Electroporation induced by internal defibrillation shock with and without recovery in intact rabbit hearts

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Wang YT, Efimov IR, Cheng Y. Electroporation induced by internal defibrillation shock with and without recovery in intact rabbit hearts. Am J Physiol Heart Circ Physiol 303: H439–H449, 2012. First published June 22, 2012; doi:10.1152/ajpheart.01121.2011.—Defibrillation shocks from implantable cardioverter defibrillators can be lifesaving but can also damage cardiac tissues via electroporation. This study characterizes the spatial distribution and extent of defibrillation shock-induced electroporation with and without a 45-min postshock period for cell membranes to recover. Langendorff-perfused rabbit hearts (n = 31) with and without a chronic left ventricular (LV) myocardial infarction (MI) were studied. Mean defibrillation threshold (DFT) was determined to be 161.4 ± 17.1 V and 1.65 ± 0.44 J in MI hearts for internally delivered 8-ms monophasic truncated exponential (MTE) shocks during sustained ventricular fibrillation (>20 s, SVF). A single 300-V MTE shock (twice determined DFT voltage) was used to terminate SVF. Shock-induced electroporation was assessed by propidium iodide (PI) uptake. Ventricular PI staining was quantified by fluorescent imaging. Histological analysis was performed using Masson’s Trichrome staining. Results showed PI staining concentrated near the shock electrode in all hearts. Without recovery, PI staining was similar between normal and MI groups around the shock electrode and over the whole ventricles. However, MI hearts had greater total PI uptake in anterior (P < 0.01) and posterior (P < 0.01) LV epicardial regions. Postrecovery, PI staining was reduced substantially, but residual staining remained significant with similar spatial distributions. PI staining under SVF was similar to previously studied paced hearts. In conclusion, electroporation was spatially correlated with the active region of the shock electrode. Additional electroporation occurred in the LV epicardium of MI hearts, in the infarct border zone. Recovery of membrane integrity postelectroporation is likely a prolonged process. Short periods of SVF did not affect electroporation injury.

Electroporation, the disruption of cell membranes by an electric shock, may play a role in these adverse effects. Cardiac studies have shown that electroporation occurs in areas of high potential gradients (14, 40, 41) and alters the flow of ions across the cell membrane (7, 13, 25). Electroporated myocytes have a depolarized diastolic potential (12, 23) and altered action potential morphology (23). However, existing studies characterizing shock-induced cardiac electroporation have several critical limitations.

First, while there are many studies of electroporation in single myocytes (9, 25, 41), strands of myocytes (7, 12, 13), and in tissue preparations (14, 23, 40), studies in intact whole hearts have been limited. Because shock-induced electroporation is a heterogeneous phenomenon, the position of the defibrillation electrodes can affect the electric field generated (48), thus altering the pattern of electroporation. Additionally, tissue structure can affect electroporation. ICDs are implanted in patients with increased risk of lethal arrhythmias, including those with a prior left ventricular (LV) myocardial infarction (MI) (32). The heterogeneity of cardiac tissues affected by an MI may increase susceptibility to electroporation (2). To begin to address this limitation, our group previously characterized the three-dimensional spatial distribution and extent of electroporation in intact normal and MI hearts (22). In that study, a single shock was delivered under paced conditions through a coil electrode placed in the right ventricle (RV), similar to clinical ICD lead placement.

Previous studies have also delivered shocks during sinus rhythm (9, 23, 25, 41) or under paced conditions (7, 12–14, 22, 40), rather than during clinically relevant SVF. While ICDs are able to rapidly detect SVF and deliver a rescue shock, patients still experience short periods of SVF before shock delivery. It is unknown whether this period of SVF before and during defibrillation affects susceptibility to shock-induced electroporation injury.

Furthermore, studies of shock-induced cardiac electroporation have focused on immediate effects. These effects are important to defibrillation success and to the susceptibility for further arrhythmias (34), and they are critical for the accuracy of computational models of defibrillation (1, 3, 11, 27). However, longer-term effects have not been studied in intact hearts, but should be considered, since electroporation can be irreversible. Theoretical models of electroporation suggest that cell membranes can close small and mid-sized holes (8), and cell studies have shown this recovery occurring over seconds to minutes (4, 37). However, cell membranes are unable to recover following severe electroporation injury, leading to cell death (10). Recent findings suggest that the cardioprotective effects of ICDs in decreasing SCD may not affect total mor-

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tality, due to increased HF deaths (16). Cell death by irreversible electroporation may contribute to the development and progression of HF.

This study sought to address these limitations by examining the spatial distribution and extent of shock-induced electroporation in intact normal and MI hearts during SVF with and without a recovery period. A single defibrillation shock with a clinically relevant intensity and electrode placement was initially delivered to terminate SVF. Electroporation with and without recovery was compared, as well as the electroporation between fibrillating and previously studied paced hearts.

MATERIALS AND METHODS

All experimental protocols were approved by the Institutional Animal Care and Use Committee of the Cleveland Clinic. All animals used in this study received humane care in compliance with the National Institutes of Health’s (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, revised 1996).

Animal model. This study used 31 adult male New Zealand White rabbits weighing 2.8–4.1 kg. The 13 rabbits that served as normal controls did not undergo any survival surgery. In the remaining 18 animals, a chronic MI was created by coronary artery ligation, as previously described (22, 29). Briefly, each animal was anesthetized and intubated. A left thoracotomy was performed to access the heart, and the major branch of the left coronary artery was ligated 45–60% of the distance from the apex to the base of the LV. Throughout the procedure, the electrocardiogram (ECG), PaO2, and expired CO2 were monitored. Animals were given analgesia for 3 days following surgery and were allowed to heal for at least 4 wk before the acute experiment was performed.

Heart preparation. The ex vivo heart preparation for acute experiments has been described previously (22, 29). Briefly, the rabbit was euthanized, and the intact heart was extracted, cannulated, and retrogradely perfused via the aorta on a modified Langendorff apparatus with temperature- and pH-controlled oxygenated modified Tyrode solution. The heart was also immersed in a heated perfusion bath filled with Tyrode solution.

A modified pediatric defibrillation electrode with a 10-mm-long, 1.8-mm-diameter active coil above a 7-mm-long inactive plastic cap was inserted in the RV cavity via the pulmonary artery and placed with the tip at the anterior apex of the RV. The 50-mm-long reference coil was immersed in the perfusion bath in a U-shape on the posterior side of the heart, not in physical contact with the heart. The shock electrode was connected to a 150-μF capacitor HVS-02 defibrillator (Ventritex, Sunnyvale, CA), which was used to deliver rescue shocks in this study. A bipolar pacing electrode was placed on the posterior RV near the apex but above the infarction. The heart was acclimatized by pacing at a basic cycle length of 300 ms for at least 30 min. ECG readings were monitored continuously using pellet electrodes placed in the perfusion bath.

Induction of sustained ventricular fibrillation. SVF was induced by dynamic pacing (15). The protocol was modified so that the heart was paced at successively shorter cycle lengths until SVF was induced, increasing pacing current to overcome 2:1 conduction block as necessary rather than considering it as an end-point. Specifically, the heart was paced for at least 30 s at each cycle length, starting at 300 ms. Initial decreases in cycle length were in 50-ms steps, with the step size decreasing to 5 ms as the cycle length for inducing SVF was approached. If 2:1 conduction block was observed on the ECG after a decrease in cycle length, the cycle length was immediately increased until 1:1 pacing was recaptured. Next, the shortening of the cycle length was resumed with an increased pacing current that allowed 1:1 pacing to be maintained at and below the cycle length that previously caused 2:1 conduction block. When ventricular fibrillation was observed on the ECG, the pacing electrode was turned off, and the arrhythmia was considered to be SVF if it lasted for at least 20 s.

Defibrillation threshold. Shock energy has a significant impact on electroporation (35), so it was important to use clinically relevant shock intensities. Defibrillation threshold (DFT) was first measured in seven MI hearts, since it was expected that DFT would be higher in MI than in control hearts. Electroporation was not measured in these hearts, since they received multiple shocks. DFT was determined using an up-down protocol (19). Briefly, for each heart, dynamic pacing was used to induce SVF. Next, 8-ms anodal monophasic truncated exponential (MTE) defibrillation shocks of varying strengths were delivered. If a shock was unsuccessful at terminating the arrhythmia, successive shocks were delivered at higher voltages until SVF was terminated. If a shock was successful, SVF was again induced by dynamic pacing and a lower voltage shock would be delivered, with this being repeated until a shock was unsuccessful. The first shock delivered to each heart was 100 V, with changes in shock strength made in 20-V increments.

This procedure was continued until at least three crossings of the threshold (from unsuccessful to successful or vice versa) were achieved. The DFT recorded for each heart was the average of the successful shocks immediately before or after a threshold crossing.

The voltage and current for delivered shocks were recorded by a sensing circuit. The resistance of the heart and the energy required to defibrillate were calculated for the successful shocks of each threshold crossing.

Shock-induced electroporation. The experimental protocol for determining shock-induced electroporation during SVF is shown in Fig. 1A. Membrane-impermeant dye propidium iodide (PI; Sigma-Aldrich, St. Louis, MO) (7) was added to the perfusate at a concentration of 30 μM after the heart was acclimatized to the Langendorff perfusion. The heart was perfused with PI for 15 min before SVF was induced via dynamic pacing. After 35–45 s of SVF, a single 8-ms 300-V anodal MTE shock (two times the determined DFT voltage) was delivered. If SVF was observed (>20 s) but then self-terminated before a defibrillation shock could be administered, shock delivery was aborted, and the heart was considered to have received a pseudoshock. Following shock or pseudoshock delivery, the heart was perfused with the PI solution for an additional 15 min. Pacing resumed 1 min after the shock (or pseudoshock) delivery with a 300-ms cycle length. The PI solution was then washed out with fresh (PI-free) Tyrode solution for 40 min while the heart continued to be paced. The heart then was removed from the Langendorff apparatus and placed in ice-cold Tyrode solution to stop contractions. The atria were excised, and the intact ventricles were embedded and frozen in optimum cutting temperature (OCT) medium (Sakura Finetek, Torrance, CA) and stored at −80°C.

Fig. 1. Experimental design. Timelines of the acute experiments for shock-induced electroporation during sustained ventricular fibrillation (>20 s, SVF) (A) and residual shock-induced electroporation after a 45-min recovery period following SVF termination (B). As indicated, each experimental protocol began with an acclimatization period of at least 30 min and ended with a 40-min washout before the whole ventricles were embedded in optimum-cutting temperature (OCT) compound and frozen. SVR was induced by dynamic pacing (DP). Hearts were paced at a 300-ms cycle length except during DP and SVF. PI, propidium iodide.
Residual shock-induced electroporation following recovery. The experimental protocol is shown in Fig. 1B. To detect residual shock-induced electroporation that remained after a 45-min recovery period, SVF was induced and then terminated by a single defibrillation shock as described above. This was done after acclimatization, while the heart was still perfused with PI-free Tyrode solution. After successful defibrillation, the heart was paced at a 300-ms cycle length and allowed to recover for 45 min before 30 μM PI was added to the perfusate. After 30 min of PI perfusion, a 40-min washout was performed, followed by embedding and freezing the whole ventricles in OCT, as described above.

Fluorescence studies. Hearts were cryosectioned with multiple 20-μm-thick transverse sections taken at each position from the apex to the base of the ventricles, with an 800-μm gap between positions. The sections were mounted on slides and 14–17 were chosen to be representative of the entire ventricle, based on the location of both PI staining and the infarct. Imaging was performed with an epifluorescence microscope (DM5000 B; Leica Microsystems, Wetzlar, Germany) with a ×5 objective and a N21 filter. Each transverse section was scanned in its entirety using a motorized stage (H101A; Prior Scientific, Rockland, MA), a cooled charge-coupled device camera (Retiga-SRV Fast 1396; QImaging, Surrey, BC, Canada), and Image-Pro Plus (Media Cybernetics, Bethesda, MD), and a mosaic image was generated.

In these images, unjured tissue was unstained but dimly autofluorescent while tissue with compromised membranes had PI bound to nucleic acids, resulting in a bright fluorescent signal (35, 42). PI was quantified as previously described (22). Briefly, the total tissue and PI staining were segmented using a custom MATLAB (MathWorks, Natick, MA) program. Because PI fluorescence was generally confined to nuclei, the staining was clustered, and the area within the boundary of these clusters was considered PI-stained tissue. For each tissue section, the ventricles were divided into quadrants: anterior RV, posterior RV, anterior LV, and posterior LV. The anterior RV was where the shock electrode was placed and was considered as a single region. The other three quadrants were each further subdivided into three regions: the epicardial side of the free wall, the endocardial side of the free wall, and the septum. The area of total tissue and the area of PI-stained tissue for each of the 10 regions were measured. Next, total tissue volume and PI-stained tissue volumes were calculated by piecewise linear integration of the areas from the serial tissue sections covering the entire ventricles longitudinally. The volume of PI staining in each region as well as for the whole ventricles was normalized as a percentage of the total ventricular tissue volume.

Histological studies. Histological studies were performed on the same tissue sections that were used for the fluorescence studies. These samples were stained using Masson’s Trichrome that rendered myocytes red and fibrotic tissues blue. The tissue sections were scanned using the same microscope setup as the fluorescence study, except under brightfield illumination. Segmentation was performed using a custom MATLAB program in the YIQ color space to obtain areas of myocytes and fibrotic tissues (22). Volumes were again calculated by piecewise linear integration, and infarct size was calculated as the percentage of total ventricular tissue volume that was fibrotic. Image registration was performed between the histological images and the PI images with a custom MATLAB program to determine the correlation between PI staining and tissue structure.

Statistical analysis. Group data are reported as means ± SD. Statistical comparisons between groups were performed using two-tailed, unpaired Student’s t-tests. Differences between groups were considered significant for P < 0.05.

RESULTS

Induction of sustained ventricular fibrillation. The dynamic pacing protocol developed for this study was successful in inducing SVF as evaluated by ECG morphology in all 13 control and 18 MI hearts. Pacing current did not exceed 1.0 mA in any heart, and no sustained ventricular tachycardia was observed. In the 7 MI hearts used to determine the DFT, a total of 22 MTE shocks that successfully terminated SVF were delivered as part of a threshold crossing. The cycle length at which SVF was induced for these shocks varied between 90 and 130 ms (mean: 115.0 ± 10.9 ms), with multiple inductions per heart. The cycle length of SVF induction was not correlated with the voltage of the shock that terminated the arrhythmia (r = 0.25, P = 0.26). In the hearts used to study electroporation where there was only a single SVF induction, the cycle length required for control hearts (116.2 ± 12.6 ms, n = 13) was shorter than for MI hearts (125.5 ± 9.6 ms, n = 11), but was not significantly different (P = 0.06).

Defibrillation shock intensity. DFT was measured in seven MI hearts with three crossings of the threshold made in five hearts and four crossings for the remaining two. The mean DFT voltage was 161.4 ± 17.1 V. From the successful shocks of the crossings, the mean resistance of these hearts was 53.4 ± 4.5 Ω, and the mean defibrillation energy was 1.65 ± 0.44 J.

Based on these DFT measurements, the 12 control and 10 MI hearts used to determine shock-induced electroporation injury, either with or without a period of recovery, each received a 300-V MTE shock, roughly two times the DFT voltage, to ensure successful defibrillation with a single shock. In all 22 hearts, this single shock was successful in terminating SVF and restoring sinus rhythm, with no arrhythmias observed after the shock. The two groups had similar resistances (control: 45.8 ± 3.8 Ω; MI: 45.4 ± 2.6 Ω, P = 0.74). Also, the single MTE shock energy was comparable between groups (5.93 ± 0.15 vs. 5.97 ± 0.10 J, P = 0.49), yielding a clinically relevant safety margin of 2.6 times the DFT energy.

In the remaining one control and one MI hearts, no shock was delivered, since SVF self-terminated after 20 s, but before the planned time for defibrillation.

Histological characterization of ventricles. Representative images from five positions of a Masson’s Trichrome-stained control heart are shown in Fig. 2A. Ventricles of the control hearts had a minimal level of fibrotic tissue (mean: 2.5 ± 1.3%, n = 12) and was generally constant along the longitudinal axis (Fig. 2C).

In the MI hearts, the majority of the ventricular tissue was fibrotic near the apex in both the LV and RV, as shown in the representative image from an MI heart in Fig. 2B. Away from the apex, most of the infarct in the MI hearts was centered on the lateral LV free wall, extending in both the anterior and posterior directions. All infarcts were transmural across the free wall, resulting in wall thinning. Qualitatively, the infarcts had a complete endocardial border zone and a thinner, scattered epicardial border zone. Additionally, there were islands of infiltrated myocytes in the infarct zone. Longitudinal fibrosis in MI hearts was very high near the apex and decreased to levels similar to the control hearts near the base of the ventricles (Fig. 2D), with total fibrosis averaging 14.7 ± 6.4% of the entire ventricular (LV and RV) volume (n = 10; P < 0.01 vs. control). These results are in good agreement with our previous reports regarding this MI model (22, 29).

Electroporation in fibrillating hearts. The spatial distribution and extent of electroporation were characterized by PI staining during defibrillation in control and MI hearts without recovery. Figure 3, A and B, shows representative fluorescence...
images from the same control and MI hearts as in Fig. 2, A and B. The approximate transverse positions of the shock electrode and reference electrode are indicated in one image. Qualitatively, the majority of the cross-sectional PI staining was found in the anterior RV, near where the active lead of shock electrode was placed in all control (n = 7) and MI (n = 7) hearts. PI staining in the shock electrode region was always transmural across the RV free wall. In three control and one MI hearts, PI staining was transseptal, whereas in the remaining hearts the PI staining did not extend beyond the middle of the septum. Compared with the shock electrode region, there was minimal PI staining in the other areas of the ventricles in both control and MI hearts. However, MI hearts had increased staining compared with controls in the epicardial regions of the LV. A majority of the epicardial PI staining in MI hearts was proximal to both infarct tissues and myocytes, suggesting that much of the staining occurred in the infarct border zone.

Longitudinally, PI staining mostly corresponded with the active region of the shock electrode in all hearts, with minimal staining near the base and apex of the ventricles, above and below the active region. The individual traces for the seven control and seven MI hearts are shown in Fig. 3, C and D. Integrated total tissue areas measured from the fluorescence images showed that the total ventricular volumes of the MI and control hearts were not significantly different (3,599 ± 349 vs. 3,862 ± 288 mm³, P = 0.15; Fig. 3, C and D).

In one control and one MI heart, SVF self-terminated, after 29 and 35 s, respectively, before defibrillation was administered. In both of these hearts, there was substantially less PI staining in the shock electrode region, found only in...
a shallow layer of the endocardium (Fig. 4, pseudoshock vs. shock).

To quantify PI staining, the volume of PI staining was normalized by the total ventricular tissue volume in each of the 10 regions and in the entire ventricles. There was no significant difference in the amount of PI staining between control and MI hearts in the shock electrode region (SE: \( P = 0.71 \)) or in the whole ventricles (total: \( P = 0.76 \); Fig. 5A). PI staining in the regions away from the shock electrode was much lower (Fig. 5B; note the difference in the y-axis scale). However, there was significantly greater epicardial PI staining in MI hearts for both the anterior LV (0.43 ± 0.07 vs. 0.04 ± 0.06%, \( P < 0.01 \)) and posterior LV (0.15 ± 0.05 vs. 0.05 ± 0.04%, \( P < 0.01 \)). The other seven regions had minimal PI staining and did not show any significant difference between groups, either individually or in aggregate (other regions: \( P = 0.19 \)).

**Residual electroporation following recovery in fibrillating hearts.** Hearts that had a recovery period of 45 min before PI staining showed qualitatively less PI staining (Fig. 6 compared with Fig. 3), particularly in the shock electrode region (Fig. 4, shock residual vs. shock). Residual PI staining remained longitudinally correlated with the active region of the shock electrode (Fig. 6, C and D).

Quantitatively, there was no significant difference in the amount of residual PI staining between control (\( n = 5 \)) and MI (\( n = 3 \)) hearts in the shock electrode region (SE: \( P = 0.97 \)) or in the whole ventricles (total: \( P = 0.25 \); Fig. 5A). However, there was a significant difference in residual epicardial staining in both the anterior LV (0.02 ± 0.02 vs. 0.14 ± 0.10%, \( P = 0.03 \)) and posterior LV (0.01 ± 0.01 vs. 0.21 ± 0.11%, \( P < 0.01 \); Fig. 5B). Residual PI staining in the other regions was minimal, with no significant differences between groups.
Compared with hearts stained without recovery, the recovery period resulted in a significant reduction of PI staining, and that reduction was the same between the shock electrode region and the entire ventricles in control (SE: 45% reduction, \( P = 0.046 \); total: 45%, \( P = 0.02 \)) and MI (SE: 40% reduction, \( P = 0.046 \); total: 40%, \( P < 0.01 \)) hearts (Fig. 5A). In MI hearts, there was also a significant reduction of PI staining in the anterior LV epicardial region after recovery (67% reduction, \( P < 0.01 \)) but not in the posterior LV epicardial region (\( P = 0.23 \); Fig. 5B). However, there

![Fig. 4. Shock electrode region. Representative images of the shock electrode region of fibrillating control and MI hearts, for shock-induced electroporation with no recovery, pseudoshock, and residual shock-induced electroporation following a 45-min recovery. All images are from longitudinal positions approximately in the middle of the active region of the shock electrode.](image)

![Fig. 5. Comparison of PI staining. PI staining (light gray bars) and residual PI staining (dark gray bars) in fibrillating hearts and PI staining in paced hearts (white bars) for control groups (solid bars; \( n = 7, 5, \) and 5, respectively) and MI groups (striped bars; \( n = 7, 3, \) and 4, respectively). The staining for the shock electrode region (SE) and the whole ventricles (Total) (A) as well as for the anterior LV epicardial (aLV Epi) region, posterior LV epicardial (pLV Epi) region, and an aggregate of the remaining 7 regions (Other Regions) (B) with an expanded y-axis scale. *\( P < 0.05 \) between control and MI groups as indicated. †\( P < 0.05 \) between matching groups with and without recovery, and error bars indicate the SD.](image)
remained a significant amount of residual PI staining postrecovery for both control (SE and total: 55% residual) and MI (SE and total: 60% residual) hearts. There was no significant difference in PI staining in the other seven regions individually, although in aggregate, there was a reduction in control hearts (control vs. control, residual: other regions: 64% reduction, \( P < 0.04 \)) but not in MI hearts (MI vs. MI, residual: other regions: \( P = 0.09 \); Fig. 5B).

Comparison with paced hearts. Data from our previous electroporation study (22), in which single 8-ms 300-V MTE shocks were delivered to hearts while being paced at a 300-ms cycle length, were reanalyzed to compensate for ischemic PI staining in some hearts, with one MI heart excluded due to severe ischemia. Mean infarct volume in the paced MI hearts was 14.1 ± 2.7%. This reanalysis did not affect the previously published trends or conclusions. In particular, there remained no difference in electroporation between the paced control (\( n = 5 \)) and paced MI (\( n = 4 \)) hearts in the shock electrode region or over the entire ventricles. Only in the epicardial region of the anterior LV was there a significant difference in electroporation (control, paced: 0.05 ± 0.04%, MI, paced: 0.57 ± 0.48%, \( P = 0.046 \)), as previously reported.

Comparisons of total and regional PI staining were made between the fibrillating hearts studied here and the paced hearts (Fig. 5). MI size was similar between the studies (\( P = 0.56 \)). There was no significant difference in PI staining in the shock electrode region between either control (SE: \( P = 0.99 \)) or MI (SE: \( P = 0.37 \)) hearts. There was also no significant difference in total PI staining between control (total: \( P = 0.79 \)) or MI (total: \( P = 0.19 \)) hearts (Fig. 5A). PI staining was similar between paced and fibrillating hearts in the other nine regions, including the LV epicardial regions (Fig. 5B).
DISCUSSION

This study shows that dynamic pacing can be used to easily and consistently induce SVF in normal and MI rabbit hearts. Electroporation injury from a single clinically relevant defibrillation shock was concentrated around the shock electrode, and the presence of an MI only increased electroporation in the border zone around the infarct. A significant amount of cells recovered membrane integrity within 45 min of defibrillation, but a significant amount of electroporation remained, indicating a prolonged healing process. SVF had no significant effect on electroporation compared with previously studied paced hearts.

Induction of sustained ventricular fibrillation and shock delivery. The dynamic pacing protocol developed for this study offers a useful, easy, and effective means of inducing SVF in both normal and MI hearts. The current of the pacing stimuli remained relatively low (<1.0 mA), even at short cycle lengths (<100 ms), and no PI staining was observed near the position of the pacing electrode. This method of SVF induction was superior to all other methods that we tried, including high-frequency burst pacing.

Shock intensity affects the depth of electroporation (35), making it important to use clinically relevant shock energies in experimental studies. For this study, anodal shocks were chosen because they result in a lower DFT (18) and are less likely to trigger postshock arrhythmias (51). Clinically, shocks with safety margins of 1–2.6 times the energy of the DFT, resulting in a total shock intensity of 200–360% of the DFT, are necessary to achieve a high probability of successful defibrillations on the first shock (21, 33, 45). Here, measurement of the DFT was used to select clinical relevant shocks. Use of roughly two times the voltage of the DFT provided a safety margin of ~2.6 times the energy. A high safety margin was chosen to ensure that SVF would be terminated by a single shock, as required by the study design.

A monophasic waveform was chosen over a biphasic waveform for its simplicity. Although biphasic waveforms are now the standard in clinical defibrillators, there are many more parameters that may affect defibrillation success and the extent of electroporation injury, including the ratios between the amplitude and durations of the two phases. In this first study of electroporation in intact hearts under fibrillating conditions, a reduced complexity in the shock waveform was desirable. Additionally, this study’s 300-V anodal MTE shocks matched those used in our previous study in paced hearts (22), facilitating comparisons between the studies.

Electroporation in fibrillating hearts. In both normal and MI hearts, shock-induced electroporation predominately occurred around the location of the active lead, in both the transverse and longitudinal axes. Compared with the shock electrode region, the remaining nine regions had relatively little electroporation, as expected since the potential gradient is greatest near the active lead.

The presence of an MI at the apex and on the LV free wall had minimal effect on the amount of electroporation observed in the shock electrode region. This was likely due to the fact that infarct tissue was not adjacent to the active region of the shock electrode. Increased heterogeneity of tissue around the infarct would likely have a greater impact on electroporation if the infarct was closer to the active region of the shock electrode. There was increased electroporation in the LV epicardial regions in MI hearts, but this did not result in a significant difference in electroporation between control and MI hearts, since the electroporation was minimal and was overshadowed by the much greater amount in the region near the shock electrode.

Pseudoshock controls. Our previous study showed that paced hearts given a pseudoshock as controls had minimal PI staining throughout the ventricles, including the shock electrode region, showing that the PI staining was not caused by the perfusion setup (22). In this study, to have pseudoshock hearts to isolate the effects of SVF on electroporation, it would have been necessary to have SVF self-terminate after a consistent period of time, which we could not control. However, one control and one MI heart had SVF spontaneously terminate after the threshold for SVF (>20 s) but before a defibrillation shock was applied. Although limited in number, these events served as pseudoshock controls. Similar to paced pseudoshock hearts, minimal PI staining was observed in the shock electrode region of the fibrillating pseudoshock hearts, indicating that most PI staining was the result of shock-induced electroporation. However, in the fibrillating pseudoshock hearts, there was a thin endocardial layer of PI staining, possibly because of mechanical damage as the tissue rubbed against the electrode during SVF. The fibrillating pseudoshock MI heart had PI staining in the LV due to ischemia. While this did not affect the RV, including the shock electrode region, it made further comparisons with the defibrillated MI hearts difficult.

Residual electroporation following recovery in fibrillating hearts. To examine the persistent effects of shock-induced electroporation, hearts were allowed to recover for 45 min after a defibrillation shock before staining with PI. This recovery period is sufficient for isolated preparations of various types of cells to recover their membrane integrity (4, 37). However, our study showed that there remained a significant amount of residual electroporation 45 min postshock.

Autopsy studies of patients who have received defibrillation shocks from ICDs show only a small amount of necrosis around the active lead (43). This suggests that 45 min is insufficient for membrane healing to be completed and that the heart requires a longer period to recovery from a defibrillation shock. This discrepancy with previous electroporation recovery studies is likely due to the differences in the cell types and to the interconnected nature of cardiac myocytes in an intact heart vs. isolated cells.

It is also possible that multiple mechanisms are involved in membrane healing. The mechanism(s) involved in electroporation and subsequent membrane recovery are still poorly understood. While there are different theoretical models to explain membrane effects of electroporation (8), the general view is that the electric shock produces holes in the membrane that expand and contract. Over time, the smaller holes will close. In these models, if a hole becomes too large, either from the initial shock or from several smaller holes merging, the cell cannot repair the membrane, leading to cell death.

However, there is evidence that membrane repair can also occur via vesicular fusion. Studies with nerve cells have shown that, when transected, nerves will close off the end by constricting and filling the gap with vesicles (24). Nerves have been observed to survive for prolonged periods of time, even if the seal is leaky to ions and current. In cardiac myocytes,
vesicles have been observed in regions of the membrane where gap junctions had been torn out following dissociation (39). If vesicles are involved in the sealing of very large membrane holes following electroporation, it may explain how the cells are able to survive and heal over a longer period than minutes, during which time the membrane remains leaky, allowing PI to enter the cell.

Comparison with paced hearts. No significant difference in shock-induced electroporation was observed between the fibrillating hearts in this study and the paced hearts of our previous study. This indicates that 35–45 s of SVF did not have an impact on the extent or spatial distribution of shock-induced electroporation. The period of SVF in this study is generally longer than those experienced by patients with ICDs. Modern ICDs are designed to be able to detect SVF, charge, and deliver a shock in 20 s or less, depending on the device and its programming (5, 31). However, much longer delays before defibrillation may affect electroporation due to SVF-induced ischemia, which can compromise membrane integrity (17).

In agreement with the previously studied paced hearts, the additional PI staining in LV epicardial regions of MI hearts was concentrated in the infarct border zone. The increased PI staining was shown to be a result of the shock in our previous study (22). However, the mechanism promoting increased electroporation of the LV epicardial regions is unclear. One factor might be that the surviving myocytes in and around the infarct are more susceptible to electroporation because of pathophysiological conditions. Additionally, the infarct has greater tissue heterogeneity (20, 38) that may create more heterogeneous electrical field gradients (47), which could result in increased electroporation where the gradients are steeper. The thickness of the border zone may also play a role, since the thicker endocardial border zones did not have significantly increased electroporation.

Study limitations and future directions. The infarcts in this study were predominately located on the LV free wall, away from the active region of the shock electrode, which was placed in the RV. Clinically, many MI patients have infarcts that extend higher in the septum, resulting in infarcted tissues located near the active region of an ICD lead. It is likely that proximity of infarcted tissue to the lead would increase the extent of electroporation in the shock electrode region and change its spatial pattern. Future studies might use different MI models to evaluate the effects of the location of an MI relative to the active region of the shock electrode on the distribution and extent of electroporation. Additionally, the location of the reference electrode in this study was different from that used clinically, which may have some impact on electroporation in the septum.

This study examined the electroporation effects of a single successful defibrillation shock used to terminate SVF. We did not evaluate the effects of exposure to multiple shocks to terminate an arrhythmia or the effects of different shock waveforms. It would be interesting to quantify these effects on electroporation in future studies. Our preliminary analysis of three control and three MI hearts suggests that multiple shocks greatly increase the extent of PI staining around the shock electrode compared with the heart presented in this study that received a single shock. These hearts each received three to seven shocks before successful defibrillation, resulting in greatly increased PI staining around the shock electrode that was not only transmural across the RV free wall but also transseptal in all cases. These results suggest that delivery of multiple shocks to terminate an arrhythmia may have a significant impact on the extent of electroporation. However, allowing multiple defibrillation attempts would also allow for a reduction in the intensity of the initial shock, which will reduce the amount of electroporation. Electroporation from multiple shocks would likely depend on the number of shocks delivered, the shock intensities, as well as the interval between successive shock deliveries. Biphasic waveforms may result in faster membrane recovery, since the excess charge deposited by the first phase of the shock can be removed by the second phase (26) and are more clinically relevant than monophasic waveforms.

Our current study of recovery following shock-induced electroporation indicates that additional studies are necessary. The significant degree of electroporation remaining after 45 min strongly suggests that the heart has not recovered by that time. Longer recovery times need to be tested to elucidate the rate and pattern of recovery and to determine the extent and spatial distribution of truly irreversible electroporation that may have long-term adverse effects. Because the mechanism of electroporation and the membrane recovery that follows is still not well understood, basic cellular studies are also warranted, particularly to determine if vesicles are involved in membrane sealing following shock-induced electroporation.

In conclusion, in this study, a single clinically relevant defibrillation shock was delivered to fibrillating intact rabbit hearts to assess the spatial distribution and extent of defibrillation shock-induced electroporation, with and without a recovery period. The majority of electroporation in both cases was proximal to the active region of the shock electrode. The presence of an MI only affected the degree of electroporation in the epicardial regions of the LV predominately in the infarct border zone. A large portion of the shock-induced electroporation is reversible; however, it is likely that recovery occurs slowly, since less than half of the affected tissue recovered membrane integrity within 45 min following shock delivery. A short period of SVF before and during defibrillation did not affect electroporation.

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DISCLOSURES

No conflicts of interest.

AUTHOR CONTRIBUTIONS

Author contributions: Y.T.W., I.R.E., and Y.C. conception and design of research; Y.T.W. and Y.C. performed experiments; Y.T.W. analyzed data; Y.T.W. and Y.C. interpreted results of experiments; Y.T.W. prepared figures; Y.T.W. drafted manuscript; Y.T.W. and Y.C. edited and revised manuscript; Y.T.W., I.R.E., and Y.C. approved final version of manuscript.

REFERENCES

1. Aguel F, DeBruin KA, Krassowska W, Trayanova NA. Effects of electroporation on the transmembrane potential distribution in a two-


