Intracellular Calcium and Vulnerability to Fibrillation and Defibrillation in Langendorff-Perfused Rabbit Ventricles

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Background—The role of intracellular calcium (Ca) in defibrillation and vulnerability is unclear.

Methods and Results—We simultaneously mapped epicardial membrane potential and Ca during shock on T-wave episodes (n = 104) and attempted defibrillation episodes (n = 173) in 17 Langendorff-perfused rabbit ventricles. Unsuccessful and type B successful defibrillation shocks were followed by heterogeneous distribution of Ca, including regions of low Ca surrounded by elevated Ca (“Ca sinkholes”) 31 ± 12 ms after shock. The first postshock activation then originated from the Ca sinkhole 53 ± 14 ms after the shock. No sinkholes were present in type A successful defibrillation. A Ca sinkhole also was present 39 ± 32 ms after a shock on T that induced ventricular fibrillation, followed 22 ± 15 ms later by propagated wave fronts that arose from the same site. This wave propagated to form a spiral wave and initiated ventricular fibrillation. Thapsigargin and ryanodine significantly decreased the upper limit of vulnerability and defibrillation threshold. We studied an additional 7 rabbits after left ventricular endocardial cryoablation, resulting in a thin layer of surviving epicardium. Ca sinkholes occurred 31 ± 12 ms after the shock, followed in 19 ± 7 ms by first postshock activation in 63 episodes of unsuccessful defibrillation. At the Ca sinkhole, the rise of Ca preceded the rise of epicardial membrane potential in 5 episodes.

Conclusions—There is a heterogeneous postshock distribution of Ca. The first postshock activation always occurs from a Ca sinkhole. The Ca prefluorescence at the first postshock early site suggests that reverse excitation-contraction coupling might be responsible for the initiation of postshock activations that lead to ventricular fibrillation. (Circulation. 2006;114:2595-2603.)

Key Words: arrhythmia ■ cardioversion ■ electrical stimulation ■ electrocardiography ■ electrophysiology

An electrical shock given during ventricular fibrillation (VF) may lead to 3 different outcomes: immediate electrical quiescence (type A defibrillation); ventricular premature beats, couplets, or tachycardia, followed by resumption of normal rhythm (type B defibrillation); and unsuccessful defibrillation.1 In both type B successful defibrillation and unsuccessful defibrillation, an isoelectric window usually is present between the shock and the first postshock activation.1 The presence of an isoelectric window after the shock suggests that VF has been terminated, but the same shock also then reinitiates VF by falling into the vulnerable period of the cardiac cycle. This hypothesis has been supported by a correlation between the defibrillation threshold (DFT) and the upper limit of vulnerability (ULV) in animal models and in humans.2-4 More recent studies showed that a shock can reduce intracellular calcium (Ca) in cultured myocytes and that the magnitude of change decreased at stronger shock strengths.5 These findings imply that Ca dynamics might play an important role in determining defibrillation success or failure. The process by which spontaneous (voltage-independent) Ca release induces nondriven electrical activity is known as reverse excitation-contraction coupling.6 It is possible that reverse excitation-contraction coupling occurs after shocks and gives rise to electrical activation. This hypothesis has not been tested in intact hearts. The purpose of the present study was to use dual optical mapping techniques to study membrane potential (Vm) and Ca during shock on T and during attempted ventricular defibrillation in intact, Langendorff-perfused rabbit hearts. We analyzed and compared only episodes in which the earliest postshock activation occurred within the mapped region. The results were used to investigate whether Ca dynamics play a role in the mechanisms of defibrillation and vulnerability.

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Methods

Surgical Preparation
New Zealand white rabbits (n = 31) weighing 3 to 5 kg were used in this study. The vendor is a USDA licensed rabbit vendor in southern California who produces rabbits in a closed colony for biomedical research. After general anesthesia, the rabbit hearts were rapidly

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excised through midline sternotomy and immersed in cold Tyrode’s solution (in mmol/L: NaCl 125, KCl 4.5, NaH₂PO₄ 1.8, NaHCO₃ 24, CaCl₂ 1.8, MgCl₂ 0.5, and dextrose 5.5, with albumin 100 mg/L added in deionized water). The ascending aorta was immediately cannulated and perfused with warm oxygenated Tyrode’s solution (36.5±0.5°C; pH 7.4±0.5) at a rate of 30 to 40 mL/min to maintain a perfusion pressure between 80 and 95 mm Hg. Two widely spaced bipolar electrodes were used for continuous pseudo-ECG monitoring. Bipolar electrodes for S₁ pacing were attached to left ventricular apex. Right ventricular endocardial electrode (cathode) and left ventricular patch electrode (anode) were placed for direct current shocks.

**Optical Mapping**

We used 0.5 mg Rhod-2 AM (Molecular Probes, Carlsbad, Calif) dissolved in 1 mL dimethyl sulfoxide containing Phutonic F-127 (20% wt/vol) to stain Ca. This solution, diluted in 300 cm² of Tyrode’s solution to achieve a final Rhod-2 concentration of 1.48 μmol/L, was infused into the heart over a 10-minute period. The heart was perfused with dye-free Tyrode’s solution for 15 to 30 minutes for de-exterification of Rhod-2 AM. The heart was then stained again by direct injection of voltage-sensitive dye (RH237, Molecular Probes) into the perfusion system. The double-stained heart was excited with laser light at 532 nm. Fluorescence was collected using 2 charge-coupled device (CCD) cameras (Dalsa, Waterloo, Ontario, Canada) covering the same mapped field. We used a grid to calibrate the locations of the field of view of these 2 CCD cameras. Using this calibration, we could compare the recordings of Vₘ and Ca from the same locations. The CCD camera for Ca was fitted with a 715-nm long-pass filter, and the CCD camera for Vₘ was fitted with a 580±20-nm band-pass filter. The digital images (128×128 pixels) were gathered from the epicardium of the left ventricle (25×25 mm² area), resulting in a spatial resolution of 0.2×0.2 mm² per pixel. Because the normal rabbit myocyte averaged 0.1917±0.0327 mm in size, each pixel contained the information from an average of 6.4 epicardial myocytes. In addition, appreciable signal arises from cells 2 mm (61.2 cell layers) below the epicardium. Therefore, each pixel registered optical signals from roughly 392 cells in this study. We acquired 1000 frames continuously with 12-bit resolution (260 to 400 frames per second, or roughly 2.5 to 4 ms per frame). The voltage-sensitive dye RH237 was used because its emission band pass differs from that of Rhod-2, thereby preventing cross-talk between these 2 signals.⁸–¹⁰ The signal-to-noise ratio of our mapping system, as estimated from the peak-to-peak time variation in fluorescence intensity, is 40 to 1 during pacing and about 5 to 1 during VF. Cytochalasin D (5 to 10 μmol/L) was added to assist perfusate to minimize motion artifacts.

Note that because 2 CCD cameras were used, the same anatomic location may appear at different coordinates on the Vₘ and Ca maps. Therefore, we implanted 4 cactus needles on the epicardium as markers. A software program then used these markers to match the pixels on the Vₘ and Ca maps to the same locations. Data analyses were performed only with aligned maps.

**Dual Optical Mapping of Ventricular Vulnerability and Defibrillation**

A total of 17 hearts were studied. After 8 S₁-paced beats, biphasic truncated exponential waveform shocks of fixed pulse duration (6 ms) were delivered from a Ventritex (Sunnyvale, Calif) HVS-02 defibrillator on T wave. When VF was induced, an up-down algorithm was used to determine the 50% probability of successful defibrillation (DFT₅₀). The same up-down protocol was used to determine ULV₅₀. We define the near-threshold shock strengths as ULV₅₀, and the DFT₅₀ as described above. We attached bipolar electrodes at the left ventricular apex for S₁ pacing. Right ventricular endocardial electrode (cathode) and left ventricular patch electrode (anode) were placed for direct current shock in 14 rabbits. In the remaining 3 rabbits, we placed a defibrillation electrode on the right ventricle (cathode) and 2 on the left ventricular lateral wall (anode) with S₁ giving to the apex. We made these changes to increase the probability that the first postshock activations occur at the center of the mapped region.¹²

**Effects of Ryanodine and Thapsigargin**

We used an abbreviated protocol to estimate the DFT and ULV with a simple step-up protocol and then perfused ryanodine (5 μmol/L) and thapsigargin (200 nmol/L) over 30 minutes to inhibit sarcoplasmic reticulum Ca cycling.¹¹ The protocol was repeated afterward. Seven hearts were studied.

**Dual Mapping After Cryoablation**

A limitation of the above protocols was that only the epicardial layer of the rabbits was mapped. It is possible that we missed the earliest postshock site originating from subepicardial locations. We therefore performed cryoablation in an additional 7 rabbits by placing a 7-cm SurgiFrost probe (CryoCath Technologies Inc, Montreal, Quebec, Canada) into the left ventricle. The probe temperature was decreased to −135°C for 5 to 10 minutes, during which the epicardium was protected by warm (37°C) oxygenated Tyrode’s solution and the entire heart was continuously Langendorff perfused. After the study, we perfused the coronary arteries with 1% triphenyl tetrazolium chloride and sectioned the heart horizontally into 5 equally spaced sections to document the effects of cryoablation. The tissues were then sampled from the left ventricle, processed routinely, embedded in paraffin, and then stained with hematoxylin and eosin to document myocardial necrosis.

**Data Analysis**

The activation maps were used to examine Vₘ and Ca patterns during induction of VF and defibrillation. The average fluorescence level (F) of the entire data window was first calculated. The fluorescence level of each pixel was then compared with this average. We assigned shades of red to represent above-average fluorescence and shades of blue to represent below-average fluorescence to generate the ratio maps. For depolarization and repolarization isochronal maps, we define the time of activation and repolarization as the fluorescence moved upward across the F or downward across the F, respectively. The beginning of the isochronal measurement was marked on each isochronal map, with the time of shock as time 0. The intervals between the beginning of the measurements to the times of depolarization or repolarization at different sites were then color coded according to depolarization and repolarization color scale bars, respectively. All data are presented as mean±SD. Wilcoxon sign-rank tests were used to compare the data from the 2 groups. A value of P<0.05 was considered statistically significant.

The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

**Results**

**Dual Optical Mapping of Ventricular Vulnerability and Defibrillation**

The ULV₅₀ and the DFT₅₀ were 217±81 and 266±79 V, respectively. We mapped 104 episodes of VF induction by shock on T. The earliest postshock activation occurred in the mapped region in 19 episodes. We also mapped 173 episodes of attempted defibrillation. The latter episodes included 109 failed, 37 type A, and 27 type B defibrillation. Among these episodes, 3 episodes of type B and 40 episodes of failed defibrillation (2.4±2.4 episodes per rabbit; range, 0 to 8 episodes) had their first postshock focal activations in the center of the mapped region. We analyzed all episodes with first postshock activation from the center of the mapped region to study the mechanisms of postshock initiation or reinitiation of VF.
**Ca, Sinkhole and Postshock Focal Discharges**

An unsuccessful defibrillation shock was followed by an isoelectric window in the \( V_m \) map and an inhomogeneous \( Ca_i \) map. Figure 1 shows a typical example. Figure 1A shows optical signals from the site marked by asterisks in Figure 1B. Figure 1B shows \( V_m \) and \( Ca_i \) maps at the time of shock (0 ms) and 3 additional snapshots roughly 25 ms apart. In this and other color panels, the right and left lower corners were outside the heart and were cropped. A bright dot at 0 ms is a light artifact used to indicate the time of the shock. After shock, the \( Ca_i \) map showed an area with continued decline of \( Ca_i \), resulting in a blue region on the \( Ca_i \) map (marked by asterisks in the frame 25 to 50 ms). Because this blue region had a lower \( Ca_i \) level than the surrounding tissues, we called this blue region a \( Ca_i \) sinkhole. These sinkholes first occurred at an average of 31±12 ms after the shock in all episodes and were most apparent on the map 20 to 40 ms after the shock. The corresponding site on the \( V_m \) map also is marked with asterisks. There was a \( V_m \) sinkhole below the asterisks on the 25-ms frame. The same region continued to have a relatively lower \( V_m \) level on the 50-ms frame than the remainder of the map. However, note that the entire \( V_m \) map was coded blue at that time, consistent with a postshock quiescent period. The site with lower \( V_m \) and \( Ca_i \), than the surrounding tissues (sinkholes) predicted the subsequent focal discharge that started at 60 ms after shock and propagated to surrounding tissues in a centrifugal pattern 73 ms after the shock. Figure 1C shows consecutive isochronal activation maps, repolarization maps, and \( Ca_i \) maps. The first discharge propagated to the peripheral region in a focal pattern (60-ms frame). Two foci were noted in the same general region in 2 subsequent beats (black arrows in the frames at 143 and 198 ms). Note that \( Ca_i \) sinkholes (white arrows; 50-, 140-, and 185-ms frames) preceded the occurrence of repetitive focal discharges. The conductive velocity toward the left lower quadrant progressively slowed, leading to wave breaks (yellow arrow; frame at 143 ms) and VF. The repolarization map shows a white area in the center, indicating that the repolarization time was out of range of the color scale. The reason is that the shock induced only minimal or no graded response at that site as seen in the upper \( V_m \) tracing of Figure 1D (red arrow on \( V_m \) tracing). Because the repolarization portion of the action potential did not cross \( F \), the computer was unable to assign a repolarization time within the 50-ms color scale; it was coded white instead. This white region corresponded to the site of focal discharge on the depolarization map. There was also a correlation between early repolarization and subsequent focal discharges (black arrows). Figure 1D shows the optical signals from a site in the sinkhole (top 2 tracings) and a site from the edge of the sinkhole (bottom 2 tracings). The continuous \( Ca_i \) decline at the upper tracing (red arrow on \( Ca_i \) tracing) accounted for the low \( Ca_i \) within the sinkhole. In comparison, there was a large graded response on \( V_m \) tracing and a large \( Ca_i \) response on the \( Ca_i \) tracing (blue arrows), resulting in relatively elevated \( Ca_i \) at the edge of the sinkhole.

A \( Ca_i \) sinkhole was associated with the subsequent development of the early activation site on the \( V_m \) map in all 40 episodes studied. Figure 2 shows 10 episodes from 6 different rabbits. In each episode of near-threshold unsuccessful defibrillation shock, a \( Ca_i \) sinkhole occurred on the \( Ca_i \) map (white arrows) 31±12 ms after the shock. However, not all sinkholes resulted in early activation sites. Therefore, a sinkhole appeared to be a necessary but not a sufficient condition for a postshock focal discharge. These \( Ca_i \) sinkholes were associated with the focal origin of the first postshock activation (black arrow on the isochronal map) that occurred 53±14 ms after the shock. This latter number also is
the isoelectric window of all unsuccessful shocks. The times on $V_m$ and Ca maps indicate the isoelectric window and the time of the Ca sinkhole, respectively.

**Ca$_i$ Levels During Unsuccessful and Successful Defibrillation Episodes**

Relative to the maximum (100%) and minimum (0%) Ca$_i$ during the entire VF episode, the level of Ca$_i$ in the Ca$_i$ sinkhole averaged 2±4%. Although the Ca$_i$ in the Ca$_i$ sinkhole was lower than most of the other sites during VF (Figure 3A), its level was still higher than the Ca$_i$ in late diastole of the sinus rhythm (Figure 3B). We found 3 episodes of type B defibrillation with a centrifugal pattern of activation originating from the mapped region. All 3 episodes had a Ca sinkhole (Figure 3B) that preceded the site of first postshock activations. However, the same was not true for type A defibrillation shocks (Figure 3C). The shock was followed by a homogeneous Ca$_i$ elevation throughout the mapped region for >38 ms. The Ca$_i$ declined afterward in a less homogeneous pattern but did not produce a Ca$_i$ sinkhole. The Ca$_i$ eventually came down to the baseline level 188 ms after successful type A defibrillation. Figure 3 also shows that VF caused the overall calcium signal to “hang up” at a high level (ie, close to systolic level), as shown by Stefaneli et al. Successful defibrillation causes the overall level to come back down.

**Induction of VF by Shock on the T Wave**

Figure 4 shows an example of shock on T that induced VF. Figure 4A shows the actual optical signal of the shock induction of VF. Figure 4B shows the $V_m$ isochronal map and Ca$_i$ map of consecutive activations. The time of the shock was time 0. Note that before the early activation (black arrow) at 124 ms, a partial Ca sinkhole (52 ms) or full Ca$_i$ sinkholes (240, 348, and 480 ms) were observed. In all 19 episodes in which the earliest postshock activation occurred in the mapped region, a Ca$_i$ sinkhole preceded the early site by 30±18 ms.

**Effects of Coupling Interval and Shock Strength on Sinkhole**

The appearance of Ca$_i$ sinkholes and the induction of VF or repetitive responses depend on both the S$_1$-S$_2$ coupling interval and the S$_2$ shock strength. Figure 5 shows typical examples of shock on T with different S$_1$-S$_2$ coupling intervals and S$_2$ shock strength. All episodes associated with induction of VF or repetitive responses had a Ca$_i$ sinkhole (white arrow) after the shock.

**The Critical Points**

In 3 rabbits, we positioned the parallel shock electrodes at the left and right sides of the mapped region to increase the probability that the first postshock activation, hence a critical point (phase singularity point), occurs in the center of the
mapped region. Figure 6A shows isochronal maps of S1-paced beat and the first postshock activation (S), which was followed by a spiral wave with clockwise rotation. Figure 6B shows that a postshock Ca sinkhole formed 45 ms after shock (yellow arrow). Figure 6C shows 2 phase maps. The first (45 ms) shows phase singularity (critical point) formed at the lower portion of the mapped field. This phase singularity meandered to the higher portion of the map 57 ms after shock. Note that the critical point formed at the edge of Ca sinkhole. Figure 6D shows the optical signals. Note that between the time of a 50-V shock (red line) and the formation of Ca sinkhole (blue line), there were different degrees of depolarization. Large depolarizations at sites 1 and 2 were associated with a small increase in Ca (red upward arrows). However, smaller depolarization (graded responses) at sites 3 through 5 was not associated with any appreciable Ca elevation (black upward arrows). The continued Ca decline in these and neighboring pixels was responsible for the appearance of a Ca sinkhole above site 5 after the shock (Figure 6B). Omichi et al previously demonstrated dissociation between Vm and Ca during VF. In Figure 6D, we show that shock on T also can result in dissociation between Vm and Ca near the sinkhole. In that figure, shock induced graded response on the Vm map without inducing Ca elevation (upward black arrows), resulting in prolongation of action potential duration (APD) but no prolongation of Ca at those sites. The continued decline of Ca underlies the mechanism by which a sinkhole is observed at those sites.

Effects of Ryanodine and Thapsigargin
Ryanodine and thapsigargin infusion caused a significant ($P=0.04$, $n=7$) increase in spontaneous sinus cycle length from $515\pm28$ to $908\pm19$ ms and a significant increase in APD90 from $154\pm20$ to $217\pm30$ ms ($P=0.03$, $n=6$) within 30 minutes after the start of infusion. The APD during S1 pacing at a 300-ms cycle length was $148\pm15$ ms at baseline and $218\pm24$ ms after drug infusion ($P=0.03$, $n=6$). VF was still inducible after ryanodine and thapsigargin administration. The combined drug infusion decreased ULV from $393\pm137$ to $171\pm99$ V ($P=0.02$, $n=7$) and DFT from $429\pm141$ to $271\pm202$ V ($P=0.02$, $n=7$).

Dual Mapping After Cryoablation
Epicardial Ca Sinkholes and the Earliest Postshock Activation After Cryoablation
To exclude the possibility that the earliest postshock activation site occurred somewhere beneath the tissue surface rather than at the Ca sinkhole, we studied 7 hearts in which endocardial cryoablation left only a thin (0.5-mm) layer of
surviving epicardial tissues (white arrow, Figure 7A). The tissues stained negative for triphenyl tetrazolium chloride show cell necrosis and contraction bands (white arrow, Figure 7B), compatible with effective cryoablation. After cryoablation, the right ventricle was usually intact. VF was still inducible after cryoablation (Figure 7D). The ULV50 and DFT50 were 244 ± 69 V and 259 ± 70 V, respectively (P = NS). We mapped 112 episodes of VF induction by shock on T. The earliest postshock activation occurred in the mapped region in 7 episodes. We also studied 201 episodes of attempted defibrillation, including 109 failed, 45 type A, and 47 type B defibrillation. Among these episodes, 6 episodes of type B and 63 episodes of failed defibrillation (9 ± 6 episodes per rabbit; range, 3 to 17 episodes) had their first postshock focal activations occurring in the center of the mapped region. In all failed defibrillation episodes, the Ca sinkholes were

Figure 4. Induction of VF by shock on T. A, Actual recordings at a site near the base before and after shock. B, Isochronal maps of consecutive activations before and after shock (left). Each early site (black arrows) was preceded by a Ca sinkhole (white arrows).

Figure 5. Ca sinkholes and the vulnerable domain. Note that the presence of Ca sinkholes (white arrows) is associated with the induction in VF or repetitive responses. Vertical white line segments indicate the time when the ratio maps were taken.
detected, occurring $31 \pm 12$ ms after the shock and followed $19 \pm 7$ ms later by the first postshock activation. Figure 7E shows 4 different episodes of failed defibrillation after cryoablation. The white arrows indicate $\text{Ca}^\text{\textsubscript{i}}$ sinkholes and the earliest postshock activations at corresponding sites. The yellow arrows on the bottom show a secondary $\text{Ca}^\text{\textsubscript{i}}$ sinkhole, followed by a secondary postshock early site. A $\text{Ca}^\text{\textsubscript{i}}$ sinkhole was also present $68 \pm 25$ ms after a shock on T that induced VF, followed $23 \pm 15$ ms later by propagated wave fronts that arose from the same site.

**$\text{Ca}^\text{\textsubscript{i}}$ Prefluorescence and Postshock Focal Discharge**

Rabbits without cryoablation showed simultaneous rise in $V_m$ and $\text{Ca}^\text{\textsubscript{i}}$ in 5 of 40 episodes of failed defibrillation. None of these episodes showed a rise in $\text{Ca}^\text{\textsubscript{i}}$ preceding the rise in $V_m$ ($\text{Ca}^\text{\textsubscript{i}}$ prefluorescence). In comparison, $\text{Ca}^\text{\textsubscript{i}}$ prefluorescence was observed in 5 of 63 failed defibrillation episodes in hearts with cryoablation. The $\text{Ca}^\text{\textsubscript{i}}$ fluorescence always occurred within the $\text{Ca}^\text{\textsubscript{i}}$ sinkhole and preceded the onset of first postshock activation. In addition, 9 episodes of failed defibrillation showed a simultaneous rise in $V_m$ and $\text{Ca}^\text{\textsubscript{i}}$ fluorescence at the postshock early site. Figure 7F shows a typical example of $\text{Ca}^\text{\textsubscript{i}}$ prefluorescence after failed defibrillation. A $\text{Ca}^\text{\textsubscript{i}}$ sinkhole was present 24 ms after the shock, followed by focal discharge 52 ms after the shock. At the 64-ms frame, the $\text{Ca}^\text{\textsubscript{i}}$ map showed a larger area of red than the $V_m$ map, indicating that $\text{Ca}^\text{\textsubscript{i}}$ fluorescence had increased above $F$, whereas the $V_m$ depolarization lagged behind. These findings are consistent with $\text{Ca}^\text{\textsubscript{i}}$ prefluorescence. Afterward, rapid outward propagation occurred with $V_m$ depolarization followed by a $\text{Ca}^\text{\textsubscript{i}}$ rise, resulting in a larger red area in the $V_m$.
map than in the Ca, map (76-ms frame). A Ca, sinkhole also was present 68±25 ms after a shock on T that induced VF, followed 23±15 ms later by propagated wave fronts that arose from the same site. Among the shock on T episodes, 1 episode showed simultaneous Vm and Ca elevation.

**Discussion**

In isolated rabbit ventricles, the first postshock activation after shock on T and after failed or type B successful defibrillation shocks consistently arose from a Ca, sinkhole. A Ca, sinkhole also was observed at the first postshock activation site in the epicardial surviving layer after cryoablation. Suppression of SR function by ryanodine and thapsigargin significantly reduced the ULV and DFT. These data suggest that Ca, dynamics are important in the mechanism of ventricular vulnerability and defibrillation.

**Relation Between Ca, Sinkhole and the Postshock Activations**

**Ca, Mediated Mechanisms**

The reason that postshock activations consistently originated from Ca, sinkholes is still a matter of speculation. Given that the first postshock activations after unsuccessful defibrillation are focal,11,15,16 triggered activity is a possible mechanism for this first postshock activity. One possible mechanism of the triggered activity is the reverse excitation-contraction coupling induced by spontaneous (voltage-independent) SR Ca release.9 The low Ca, in the sinkhole reflects more complete SR Ca uptake. With the SR more Ca loaded and more fully recovered, it may be more susceptible to spontaneous Ca release compared with regions with persistently high Ca,. Thus, triggered activity is a possible mechanism for consecutive activations from the Ca, sinkhole. A second possible explanation is the electrotonic interaction with surrounding cells. In the Ca, sinkholes, APD was shorter than in the surrounding sites. Electrotonic currents from the surrounding depolarized regions might assist impulse formation in the sinkhole region. This mechanism is similar to phase 2 reentry or reflection induced by heterogeneous APD among neighboring cells.17

**Dispersion of Refractoriness and Defibrillation**

However, not all postshock focal discharges were associated with Ca, prefluorescence. It is possible in those episodes, heterogeneous distribution of APD after the shock18 resulted in virtual electrode effect, which triggered subsequent Vm elevation and focal discharge.19 One can argue that this mechanism does not require SR Ca dynamics. However, in the simultaneous Ca recordings in Figure 6D, the upright black arrows indicate no Ca, elevation after the shock at those sites. Because of the bidirectional coupling between Ca, and APD,20,21 the reduced Ca, level in the sinkholes might have contributed to the shortening of APD at those sites. This example suggests a possible contribution of Ca, dynamics to the formation of heterogeneous APD distribution. This alternative explanation, however, is not compatible with the patterns of activation after unsuccessful defibrillation. As documented in a previous study,11 a near-threshold unsuccessful defibrillation shock usually is associated with an isoelectric window and at least a transient homogeneous Vm distribution before the onset of focal discharge. The same phenomenon is seen in Figure 1B (50-ms frame).

**Focal Discharge Versus Reentry**

In this study, we confirmed that a focal discharge pattern usually was present after unsuccessful defibrillation shocks,11,15,16 whereas shocks on T wave usually induced reentrant excitation.12,18 The discrepancy can be explained by the preshock distribution of refractoriness. When shock was given on the T wave, the tissues near S1 had more time to recover than tissues away from it. As a result of the differential refractoriness, the activation induced by the shock first propagated into fully recovered tissue before turning around to excite the less refractory region, forming reentrant excitation. In comparison, the preshock distribution of refractoriness in an unsuccessful defibrillation shock is not predictable and is likely to be highly heterogeneous. Therefore, the first postshock activation is usually focal, although reentrant excitation also might be observed.15

**Study Limitations**

We have mapped only the epicardial surface of the rabbit ventricles in the first 17 hearts. It is possible that Ca, prefluorescence occurs in the subepicardium and was not detected by the epicardial mapping techniques used in this study. This is the reason that we performed additional experiments with cryoablation. Because the left ventricle had only a thin layer of surviving myocytes, the earliest postshock activation can occur only from this thin layer of tissues. We found that Ca, sinkholes also preceded the occurrence of focal discharges in these hearts. A second limitation is the signal-to-noise ratio. The data obtained from the CCD camera have a high spatial resolution, which is needed to perform this study. However, the signal quality from individual pixels might vary. The conclusion of the study was supported by the 2-dimensional maps with information obtained from multiple pixels, not by data from any single pixel.

**Conclusions**

We conclude that the first and subsequent repetitive activations after a vulnerable shock on T and after an unsuccessful defibrillation may arise from an area of low Ca, surrounded by areas of high Ca, (a Ca, sinkhole). A Ca, related gradient of repolarization, spontaneous SR calcium release, or reactivation of ICa,L from a Ca, sinkhole may initiate postshock activation through reverse excitation-contraction coupling or phase 2 reentry to reinitiate VF. Whether the postshock activation propagates in a focal discharge pattern or in a reentrant pattern depends on the distribution of refractoriness at that time.

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References

CLINICAL PERSPECTIVE
Mechanisms by which electrical defibrillation succeeds or fails are still being elucidated. Previous studies have shown that some near-threshold shocks terminate all wave fronts during ventricular fibrillation but initiate new wave fronts that reinitiate ventricular fibrillation. The mechanism by which a new focal wave front appears after an unsuccessful shock remains unclear. The present study performed optical mapping of intracellular calcium and membrane potentials during shocks for ventricular fibrillation. The distribution of intracellular calcium during the failed postshock period was highly heterogeneous despite shock-induced electrical quiescence over the preparation. The first postshock activation arose from a region of low intracellular calcium surrounded by regions of elevated intracellular calcium (calcium sinkhole), suggesting that these sinkholes might be related to the first postshock activation that reinitiates ventricular fibrillation. We also demonstrated that the rise in intracellular calcium in these sinkholes preceded membrane potential depolarization, suggesting that the first postshock activation is calcium mediated. Future studies in this area might improve the understanding of the mechanisms of ventricular defibrillation and potentially improve the clinical efficacy of implantable cardioverter-defibrillators.
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