a decrease in vulnerability. Because our experimental approach renders such a high spatial resolution with tens of thousands of sites, we were able to calculate the spatial dispersion over the majority of the epicardium (measured as the spatial SD of $V_m'$) during every recorded frame (every 2.1 ms). By comparison, the traditional measure of dispersion estimates the width (in time) of the second peak of the curve, occurring during repolarization (Figure 1D). According to our study, arrhythmia induction occurred when the spatial dispersion of repolarization was high, but the spatial dispersion of $V_m'$ at the time of the shock was not linearly related to inducibility. The highest probability of inducing arrhythmias (Figure 1D, coupling intervals of 130 and 150 ms) did not occur at the peak of repolarization dispersion, but before the peak.41,42 The relationship between dispersion of repolarization and vulnerability to induce arrhythmias in the normal heart is consistent with the CP hypothesis. Figure 1D shows a decrease of inducibility starting at the peak of the dispersion of $V_m'$ during repolarization. This suggests that other factors such as reentry through the Purkinje system or rapid firing, and shock-induced hyperpolarization and depolarization, as predicted by the virtual electrode hypothesis, may contribute to heart vulnerability.

Outcome

The 6 coupling intervals studied resulted in outcomes ranging from VF induction to single shock-induced activations. The number of episodes that induced arrhythmias in the Table suggests that our strength/coupling interval pairs were focused in the lower right corner of the area of vulnerability.7 Therefore, our shocks were below the ULV and longer than the shortest coupling interval that induces VF.

The difference between sustained and nonsustained arrhythmias cannot be discerned from isochrones of the first beat after the shock, although our results suggest that the first beat of sustained rhythms is most often reentrant. But episodes in which the second beat was focal were most often
nonsustained ($P<0.01$). For episodes in which reentry occurred, the phase maps (Figure 6) after the shock all contained phase singularities 33 ms after the shock. We have observed that at short coupling intervals, the most common outcome was a pair of counterrotating waves, one on each side of the heart (anterior and posterior). For episodes of nonsustained arrhythmias, reentry was not always present and the phase distribution was mostly uniform. We observed that phase singularities did not always form during the shock, but developed after the shock. Phase singularities moved with time along the line of block represented on the isochrones (Figures 4 and 6). Although isochrone maps are helpful in describing the propagation of the wavefront, phase maps are localized in time and therefore can describe the state of the heart at any time.\textsuperscript{36}

**Critical Point**

We have observed the formation of a pair of counterrotating wavefronts (Figure 4) for many of the episodes that induced arrhythmias. Consistent with the CP hypothesis,\textsuperscript{8,12} increasing the coupling interval moved the phase singularities toward the base of the heart following the movement of the 43% repolarization isorecovery line ($V_{\text{m}} = -23.5$ mV; Figure 7D). By increasing shock strength, the phase singularities moved away from the edge of the RV, which is also consistent with the CP mechanism. Although we did not measure the extracellular potential gradient caused by the shocks, models\textsuperscript{43,44} and experimental evidence\textsuperscript{8,45,46} suggest that increasing shock strength will move the critical gradient further away from the electrodes. Our results enable us to extend the explanation of the CP mechanism\textsuperscript{8,12} to include the effects of hyperpolarization. Originally, CP suggested that shocks producing depolarization cause direct excitation to a larger region when increasing shock strength. The shock-induced hyperpolarization observed presumably caused all-or-nothing repolarization,\textsuperscript{47} therefore resetting the sodium channels in certain areas and restoring excitability. We believe that in our experiments, the movement in phase singularity location is caused by the fact that the apical region was hyperpolarized by the shock, enabling faster propagation toward the residually refractory area in which block occurred. At sites where phase singularities occurred, $V_{\text{m}}$ was $-23.5$ mV immediately preceding the shock. This value is similar to the value of $V_{\text{m}}$ at the center of reentry (2405 or $-21.3$ mV; see Materials and Methods) during arrhythmias. The occurrence of phase singularities in the center of reentrant patterns suggests that the threshold for propagation and block in this preparation may be $-20$ mV. The complex patterns of hyperpolarization and depolarization (such as virtual electrodes) throughout the wall may provide an alternate explanation to the influence of shock strength on the location of phase singularities.\textsuperscript{48}

**Shock-Induced Changes in $V_{\text{m}}$**

By using optical methods to record the electrical activity during a shock, we observed hyperpolarization occurring near the RV cathode followed by rapid conduction causing the wavefront to propagate into recovered regions and block in regions exhibiting residual refractoriness or shock-induced prolongation of refractoriness. In earlier studies,\textsuperscript{8,12} investigators were unable to map during the shock because of the amplifier-switching delay of the mapping system. The first activations that could be detected were away from the shock location, which led to the assumption that the region between the new activation and the electrode was directly excited by the shock.

Electrical mapping studies such as the ones leading to the CP mechanism could not address the issue of shock-induced hyperpolarization/depolarization because of the limitations of the technique. Studies using microelectrodes, optical mapping, and monophasic AP recordings (MAPs) have measured the effects of shocks on $V_{\text{m}}$ from a limited area. The magnitude of change of $V_{\text{m}}$ is related to strength and polarity; anodal shocks cause greater changes in $V_{\text{m}}$ than cathodal shocks. Conversely, passive models predict that a greater shock will induce a proportionally greater change in $V_{\text{m}}$. The shock-induced depolarization has been shown to prolong the APD, therefore extending the refractory period.\textsuperscript{13,15,49–52} This suggests that the spatial effects of the shock on $V_{\text{m}}$ (such as virtual electrodes) influence the outcome of the shock.

The mechanism of origination of the new activation wavefronts arising during or after the shock cannot be determined by our experiment. Several hypotheses have been proposed. The anode-break excitation mechanism predicts new wavefronts originating where a sufficiently large gradient in $V_{\text{m}}$ causes diffusion between depolarized and hyperpolarized regions.\textsuperscript{18,53} Recent simulations and experiments by Ranjan et al\textsuperscript{54,55} propose that excitation can originate at the site of stimulation-induced hyperpolarization (cellular anode break) because of the combined effect of the $I_{\text{f}}$ and $I_{\text{k1}}$ currents driving $V_{\text{m}}$ toward threshold and initiating an AP.

Many researchers have published experimental data of shock-induced virtual electrode patterns near electrodes\textsuperscript{14,17,18,23,24} similar to the ones obtained with the anisotropic bidomain model by Sepulveda et al\textsuperscript{25} and Roth.\textsuperscript{56} We did not observe the depolarization and hyperpolarization in a dog bone shape. We have only observed hyperpolarization of $V_{\text{m}}$ in refractory tissue near the cathode. Our experimental protocol was not designed to study virtual electrodes. It is possible that virtual electrodes were present outside the field of view (eg, transmurally, at the boundary of the field of view on the RV and/or LV free walls). Several differences in experimental procedure may explain why we did not observe the same patterns as Efimov et al\textsuperscript{18,27} We did not press the heart against a window, and the electrode geometry and placement (9-mm coil inside the RV and 6-cm coil above the heart) are different. Recent modeling simulations\textsuperscript{58,57,58} predict a difference in epicardial measurements of $V_{\text{m}}$ when experimental conditions such as conductivity of the medium surrounding the heart are changed. Entcheva et al\textsuperscript{46} predict that the dog bone pattern of virtual electrodes will not be observed on the epicardium when conductive fluid is present in the ventricular chambers and surrounding the heart. By restricting the fluid layer, the virtual electrodes become visible from the epicardium. According to this hypothesis, our data may be consistent with the virtual electrode mechanism, except that the pattern driving the epicardial layer is below the epicardium. In this case, increasing the shock.
strength will also increase the size of the virtual electrodes produced,8,24 therefore affecting the location of phase singularities, as observed.27 The prediction that large polarization occurs in the midwall48 may explain the large epicardial RV area depolarized by the shock at long coupling intervals (Figure 3B, 210 and 230 ms) in contrast to the smaller apical area hyperpolarized at shorter coupling intervals (Figure 3B, 130 to 170 ms). The virtual electrode mechanism also predicts that changing the time of application of the shock from the plateau to the resting phase of the AP will not reverse the sign of the shock-induced changes in Vm,17,25,56 as we have observed. The magnitude of shock-induced polarization is affected by coupling interval because of the nonlinear properties of cardiac tissue.15 This will affect the spatial pattern of shock-induced polarization due to the preshock repolarization gradient. It is possible that at long coupling intervals, the sites that we report as being depolarized by the shock were initially hyperpolarized, but propagation from depolarized sites in deeper layers of the myocardial wall occurred so rapidly (in <2 ms) that we were unable to record it. At short coupling intervals (ie, during the refractory period), the shock fails to hyperpolarize all sites sufficiently to restore excitability, leading to slow conduction or block.

Although many studies suggest that 2 CPs will produce a figure-of-eight reentry pattern,8,59 some have suggested that 4 CPs can induce quatrefoil reentry by applying S1 and S2 stimuli from the same location of an anisotropic medium.28,60 We did not observe quatrefoil reentry patterns but only figure-of-eight reentry patterns. We believe that both cathodal make and break excitations are involved in the induction of fibrillation in our experiment; at short coupling intervals (<130 ms; ie, in refractory tissue), cathodal break occurs, whereas at long coupling intervals (>190 ms; ie, in recovered tissue), cathodal make is responsible for the rapid depolarization. At intermediate coupling intervals, break may occur in the basal portion of the heart that is refractory, whereas make will occur in the apical portion of the heart that is recovered.60 Accordingly, we believe that residual refractoriness plays an important role in the pattern of activation ensuing from the shock. In this study, the shock did not seem to prolong the APD of sites that did not hyperpolarize during the shock (at short coupling intervals), but these sites remained refractory long enough to support reentry. Additional studies using reversed polarity are necessary to generalize our findings and to further distinguish whether the CP or the virtual electrode mechanism is responsible for inducing fibrillation.

Limitations
Because of the decrease in SNR caused by dye bleaching, we limited our experiments to the use of one waveform, which produced ~20 episodes per heart. The RV cathode/simulated superior vena cava anode electrode configuration was selected because of its clinical relevance. The polarity chosen is reported to induce reentry more easily.30 Similar electrode configurations have been shown to produce a nonuniform shock field of complex spatial distribution.3,6 The RV cathode/simulated superior vena cava configuration increased the complexity of the analysis and limited detailed comparisons with the CP12 and virtual electrode18,24 experiments, which were observed with a more specific electrode configuration. We did not measure the extracellular field during the shock.

To allow spatial measurements of Vm*, we assumed all sites to have the same AP amplitude.61 Although the effects of the drug DAM have not been characterized in the rabbit ventricles, it has been shown to have some effect in sheep and guinea pig on electrical activity, such as shortened APD and refractory period.62,63 DAM reversibly blocks cardiac contraction without causing damage to the cardiac cells. Other factors such as pacing cycle length and species also affect APD and refractoriness. The reported outcomes should not be strictly associated with the reported coupling intervals, because these factors influence the timing of the vulnerability window within the cardiac cycle. For example, Fabritz et al7 have reported vulnerability on the isolated rabbit heart to occur at coupling intervals ranging from 170 to 210 ms when pacing at 600 ms. Our pacing rate was 250 ms, yet the vulnerability window remained in a similar range from 130 to 190 ms. This difference in vulnerability window is probably a result of the slower pacing rate as well as the use of DAM. Comparisons with structurally diseased human hearts must be made with caution because of differences in size and electrophysiological properties. The hearts studied here were not diseased or ischemic and were much smaller than typical human hearts.

In this study, we identified the CP as the location where phase singularities occurred. For several reentrant episodes, the phase singularities did not form immediately after the shock, whereas in all episodes, they were clearly established 33 ms after the onset of the shock (Figure 6B, 150 ms). The effect of shock strength and coupling interval on the location of phase singularities identified immediately at the end of the shock and at the end of the first reentrant cycle showed relationships similar to the ones shown in Figure 7B and 7C.

The activations we measured are averages of a cluster of cells that extend ~300 μm wide and at a depth of 300 to 500 μm; our high spatial resolution combined with our temporal resolution (2.1 ms) is adequate to measure activation times.14,64 Because the recordings are from the epicardium and the heart is a 3-dimensional structure, the patterns observed were influenced by regions within the deeper layers of the myocardium. Therefore, we cannot exclude the possibility that patterns that were classified as focal were produced by intramural reentry. It is also possible that the Purkinje system provided a pathway for transmural reentry or a rapid firing trigger.65 The temporal continuity of the activation patterns observed on the epicardium in most instances (see Figures 1, 4, 5, and 6) suggests that we accurately captured the dynamics of events. Lee et al66 reported that chemical ablation of subendocardial layers of myocardium did not affect the incidence, life span, cycle length, or core size of reentrant wavefronts.

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Methods

Isolated Rabbit Heart

All pre-operative and operative animal care complied with the guidelines of the National Institutes of Health and followed institution Standard Operating Procedures. New Zealand white rabbits were anesthetized using intramuscular injections of ketamine (44 mg/kg) and xylazine (10 mg/kg), followed by intravenous injections of pentobarbital (2.5 ml) and heparin (2.5 ml). A median sternotomy was performed to expose the heart. After excision, the heart was quickly connected to the perfusion system and continuously perfused through the coronaries via the aorta with warm (36° to 38°C) Tyrode’s solution at a constant flow of 50 ml/min provided by a roller pump (Masterflex/Cole Parmer, Vernon Hills, IL). The solution consisted of the following (mmol/l): NaCl, 126; KCl, 4.4; CaCl₂, 1.08; MgCl₂, 1.0; NaHCO₃, 23.8; NaH₂PO₄, 1.0; dextrose, 22; taurine, 20; creatine, 5; pyruvic acid, 5. The solution was saturated with a gaseous mixture of 95% O₂, 5% CO₂. The excitation-contraction decoupler 2,3-butanedione monoxime (diacetyl monoxime, Sigma-Aldrich, St-Louis, MO) was added to the perfusate (approximately 15 mmol/l) to eliminate contraction artifacts in the optical recordings.¹ Subsequently, the heart was immersed in a chamber filled with warm Tyrode, which acted as a volume conductor for recording the ECG.

The heart was left to acclimate for at least 15 minutes during which the rhythm was monitored, and the diacetyl monoxime took effect. An ECG was recorded by electrodes placed in the bath connected to an ECG isolation amplifier module (Serena Medical Electronics Co., San Jose, CA), high pass filtered at 1 Hz and low pass filtered at 30 Hz. The analog signal was acquired at 2 kHz through a A/D board (PC-TIO-10, National Instruments, Austin, TX) using software created using Labview (National Instruments, Austin, TX).
A stock solution of the voltage-sensitive dye di-4-ANEPPS (Molecular Probes, Eugene, OR) was prepared by dissolving 5 mg of the dye in 1 ml of dimethyl sulfoxide (Fisher Scientific, Pittsburgh, PA). The final solution consisted of 25 µl of the stock solution dissolved in 25 ml of Tyrode for a bolus concentration of 10.4 µmol/l. A first dose of 10 ml was injected into the coronary arteries through the cannula. Additional injections of 5 ml were given throughout the experiment (the total volume of di-4-ANEPPS typically injected was 20 ml).

Stimulation Protocol

A bipolar pacing electrode lead (Sweet tip model #4269-59, Guidant, St-Paul, MN) was screwed into the apex. To determine the pacing electrode threshold, a stimulator (World Precision Instruments, Sarasota, FL) delivered a 10 ms square wave pulse at a rate of 250 ms with a starting current magnitude of 0.05 mA. If the stimulus did not capture, the strength was gradually increased until 1:1 capture occurred. The minimum value for which the stimulus captured 1:1 was noted as the diastolic threshold. A 5 second episode of pacing was recorded for every heart before the application of shocks. This unfiltered pacing sequence of 20 beats was used to compute an ensemble average of a paced beat. By averaging 20 consecutive action potentials, the noise is reduced by √20. A pulse train of 10 S1 stimuli at twice diastolic threshold was delivered via the pacing electrode, and a 10 ms monophasic S2 square wave was delivered via the shock coils. The cathodal shocking coil was inserted into the right ventricle (RV) via the vena cava. The anodal shocking coil was laid on top of the left atrium posterior to the cannulated aorta, to simulate the clinical position into the superior vena cava (SVC). Both coils were 1 cm long with an outer diameter of 2 mm (see figure 1 for electrode locations). In regards to our acquired images, the RV cathode is near the apical RV free wall (located on the bottom left of the
anterior image and the bottom right of the posterior image) and the anode is atop the left atrium (upper right of the anterior image and upper left of the posterior image).

S2 shocks were delivered at three strengths, 0.75, 1.5 and 2.25 amperes (A) and six different coupling intervals (CI), 130, 150, 170, 190, 210, 230 ms, via a custom-made constant current stimulator. CI is defined as the time from the beginning of the 10th S1 pacing stimulus to the onset of the S2 shock. The pacing and S1-S2 stimuli timings were controlled by Labview utilizing counters (PC-TIO-10, National Instruments, Austin, TX). The 18 S2 strength/CI pairs were applied in random order. Fluorescence was recorded uninterrupted for 3 seconds starting from the 9th S1 pulse. The outcomes of the premature stimuli were classified into three categories: 1) sustained arrhythmia, 2) non-sustained arrhythmia and 3) single beat. Single beats were followed by sinus rhythm. An event was considered sustained if it lasted more than 2 seconds. The epicardial patterns observed following shocks were classified among four categories: reentrant, focal, global depolarization, undetermined. Reentrant beats were characterized by curving isochrones around a point or line. Focal patterns were characterized by several concentric isochrone lines. Global depolarization waves are shock-induced waves that propagate throughout the heart without reentry or foci within the field of view. Episodes where both a focal and a reentrant pattern were observed were classified as undetermined.

*High Resolution Optical Mapping*

Figure 1 is a schematic diagram of the dual camera video imaging system used to acquire the data. The light from a 450 W Xenon arc lamp (Instruments SA, Edison, NJ) passed through a 520 ± 60 nm filter (Chroma Technologies, Brattleboro, VT) and was reflected by a dichroic mirror (reflection bandwidth: 400-560 nm, transmission bandwidth 590-800 nm; Chroma
Technologies, Brattleboro, VT) onto the heart’s anterior surface. The green light excited the voltage-sensitive dye di-4-ANEPPS (Molecular Probes, Eugene, OR) present in the heart. Changes in $V_m$ produced a shift in the spectral properties of the dye. The emitted photons passed through the dichroic mirror and a 610 nm long pass optical filter (Chroma Technologies, Brattleboro, VT) and the resulting red light was focused onto a 128x128 pixel charge-coupled device (CCD) camera (Dalsa, Ontario, Canada) using a Fujinon TV lens (CFL 25L, 1:0.85/25). For the posterior side, a 250 W Xenon arc lamp was used with the same optics and camera as the anterior side. The two CCD cameras output the digital data in two 12 bit data streams. Two frame grabbers (ITI-AMDIG-16 and IC-PCI, Imaging Technologies Inc., Bedford, MA) mounted inside two PCs (Micron, Nampa, ID) collected and stored the raw data at a rate of 480 frames per second through custom-made software. The recordings from the two cameras were time aligned based on a light emitting diode (LED) in the fields of view illuminated during S1 pulses. The spatial resolution was determined from a recorded image of the heart with a superimposed ruler.

*Image and Signal Processing*

Image processing of the raw data was done using custom software and PVwave image processing libraries (Visual Numerics, Houston, TX) through a graphical user interface or in batch mode. To measure signal quality we computed the signal-to-noise ratio (SNR) and $\Delta F/F$. The SNR for each animal was calculated by dividing the upstroke magnitude (averaged over approximately 20 consecutive paced beats) by the standard deviation of the noise (calculated at baseline during diastole) for pixels within a square area (40x40) in the middle of the field of view where illumination was fairly uniform. The SNRs were lower at the edges of the field of view and at the edge of the heart. Heart curvature increases light scattering therefore reducing SNR.
The magnitude of the fluorescence signal is quantified as \( \Delta F/F \), with:

\[
\frac{\Delta F}{F} = \frac{F_{\text{max}} - F_{\text{min}}}{F_{\text{max}}} \tag{1}
\]

The first processing step was to subtract the raw signal for each pixel from its two-second temporal average. The action potential amplitude during S1 beats varied at each site; therefore, we normalized the action potential amplitude of paced beats for each pixel to 12 bits (4096 levels) denoted by \( F' \). Background subtraction and normalization do not affect spatial and temporal resolution or signal-to-noise ratio (SNR). As a first approximation, we have chosen to represent the signals (\( V_m' \)) in pseudo millivolts (mV'). For paced action potentials, we assume a resting membrane potential of -80 mV with an action potential amplitude of 100 mV at each site (see Figure 2).

\[
V_m' = \frac{F'}{40.95} - 80 \tag{2}
\]

After background subtraction and normalization, a 5 point (10.4 ms) median temporal filter was applied to each pixel to remove system noise spikes. A spatiotemporal (x,y,t) conical filter (3x3x3 mask) was applied to improve signal quality\(^4\). This filtered signal was re-normalized.

Activation times were defined as the time when the signal first reached 50\% of the maximal amplitude\(^5\) (i.e., level 2048 or \(-30\) mV', see Figure 2C). For pacing episodes, activation times were calculated relative to the time of onset of the pacing stimulus. For S2 stimulation, activation times were calculated relative to the onset of the S2 stimulus. Repolarization times were defined as the time when the action potential was 75\% recovered relative to the time of the onset of the pacing stimulus. Action potential duration at 75\%
repolarization (APD75) was obtained by subtracting the time of activation from the time of repolarization. Hyperpolarization/depolarization was defined as a decrease/increase of Vm' at the end of a S2 shock compared to the Vm' of the 9th S1 at the same time relative to the onset of the (9th and 10th ) pacing stimulus.

Potential maps were created by assigning a color to each Vm' level using a 256 color map. "Isochrone maps" were produced by assigning a value for the time of activation to each pixel. Contour lines were generated using linear interpolation after applying a 5x5 spatial median filter to remove any outlying values. Dark lines delineate regions where block occurs. The atria were removed from the figures for clarity.

The uniformity index6 was calculated for all shock strengths at each CI. The uniformity index is a global measure of the effect of the shock on Vm. Vm* is the difference between the shock-induced change in Vm (Vm at the beginning of the shock subtracted from Vm at the end of the shock) and the change in Vm normally occurring during that 10 ms interval when no shock is given (measured during the previous paced beat). We computed the uniformity index as the sum of the number of sites having a Vm*> 5 mV' minus the number of sites having a Vm* < -5 mV' divided by the total number of sites with |Vm*| > 5 mV'.6 A positive uniformity index indicates that the depolarized region was larger than the hyperpolarized region during the shock while a negative index represents the opposite.

Phase (θ) at each site was determined using the following equation:7

\[ \theta(t) = \arctan\left( \frac{F'(t + \tau) - F'_{\text{mean}}}{F'(t) - F'_{\text{mean}}} \right) \] (3)

Where τ is a delay factor (12 frames or 25 ms) and F_mean is the average fluorescence value calculated for each heart during VF from an episode that was normalized before induction i.e.
during pacing. The average $F_{\text{mean}}$ for all animals was 2405 ± 121. The location of phase
singularities are defined as sites around which all phase values (-\pi to \pi) converge.\textsuperscript{8} The location
of the phase singularity was measured for each heart relative to the boundary (apex and RV edge)
of its projection onto the CCD as recorded in the field of view.

Statistics

Values are presented by means ± standard deviations, unless otherwise indicated. Curves
were fitted by linear regression using the least square methods to minimize the error and to
determine the significance of the association. P values < 0.05 are considered significant.
Statistics were computed using Origin (Microcal Software, Northampton, MA). Comparisons of
proportions were computed using an unpaired comparison test to the standardized normal.
Conditional probabilities were computed to determine the predictive value of a test (here, we
predicted outcomes based on activation patterns).
REFERENCES:


Two CCD digital cameras were used to map electrical activity from the rabbit heart using a voltage sensitive dye (di-4-ANEPPS). One camera mapped the anterior surface of the heart (A), while the other mapped the posterior surface of the heart (B). The excitation filters (520 ± 60 nm) pass green light and the dichroic mirrors reflect light onto the respective side of the heart. The emission filter allows the wavelengths greater than 610 nm to reach the 128x128 pixel CCD camera. Pictures A and B were taken during the experiment to show positioning in the field of view. (*, pacing site)