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Atria are more susceptible to electroporation than ventricles: Implications for atrial stunning, shock-induced arrhythmia and defibrillation failure

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Abstract

BACKGROUND—Defibrillation shock is known to induce atrial stunning, which is electrical and mechanical dysfunction.

OBJECTIVES—We hypothesized that atrial stunning is due to higher atrial susceptibility to electroporation versus ventricles. We also hypothesize that electroporation may be responsible for early recurrence of atrial fibrillation.

METHODS—We investigated electroporation induced by 10 ms epicardial high intensity shocks applied locally in atria and ventricles of Langendorff-perfused rabbit hearts ($n = 12$) using optical mapping.

RESULTS—Electroporation was centered at the electrode and was evident from transient diastolic depolarization, and reduction of action potential amplitude and upstroke $dV/dt_{\max}$. Electroporation was voltage and polarity dependent and significantly more pronounced in the atria versus ventricles ($p < 0.01$), with a summary ED50 for main measured parameters of 9.2 ± 3.6 and 13.6 ± 3.2 V/cm in the atria versus 37.4 ± 1.5 and 48.4 ± 2.8 V/cm in the ventricles, for anodal and cathodal stimuli, respectively. In atria ($n = 5$), shocks of both polarities (27.2 ± 1.1 V/cm) transiently induced conduction block and reentry around the inexcitable area. Electroporation-induced ectopic activity was a possible trigger for reentry. However, in the thicker ventricles, electroporation and resulting conduction slowing and block were restricted to the surface only, preventing complete block and arrhythmia. The upstroke morphology revealed that the wavefront “dove” below the electroporated region and “resurfaced” into unaffected epicardial tissue.

CONCLUSION—We showed that the atria are more vulnerable to electroporation and resulting block and arrhythmia than the ventricles.

Keywords

electroporation; atrial stunning; conduction block; atrial fibrillation; optical mapping; defibrillation

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INTRODUCTION

Electrical shock is an effective therapy against atrial and ventricular fibrillation (AF and VF). However, despite significant improvement in defibrillation efficacy, which has resulted in a radical reduction of defibrillation thresholds and myocardial damage, there is still extensive clinical and basic electrophysiology data indicating that defibrillation shocks are accompanied by adverse effects: (1) transient ectopy, tachycardia or reinduction of VF(1–5) and AF;(6) (2) bradycardia, complete heart block and increased pacing thresholds;(4;7;8) (3) atrial and ventricular electrical and mechanical dysfunction (stunning), which is directly related to the strength of shocks. (9–17)

These effects are likely to be associated with shock-induced electroporation which is characterized by the formation of aqueous pores in the cellular lipid matrix.(18) This may disrupt ionic homeostatic mechanisms, causing loss of intracellular potassium and calcium overload.(19) Both clinical and basic experimental evidence suggests that electroporation is induced by defibrillation shocks, and plays a role in defibrillation,(20) post-shock metabolism and electromechanical function of the heart.(11;12) Functionally, some, but not all, studies have shown energy related post-shock myocardial stunning, presumed secondary to these mechanisms.(21)

The main limitation of cardioversion success of AF is not failure to defibrillate or “shock failure,” but early recurrence of AF (ERAF).(6) Because ERAF can occur within seconds of cardioversion, it may be difficult to distinguish from failure to convert. Within the first minutes after external cardioversion, a recurrence rate of 9–26% has been reported.(22;23) The mechanism of ERAF remains unclear.(24)

We hypothesized that electroporation-induced conduction block and abnormal focal activity could be responsible for defibrillation failure and atrial stunning. To test this hypothesis we investigated the effect of monophasic shock-induced electroporation on atrial and ventricular electrical activity using high-speed high-resolution optical mapping of Langendorff-perfused rabbit hearts.

METHODS

Animal protocols were approved by the Animal Care and Use Committees of Washington University. All animals used in this study received humane care in compliance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. The experiments were performed on Langendorff-perfused New Zealand rabbit hearts ($n = 12$).

The heart was perfused with oxygenated modified Tyrode’s solution at 36°C as previously described.(11) In brief, after administration of anesthesia, the heart was removed and placed on a Langendorff apparatus, where it was retrogradely perfused under a constant pressure of 70 ± 5 mm Hg with modified Tyrode’s solution. Temperature and pH were continuously maintained at $36 \pm 0.5^\circ\text{C}$ and 7.35 ± 0.05 .

We designed an electrode to achieve high and homogeneous current density over a relatively large area at the epicardium without electrochemical electrode-saline interface artifacts in the optical recordings as described previously.(25) A transparent plastic box filled with Tyrode’s solution with a 6 mm round hole was gently pressed against the left or right atria or ventricular free walls to seal the opening. A 16×16 photodiode array optical recording system with a sampling rate of 5 kHz was focused on the 11×11 mm area of epicardial surface with the hole in the center. A 20×20 mm silver wire loop was positioned inside the box 10 mm from the heart surface around the opening allowing unobstructed optical recordings from the field of view. Stimulating current entered the box through this wire. The reference electrode was a steel

mesh placed in the bath 5 cm away from the heart. Hearts were stained with the voltage-sensitive dye di-4-ANEPPS (1 $\mu\text{mol/L}$). Motion artifacts in optical recordings were suppressed by 15 mmol/L of the excitation-contraction uncoupler 2,3-butanedione monoxime (BDM, Sigma, MO).

Figures 1A and B show a representative example of heart images with fields of view and activation maps from right and left ventricles (RV and LV), and right and left atria (RA and LA).

In each experiment, the heart was equilibrated in the chamber for 30–40 min before the start of the experiment. The heart was paced at a cycle length of 300 ms through a bipolar electrode placed at the region close to the optical mapping field of view.

We optically mapped the RV, LV, LA and RA. The sequence was randomized in each heart. Our experiments lasted no more than 4 hours, during which the control values of the main electrophysiological parameters (conduction velocity and action potential duration) did not change.⁽²⁶⁾ We also did not find a correlation between electroporation effects and time since the beginning of the experiment.

Before atrial experiments were conducted, ventricular electrical activity was reduced by AV nodal ablation, induction of VF, or placing a piece of black plastic sheet between the atrial appendages and the ventricular epicardium. However, in some cases the ventricles were still activated and the resulting optical signal reached the PDs through internal optical scattering.⁽²⁷⁾ In almost all cases though, this signal was far smaller than the atrial signal and therefore did not significantly affect the measured parameters (for example see Figure 3). Rectangular stimuli with a duration of 10 ms were generated by a bipolar operational power supply/amplifier (Kepco, NY). These shocks were 5, 10, 30, 50, 70, and 90–100 volts in strength and of both polarities. The shocks were applied from lowest to highest strength at about 2 minute intervals to allow for recovery. A maximum of two shocks with the same polarity and intensity were applied on the same tissue. Data with electroporation induced extrasystolic activity and stunning were omitted. In order to maintain independence between time and shock order and electroporation effects, the order of polarity and tissue protocols (RV, LV, LA and RA) were varied. The interval between tissues was about 10–15 minutes.

We continuously paced tissue every 300 ms. Shock was applied immediately after all recording sites were depolarized (30–60 ms after pacing) in order to prevent induction of arrhythmia (source) by virtual electrode induced phase singularity.⁽²⁸⁾ We measured parameters from the first action potential after shock (EP1) 240–260ms after shock to investigate the effects of electroporation and for the tenth action potential after shock (EP10) about 3 sec after shock to investigate recovery of tissue.

Applied shock voltage and current waveforms were recorded simultaneously with optical recordings. The average current density of the stimulus was determined as the ratio between applied current and total cross-sectional area of the hole. The total impedance between the electroporation and reference electrodes was 140–250 Ω .

Electrical field values in the Tyrode's solution near the heart were calculated using the equation $E = J\rho$ as done previously,⁽²⁵⁾ with ρ (resistivity) of the Tyrode's solution estimated to be 50 cm, the value found by Weidmann for extracellular Tyrode's solution.⁽²⁹⁾ We did not directly measure field gradient values within the heart tissue because Woods et al.⁽³⁰⁾ have shown that measuring devices, such as plunge electrodes, cause unintended experimental artifacts and we did not attempt to calculate them because virtual electrode polarization,⁽²⁸⁾ and tissue heterogeneity make it impossible without exceedingly sophisticated modeling using real anatomical measurements for each preparation.

A customized Matlab-based computer program was used to analyze the optical signal in the ventricles and atria. First, we filtered the signal using the low-pass Butterworth algorithm at 200 Hz. Then, the two control beats of the optical signal were averaged and normalized to range from -85 to 15 mV. Finally, action potential amplitude (APA), shock-induced diastolic depolarization (DD), AP duration at 30% and 90% of repolarization levels (APD30 and APD90), maximum upstroke derivative ($dV/dt_{\max}$), and derivative duration at 50% amplitude level of $dV/dt_{\max}$ (VD50) were calculated for each AP using the normalized optical signal and its derivatives (Figure 1C).

Control $dV/dt_{\max}$ values for all tissues were from 5 V/s to 12 V/s which of course is significantly lower than the $dV/dt_{\max}$ of AP recorded by microelectrodes due to the averaging of optical signals from different cell layers and filtering.

We determined the % level of APA at which the rate of rise of the optical upstroke reaches its maximum, defining 0% as the level of maximum depolarization and 100% as the resting potential ($dV/dt_{\max, \%APA}$, see Figure 1C). Analysis of optical AP upstroke morphology ($dV/dt_{\max, \%APA}$) has been shown to be useful in determining subsurface wavefront orientation. (31;32) According to recent modeling and experimental study, upstroke morphology of optical APs is sensitive to the local subsurface orientation of the excitation wavefront. (32) Thus, we assumed that a value of $dV/dt_{\max, \%APA}$ on the epicardial optical AP ranges from 40% to 60% percent when excitation propagates predominantly parallel to the epicardium. A $dV/dt_{\max, \%APA}$ from 0% to 40% percent reflects movement of the excitation wavefront from endo to epi and a $dV/dt_{\max, \%APA}$ from 60% to 100% reflects movement of the excitation wavefront from epi to endo.

Maps were constructed of all of the above parameters. These data were then averaged over a 9 photodiode area before the minimum or maximum APA, $dV/dt_{\max}$, DD, APD30, APD90, and VD50 was found from within a circle centered at the center of electroporation with a radius of 8.94mm (3 photodiodes) corresponding to the region directly exposed to current. No averaging was done for $dV/dt_{\max, \%APA}$, because we hypothesized that the maximum and minimum should be adjacent to each other since they represent a change in the tissue layer with the largest $dV/dt_{\max}$.

We also identified electroporation-induced conduction block when apparent, defined as the number of channels with a $dV/dt_{\max}$ below a threshold value for each tissue. This threshold was determined by averaging the minimum $V_{\max}$ in the first sample of each series that had a conduction delay of 6.9 ms in the electroporated region, corresponding to a conduction velocity below 10 cm/s (see Figure 1 in Online Data Supplement). Manual correction was used when needed.

In summary, we analyzed the following parameters of myocardial activation in the electroporated region: maximum % changes in APA and $V_{\max}$, maximum and minimum APD30 and APD90 (ms), minimum $dV/dt_{\max}$ (V/s) and maximum VD50 (ms), minimum and maximum values of $dV/dt_{\max, \%APA}$ (%), minimum CV (cm/s) in the electroporated region, activation time across all photodiodes (ms), and number of blocked channels for the second control AP (Control), the first non-arrhythmic AP after shock (EAP1), and the last AP recorded (EAP10).

A recovery index ranging from -1 to 1 was calculated as $(EAP10 - EAP1) / \max(\text{abs}(EAP10, EAP1))$ for the EAP10 and EAP1 values of % change in APA and $dV/dt_{\max}$.

In cases when atrial arrhythmia was induced by shock, we repeated the same shock strength after 2 minutes. All ATs lasted less than 2 minutes, which was probably related to recovery of

atrial tissue excitability. We classified arrhythmia events on atrial premature beats (APBs) with <10 extra beats and atrial tachyarrhythmia (AT) with >10 extrabeats.

In atria we could not estimate electroporation-induced changes after shock >70 V/cm due to persistent myocardial damage and stunning.

To determine the role of cardiac structure in electroporation during the shocks, we examined $32\mu\text{m} \times 1\text{mm}$ sections from a $5\text{mm} \times 5\text{mm}$ square centered at the electroporated region from all four preparations (RA, LA, RV and LV) from six hearts. Standard histology and cryosectioning methods (33) were used and sections were analyzed to find the minimum and maximum transmural thicknesses (Figure 1D).

Statistics

Curve fits. Data for % change in APA and dV/dt_{max} , CV, $dV/dt_{\text{max},\% \text{APA}}$ dV/dt duration at 50%, APD30 and APD90 followed the sigmoidal relationship $f(E) = 1 - aE^b / (E^b + c)$, where E is electrical field, a is the fraction of decrease, and c is the value of E at half-maximum (ED50). (10) Data for recovery index versus E value of % change in APA and dV/dt_{max} and tissue thickness versus % change in APA and dV/dt_{max} were fit using the exponential decay equation $y = Ae^{-bx}$. Fits were obtained using a least squares method of all data points for all parameters except CV, for which averages at each voltage were used because a few outliers prevented an accurate curve fit of all data points.

Comparisons between groups of data were performed using a t-test for non-paired measurements and Kolmogorov-Smirnov test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Figure 2 shows graphs that were constructed for changes of APA, dV/dt_{max} , DD, and CV versus electric field using average values of the parameters and electric field for each shock voltage. Electrical shock induced strength and polarity dependent changes in these electrophysiological parameters of optical APs, which were related with electroporation.

Electroporation was centered at the electrode and was evident from transient DD, reduction of APA and dV/dt_{max} and conduction slowing until full block. ED50 and electroporation threshold (first shock data point that has statistical differences vs control data) for APA, dV/dt_{max} , and CV was significantly lower for anodal than cathodal shocks in all tissues (see statistics in Tables 1–2 and Online Data Supplement). The area and magnitude of these changes were directly related to shock strength, but were also polarity and tissue dependent. We found that these ED50 values were significantly higher for the right ventricle than the left, but more importantly, we found a large difference between the ED50 values for the ventricles and the atria (Figure 2). DD values could not be fit by a sigmoidal function because shock-induced atrial contractions at higher shocks (>25V/cm) changed the volume of tissue being recorded from and by extension of the baseline fluorescence.(34)

Both atria were significantly more sensitive than ventricles to occurrence of full conduction block in the center part of the electroporated region after shock. Figures 3 and 4 show representative examples of electroporation-induced conduction block in LA and LV at 5.4 V/cm and 101.6 V/cm, respectively. Panels A and B show optical AP recordings obtained from the middle of the conduction block and panel C shows maps of activation time and $dV/dt_{\text{max},\% \text{APA}}$ before and after shock, and changes of APA and dV/dt_{max} in LA appendages and LV epicardium. From our analysis of $dV/dt_{\text{max},\% \text{APA}}$ on AP upstroke, we found that the range of $dV/dt_{\text{max},\% \text{APA}}$ values significantly increased at the same electrical field strength during

which we found conduction slowing and block (Figure 2A in Online Data Supplement). This may suggest transmural propagation after shock as a result of the wave front “diving” below the electroporation induced epicardial conduction block and “resurfacing” into epicardial tissue. Moreover, we found that a second hump appeared in the $dV/dt_{\max}$ signal in the blocked area resulting from the splitting of the wavefront (Figures 3B and 4B) which induced increasing of $dV/dt_{\max}$ duration. Electroporation also induced a significant decrease in ventricular APD30, but did not significantly change APD90 (Figure 2B, C and D in Online Data Supplement).

To calculate the area of full epicardial conduction block, we calculated threshold values of minimal $dV/dt_{\max}$ for RV, LV, LA and RA under which block occurred (Figure 1 in Online Data Supplement). We did not find a statistically significant difference between these values. We suggest that $dV/dt_{\max}$ values below this threshold (2.2 V/s for ventricles and 2.4 V/s for atria) represent scattering signal and electrotonic depolarization from surrounding tissue. These values were used as $dV/dt_{\max}$ thresholds in determining conduction block area. Area of conduction block was significantly larger in atria compared to ventricles. We did not find a statistical difference between anodal and cathodal shock for block area size in all tissues.

We found a statistical difference in several parameters between RV and LV, and RA and LA, showing that the left atrium and left ventricle are more susceptible to electroporation. However, we believe that this may not actually be the case in the atria because many shocks induced arrhythmia and stunning in the right atrium (see Figure 5), and these were probably more electroporated, but were omitted.

We found that there was not complete recovery of studied parameters during the 3 sec after shock that were recorded. We also found that there was not a significant difference between the extent of atrial and ventricular recovery of APA and $dV/dt_{\max}$ after 3 seconds (see Figure 3A and B in Online Data Supplement).

However, we found the conduction slowing and block completely recovered after 1–2 minutes at less than 70 V/cm shocks, which is consistent with the fact that electroporation is a transient effect that lasts for several seconds until pores are sealed (Figure 3C and D in Online Data Supplement). However, higher intensity shock (>70 V/cm) could induce irreversible depression of conduction until full block which is related with persistent myocardial damage.

We found that in RA 102 ± 4 V/cm shock induced transient full conduction block not only in the electroporated area under the hole but in the entire RA in 4 out of 7 experiments. Figure 5 shows an example of full conduction block in the RA which was induced by 100 V/cm shock. Optical recordings from different regions and activation maps show that electroporation induced not only block near the electrode (hole) but also outside (see wave EAP1). Thus excitation could not propagate through the entire RA and excite the RA appendage (photodiode #3). AP propagation through the superior part of RA and the RA appendage resumed at wave EAP7. However, tissue in the center of the electroporated area was still inexcitable for at least 1–2 minutes after the shock.

Atrial tachyarrhythmias

Atrial premature beats (APBs) were induced after atrial shocks in some preparations ($n = 4$ for LA and $n = 4$ for RA) by shocks of both polarities and were mostly related with electroporation-induced focal activity from the border of electroporated tissue. No ventricular premature beats were observed. In 33% of cases, the APBs would initiate reentrant AT around the area of electroporation-induced conduction block with a cycle length of 107 ± 7 ms (Figure 6). The APBs which induced AT had significantly ($p < 0.05$) shorter CL's compared to APBs which did not induce AT – 92 ± 7 vs 111 ± 5 ms. Figure 6 shows an example of AT initiation by 27 V/cm LA shock. The first arrhythmic beats (maps a–c in Figure 6C) originated from the region

near the electroporation-induced inexcitable area (photodiode #1 on Figure 6A and B) 70, 145, and 223 ms after shock. These ectopic waves induced sustained counterclockwise reentry around the electroporation-induced inexcitable region. All ATs lasted less than 2 minutes, which was probably related to recovery of atrial tissue excitability. The probability of AT was significantly higher at 27 ± 2 V/cm compared to other shock ranges (Figure 6D).

We found that several 25–75 V/cm shocks in RA ($n = 4$) induced episodes of extrasystolic activity with cycle length 133 ± 9 ms which lasted 1–3 minutes after shock and were probably not directly related to electroporation. These were likely due to shock-induced release of neuromediators from intracardiac nerves in the highly innervated intercaval pacemaker region (33) outside of the field of view and therefore were excluded from the statistics in Figure 6D.

Histology

Histological sections showed significant variability of atrial wall thickness and morphology compared to ventricles. We found that the minimum thickness of the LV, RV, LA, and RA in the electroporated region had minimum and maximum thicknesses of 2.40 ± 0.32 – 5.32 ± 0.97 mm, 1.84 ± 0.51 – 2.01 ± 0.50 mm, 0.40 ± 0.11 – 1.33 ± 0.25 mm, and 0.27 ± 0.16 – 1.33 ± 0.25 mm, respectively. The minimum thickness was plotted against % change in APA and $dV/dt_{\max}$ and regressed against exponential decay. We found that thinner tissue was more susceptible to electroporation induced changes in these electrophysiological parameters (Figure 4 in Online Data Supplement).

DISCUSSION

We demonstrated that the atria are more vulnerable to electroporation than the ventricles in the rabbit heart with an APA, $dV/dt_{\max}$, CV ED50 of 16.4, 10.2, 9.7 V/s versus 37.7, 35.4 and 46.7 V/s respectively, which may explain atrial predisposition to stunning after defibrillation. Electroporation was voltage and polarity dependent and significantly more pronounced in the atria versus ventricles ($p < 0.01$). Importantly, the threshold of electroporation in the atria was found to be close to the commonly accepted defibrillation threshold of 5.4 V/cm in the ventricles, suggesting that atria could be electroporated during ventricular defibrillation.

Our results demonstrate a possible mechanism of early recurrence of atrial fibrillation (ERAF). It is likely to be caused by electroporation-induced conduction block and focal activity. These results may also explain phenomenon of the “iso-electric window”, which was observed after failed defibrillation shocks.(35)

Differences in electroporation threshold between atria and ventricular

The main interest in studying the effects of electric shocks on heart physiology was driven by the ability of strong shocks to terminate arrhythmias. Despite significant improvement in defibrillation efficacy, which has resulted in a radical reduction of defibrillation thresholds and myocardial damage, there are still extensive clinical and basic electrophysiology data indicating that high-intensity defibrillation shocks are accompanied by adverse effects, such as cardiac tissue damage and reinitiation of atrial and ventricular tachycardia.(1–4;6) Thus, measuring the electrical-induced cardiac dysfunction threshold is highly desirable to assess safety of defibrillation therapy used to terminate AF and VF.

To estimate the electroporation threshold, many electrophysiological parameters have been used: membrane conductance, depolarization of the cellular membrane during diastolic interval resulting in depression of excitability,(1;10;11;36) reduction of APA(25) and $dV/dt_{\max}$ (11), and elevation of intracellular calcium concentration.(36)

Additionally, information about electroporation can be indirectly inferred by staining tissue with fluorescent dyes such as Propidium Iodide(37) or Ethidium bromide,(38) which penetrate cells only through the electropores, and subsequent histological imaging of intracellular space. Electroporation threshold levels of local electrical field sufficient to induce dysfunction have been estimated in previous experimental studies to be in the range of 15–100 V/cm.(1;2;10; 25;39;40) This is a large range because the electroporation threshold depends on many other factors that varied in each study, such as preparation (from single isolated cell to whole heart), shock parameters (e.g., duration, waveform shape, and number of shocks), and the type of dysfunction. However, the electroporation threshold is definitely within the range of the electrical field generated during defibrillation shock (6–100V/cm).(41;42) Neunlist & Tung (10) demonstrated polarity dependence of electroporation in the frog epicardial ventricular muscle.

Thus, It is reasonable to believe that the observed shock-induced changes in optical APs from atria and ventricles are in fact a result of electroporation and it is unlikely that electric shock-induced lysis of the dye di-4-ANEPPS contributed to observed changes in fluorescence.(43)

Kodama et al showed using 7 photodiode optical recordings that the changes in post shock action potential configuration in rabbit RV epicardium were significantly greater than those observed in LV epicardium and ventricular septum when compared at the same levels of 10 ms monophasic shock intensity.(44) They suggested that RV epicardium is more susceptible than LV epicardium and septum for arrhythmogenic aftereffects of high intensity electrical shock stimulation. However, our data suggest the opposite: that the LV epicardium is more susceptible to electroporation than the RV (see Tables 1 and 2). Our study is based on higher spatial resolution (256 vs 7 photodiodes) and the larger field of view that allowed us to identify the most affected area.

Our results on the response of ventricular APA and $dV/dt_{\max}$ to electrical shocks are consistent with these experimental and modeling studies. However, all previous studies have investigated electroporation effects exclusively in ventricular tissue. We found for the first time that the ED50 for electroporation-induced effects is 3–10 times lower in atria than in ventricles (Figure 2–4 and Table 11 in Online Data Supplement). This difference can be related with tissue structure and thickness (see Figure 1 and Figure 4 in Online Data Supplement). It has been shown that myocardial structure contributes to activating function,(45) producing local secondary sources,(46) and resulting in virtual electrode polarization during point electrical stimulation(47) or defibrillation shocks.(48)

Recently, we conducted a series of experiments to investigate the role of cardiac structure in electroporation distribution in intact ventricles by a simulation/experimental approach.(49) Computer simulations and propidium iodide fluorescence analysis demonstrated that electroporation after external homogeneous 25 V/cm electrical shock occurred not only at the surface epicardial and endocardial layers, but also inside the ventricular walls near blood vessels. The 100V/cm shock caused transmural electroporation that was particularly pronounced close to the apex, where fiber orientation rapidly changes. Previously, we have shown that the endocardium is more sensitive to shock-induced electroporation than the epicardium, which may also be explained by structural differences.(11) Epicardial electroporation was localized to a small area near the electrode, whereas endocardial electroporation was observed at bundles and trabeculas throughout the entire endocardium. Atrial tissue is significantly thinner and more heterogeneous/trabeculated than ventricular tissue (Figure 1). Atrial pectinate muscles and trabeculas can be sources for virtual electrode polarizations that could increase local electrical field gradient and thus pronounce electroporation. Thus, the increased susceptibility to electroporation in the atria compared to

ventricles can be explained by their increased heterogeneity resulting in dramatically increased local field gradients through virtual electrode polarization.

Optical mapping allows us to map excitation in cardiac tissue to a depth of 1–2 mm.(50) Light scattering causes optical recordings to be made up of signals from a depth of up to 2mm.(8) Therefore, our optical recordings conducted from the atrial epicardium are likely to carry contribution from endocardial trabeculas and represent a weighted average of transmembrane recordings throughout the entire rabbit atrial wall because of its thinness. However, ventricular optical signal represents average signal almost exclusively from the subepicardium which are more resistant to electroporation than the endocardium, due to its relatively homogeneous structure.

Another explanation for the difference between the electroporation threshold for the atria and ventricles as well as between the RV and LV may be related to cell membrane structure, differences in ion channels and membrane lipid species.(38;51) It has been shown that the inward rectifier K⁺ current (I_{K1}) density is significantly lower in the atria than the ventricles and lower in the RV than the LV in mammalian hearts. (51;52) Because I_{K1} is the primary ionic current responsible for maintaining resting potential, atrial cardiomyocytes are more affected by electroporation-induced depolarization current because of a relative lack of the corrective influence of I_{K1} . Akuzawa-Tateyama et al have shown that decreasing I_{K1} increases sensitivity to electroporation in isolated ventricular cardiomyocytes.(53)

Mechanism of electroporation-induced conduction block

Severe electroporation has been previously shown to cause conduction block.(11;12) However, it has not been previously demonstrated that a moderate degree of electroporation can result in slowing of conduction. We used our localized field protocol to characterize the effects of electroporation on cellular action potential and on conduction properties. We found that electroporation induced transient conduction slowing and block in atria (>5 V/cm, Figure 3) and ventricular epicardium (>50 V/cm, Figure 4). The conduction slowing and block completely recovered after 1–2 minutes, which is consistent with the fact that electroporation is a transient effect that lasts for several seconds until pores are sealed.(18) However, higher intensity shock (>70 V/cm) could induce irreversible depression of conduction which is related with persistent myocardial damage (Figure 5). This electroporation-induced long-lasting conduction block can be one explanation of the depression of electrical and mechanical atrial functions (“stunning”) after defibrillation shock.(21)

Previously, we used the same experimental approach to demonstrate that LV epicardial shock induces electroporation mostly in the first several cell layers of the epicardium.(25) As mentioned above, our optical signal represents an average fluorescence collected from many layers of cells. Surface layers of cells are more likely to be electroporated compared to deeper layers as a result of the tissue-bath interface, governed by a space constant.(54) Thus, our optical recordings in Figures 3 and 4 could represent many layers of cells, which were completely electroporated at the surface of myocardium (epi- or endocardium), as well as deeper layers, which underwent partial electroporation or no electroporation at all. Their contribution to the signal will be of a lower weight because of the scattering and absorption of light. This effect can explain that even after high intensity shock of up to 100V/cm we still recorded optical APs from areas of electroporation-induced conduction block in the ventricles but not in the atria (see Figures 4 and 8). These APs had significantly lower amplitudes and two components of upstroke (Figure 4B) which reflected activation of the deeper layers and scattered light signal from tissue outside of electroporated area (Figure 4B). We could derive the transmural waveform orientation from the morphology of upstroke of optical AP (Figure 2A in Online Data Supplement) using the algorithm proposed by Hyatt et al.(32) We found increased transmural propagation after shock as a result of the wave front “diving” below the

electroporation-induced conduction block and “resurfacing” after passing through the electroporated region (see maps of $V_{\max, \%APA}$ in Figure 3 and 4).

The mechanism of transient conduction slowing and block may be explained by electroporation-induced diastolic depolarization and subsequent depression of fast sodium currents (I_{Na}) as well as gap junction uncoupling. Depolarization can create a region of conduction slowing or block. This region could act as a focal point for reentrant-type arrhythmias (Figure 6) and could also be responsible for the transient S-T changes sometimes observed after cardioversion or defibrillation.(7)

The intracellular Ca^{2+} overload caused by electroporation causes the gap junctions to be degraded(55) and become virtually impermeable(35;56), eliminating the low resistance path between cells, causing conduction slowing or even block. However, this response contains electroporation-induced damage to a small area, allowing non-electroporated cells to maintain the proper ion concentration and not sustain secondary damage. Yet, the decrease of conduction velocity caused by gap junction uncoupling can cause regional slowing and block of wave propagation that can result in re-entry around the blocked area. (Figure 6)

Electroporation-induced atrial tachyarrhythmias

Microelectrode experiments on isolated canine papillary muscles with attached Purkinje fibers and isolated guinea pig papillary muscles have shown that electroporation (20–75 V/cm) induced significant diastolic depolarization which can lead to afterdepolarization-like oscillations that cause spontaneous ectopic activations.(1;5) Conduction in papillary muscles of the intact rabbit heart can be transiently inhibited by strong electric shock as well. Such transient block can last from one beat to many seconds, depending on the shock strength.(11)

We found that electroporation induces re-entrant tachyarrhythmia in atria, due to depolarization which leads to conduction block and APBs originating near the border of the electroporated region (Figure 6). The mechanism of initiation of APBs and AT was related with sustained depolarization of an atrial bundle still electrically coupled with the normal excitable myocardium. Thus, the focal activity region could act as a trigger for reentry around electroporation-induced arrhythmias (Figure 6). This mechanism is supported by the observation that the probability of atria tachyarrhythmia in our experiment was significantly strength-dependent with maximum at 27.2 ± 1.1 V/cm. No APBs or ATs were induced at shock strengths below 10 V/cm or higher than 100 V/cm. However, we cannot fully exclude possible contribution of neuromediators released from intracardiac nerves by direct shock-stimulation (57) and possibly neural electroporation.(58) In fact, in 4 out of 7 RA preparations, we found APBs most likely originating from the highly innervated intercaval pacemaker region(33) with 133 ± 9 CL which lasted 1–3 minutes after shocks (25–75 V/cm), supporting this hypothesis.

LIMITATIONS

Our rabbit model cannot be directly scaled to the human heart. Conduction block and ATs were clearly evident in the atria of the rabbit heart, yet the larger size of human atrial wall might prevent this effect from occurring to the same extent. However, there is extensive clinical evidence for depression of cardiac electrical and mechanical function after the delivery of shocks. In addition, mechanical dysfunction (stunning) has been demonstrated more often in the atria than in the ventricles and appeared to be directly related to the strength of shocks. (21)

We did not investigate the amount of transmembrane potential change (ΔV_m) that was caused by the different shock strengths as in our previous study.(25) The absence of a plateau phase

and shock-induced motion artifacts in the atria did not allow us to determine if the same strength shock caused more transmembrane polarization in the atria than in the ventricles.

The use of BDM could affect the observed transients due to partial ion channel blockage. However, previously we showed that BDM did not change incidence of electroporation-induced conduction block.(11)

Changes in upstroke morphology may be attributed to other effects of electroporation, such as changes in APA or $dV/dt_{\max}$, rather than the diving or surfacing of the wave due to inexcitable tissue.

CONCLUSION

In conclusion, this study showed for the first time that the atria are more vulnerable to electroporation-induced depression of excitability than the ventricles. This may result from their decreased thickness, increased heterogeneity resulting from complex trabeculated structure, and different membrane shape and structure, including a lower density of I_{K1} channels. This vulnerability may explain the increased susceptibility of atria to stunning after defibrillation. Moreover, our results demonstrate a possible mechanism of early recurrence of AF (ERAF) after shock by electroporation-induced conduction block and focal activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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LIST OF ABBREVIATIONS

AF	atrial fibrillation
AP	action potential
APA	action potential amplitude
APB	atrial premature beat
APD	action potential duration
AT	atrial tachycardia
AV node	atrioventricular node
BDM	2,3-butanedione monoxime

CT	crista terminalis
CV	conduction velocity
DD	diastolic depolarization
EAP	post-electroporation action potential
ERAF	early recurrence of AF
ED50	the half maximal effective dose of electrical field
IVC	inferior vena cava
LA	left atria
LV	left ventricle
PD	photodiode
PVs	pulmonary veins
RA	right atria
RP	resting membrane potential
RV	right ventricle
SAN	sinoatrial node
SVC	superior vena cava
dV/dt_{max}	maximum upstroke velocity of AP
dV/dt_{max}%APA	% level of APA at which the rate of rise of the optical upstroke reaches its maximum
VD50	derivative duration at 50% amplitude level of V _{max}

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Figure 1. Activation maps and histology sections of the rabbit atria and ventricles

A. Photographs of an isolated Langendorff-perfused rabbit heart. The heart was stained with the voltage-sensitive dye di-4-ANEPPS. Electrical activity was mapped from the field of views shown with rectangles (11 × 11 mm). The 20-mm silver wire loop was positioned inside the box 10 mm from the heart surface around the 6-mm-diameter opening (superimposed circle). RA and LA: right and left atria. RV and LV: right and left ventricles. Square waves show position of pacing electrode. Dashed lines show location of sections shown on Figure 1D. Circled "1" shows location of optical AP recording in Figure 1C.

B. Control activation maps during 300 ms pacing. Isochrones are separated by 3 ms for LA, RV and LV and by 2 ms for RA.

C. Experimental protocol and terminology. An example of a left atrial optical action potential (AP) recording and its derivative (dV/dt) during constant pacing with 300 ms cycle length before (control) and after (EAP1 and EAP2) a cathodal shock of 28 V/cm. Optical control AP was normalized to range from -85 to 15 mV. Each AP was analyzed to calculate action potential

amplitude (APA), shock-induced diastolic depolarization (DD), AP duration at 30% and 90% of repolarization levels (APD30 and APD90), maximum upstroke derivative ($dV/dt_{\max}$), and derivative duration at 50% amplitude level of $dV/dt_{\max}$ (VD50) using the normalized optical signal and its derivatives

D. Masson's trichrome histology tissue sections from right and left ventricles and left atrium. See text for details.

Figure 2. Voltage and polarity dependent DD and conduction velocity (CV) and changes in APA and dV/dt_{max} in atria and ventricles

A, B and D - APA, dV/dt_{max}, and CV values were fit to a sigmoidal function using a least squares method.

C - Diastolic depolarization (DD) values could not be fit by a sigmoidal function because of motion artifacts at higher shocks (>25V/cm).

E - Half-maximum points of curve fits (ID50) are indicated by perpendicular lines on **A, B** and **C**

Figure 3. Electroporation-induced block of conduction in the left atria (same preparation as Figure 1)

A - Representative trace of optical AP from photodiode (PD) #3 and its derivative (dV/dt) from the center of the electroporated region before and after anodal shock (5.4 V/cm). The small amplitude ventricular APs (Ventr. APs) seen in the optical APs recorded from LA epicardium is due to light scattering from ventricles.(27)

B - Superimposed upstrokes of optical APs and their derivatives in control and after shock (EAP1) corresponding to photodiodes 1–6 near electroporation-induced conduction block (black line) in maps on panel C

C, Left - maps of activation and dV/dt_{max}, %APA on AP before (Control) and after (EAP1) electroporation shock. **Right** - maps of electroporation-induced changes of APA and dV/dt_{max} (%) compared to control values. Black line shows conduction block.

Figure 4. Electroporation-induced block of conduction in the left ventricular epicardium

A - Representative trace of optical AP and its derivative (dV/dt) from the center of the electroporated region before and after 10 ms anodal shock (101.6 V/cm).

B - Superimposed upstrokes of optical APs and their derivatives in control and after shock (EAP1) corresponding to photodiodes 1–6 near electroporation-induced conduction block (black line) in maps on panel C.

C, Left - maps of activation and dV/dt_{max}, %APA on AP before (Control) and after (EAP1) shock. **Right** - maps of electroporation-induced changes of APA and dV/dt_{max} (%) compared to control values. Black line shows conduction block.

Figure 5. Electroporation-induced full conduction block in the right atria (atrial stunning)

A – Photographs of an isolated Langendorff-perfused rabbit heart with superimposed PDA field of view (11×11 mm) and electroporation electrode ($\phi 6$ mm). SVC and IVC – superior and inferior vena cavae. White dotted line shows electroporation-induced conduction block.

B – Optical Action potentials recordings before and after electrical shock from photodiodes shown by 1–3 on activation maps.

C – activation maps in control and after shock EAP1 and EAP 10. Black color (map B–C) shows the electroporation-induced conduction block zone. Isochrones are separated by 4ms.

Figure 6. Electroporation-induced atrial tachyarrhythmias

A- Photographs of an isolated Langendorff-perfused rabbit heart with superimposed PDA field of view (11 × 11 mm) and electroporation (EP) electrode (6 mm). LAA – left atria appendage, LV – left ventricular, PV – pulmonary vein.

B- Optical APs from photodiode shown by 1–3 in activation maps.

C- activation maps during atrial pacing (control) and during electroporation-induced arrhythmia (A–H). Black area (map B–H) shows the electroporation-induced conduction block zone region. White area shows absence of activation during the map time interval. Isochrones are separated by 2 ms.

D- Probability of atrial premature beats (APBs) and tachyarrhythmia (AT) at different shock strength.

Table 1

Maximum difference in amplitude for first post-shock paced AP (%)

Current Density (mA/cm ²)	Control	105 ± 1.5	213 ± 3.6	639 ± 11.7	1101 ± 160	1574 ± 223	2188 ± 326
Electric field (V/cm)	Control	4 ± 1.8	10 ± 1.7	30 ± 5.5	53 ± 7.5	74 ± 10.5	103 ± 15.3
RV-	-0.3 ± 0.1	-	-0.7 ± 0.2	-5.1 ± 1.2 ^{*,#}	-21.6 ± 2.4 ^{*,#}	-39.1 ± 3.2 ^{*,#}	-43.4 ± 2.5 ^{*,#}
RV+	-0.3 ± 0.1	-	-1.0 ± 0.3	-10.8 ± 1.0 ^{*,#}	-30.4 ± 2.2 ^{*,#}	-40.6 ± 2.1 ^{*,#}	-43.7 ± 3.5 ^{*,#}
LV-	-0.2 ± 0.1	-	-1.4 ± 0.6	-12.2 ± 3.4 ^{*,#}	-31.4 ± 5.3 ^{*,#}	-44.8 ± 5.4 ^{*,#}	-45.6 ± 2.6 ^{*,#}
LV+	-0.2 ± 0.1	-	-2.0 ± 0.4	-18.7 ± 3.2 ^{*,#}	-34.5 ± 5.5 ^{*,#}	-42.9 ± 3.8 ^{*,#}	-46.0 ± 4.8 ^{*,#}
LA-	-0.7 ± 0.9	1.1 ± 1.0 ^{*,#}	-13.8 ± 3.1 ^{*,#}	-62.8 ± 2.4 ^{*,#}	-67.6 ± 2.5 ^{*,#}	-67.4 ± 2.7 ^{*,#}	-
LA+	-0.7 ± 0.9	-15.1 ± 5.5 ^{*,#}	-29.5 ± 4.8 ^{*,#}	-56.4 ± 2.5 ^{*,#}	-63.8 ± 2.7 ^{*,#}	-61.7 ± 4.1 ^{*,#}	-
RA-	-1.8 ± 2.1	-3.2 ± 2.1 ^{*,#}	-7.3 ± 4.0 ^{*,#}	-36.7 ± 5.5 ^{*,#}	-61.4 ± 4.7 ^{*,#}	-59.4 ± 6.9 ^{*,#}	-
RA+	-1.8 ± 2.1	-8.4 ± 1.6 ^{*,#}	-15.8 ± 2.8 ^{*,#}	-42.9 ± 5.3 ^{*,#}	-51.4 ± 6.0 ^{*,#}	-52.4 ± 7.7 ^{*,#}	-

Group data represented as mean ± SEM.

* p < .05,

** p < .001 vs. control (0V/cm shock)

p < .05 cathodal vs. anodal shock for same tissue and voltage

\$ p < .05 right vs. left chamber for same polarity

Table 2

Maximum difference in $dV/dt_{\max}$ for first post-shock paced AP (%)

Current density (mA/cm ²)	Control	105 ± 15	213 ± 36	639 ± 117	1101 ± 160	1574 ± 223	2188 ± 326
Electric field (V/cm)	Control	4 ± 1.8	10 ± 1.7	30 ± 5.5	53 ± 7.5	74 ± 10.5	103 ± 15.3
RV-	-0.7 ± 0.0	-	-1.1 ± 0.1	-8.3 ± 2.8 ^{*,#}	-49.6 ± 6.2 ^{**,§}	-70.1 ± 3.3 ^{***}	-69.0 ± 1.8 ^{***}
RV+	-0.7 ± 0.0	-	-1.9 ± 0.3 [§]	-26.2 ± 3.8 ^{*,§}	-60.8 ± 3.5 ^{***}	-70.8 ± 2.5 ^{***}	-70.8 ± 0.4 ^{***}
LV-	-0.9 ± 0.2	-	-1.7 ± 0.7	-20.2 ± 7.5 ^{*,#}	-50.7 ± 8.4 ^{***}	-71.3 ± 4.2 ^{***}	-74.2 ± 3.8 ^{***}
LV+	-0.9 ± 0.2	-	-3.4 ± 0.4 ^{*,#}	-37.1 ± 5.3 ^{*,#}	-62.5 ± 5.2 ^{***}	-72.6 ± 3.4 ^{***}	-74.0 ± 4.5 ^{***}
LA-	-0.8 ± 0.2	-0.9 ± 1.0 ^{#,§}	-29.6 ± 6.8 ^{*,#}	-79.5 ± 2.4 ^{*,§}	-79.3 ± 3.1 ^{***}	-76.3 ± 3.7 ^{***}	-
LA+	-0.8 ± 0.2	-35.0 ± 10.5 [*]	-53.3 ± 6.4 ^{***}	-76.6 ± 2.7 ^{***}	-79.9 ± 2.8 ^{***}	-80.0 ± 3.0 ^{***}	-
RA-	-1.0 ± 0.3	-1.4 ± 0.7 ^{#,§}	-17.3 ± 7.8 ^{#,§}	-66.3 ± 4.3 ^{***}	-75.3 ± 4.3 ^{***}	-72.4 ± 10.7 ^{***}	-
RA+	-1.0 ± 0.3	-14.1 ± 1.9 ^{***}	-41.8 ± 5.9 ^{***}	-68.5 ± 4.9 ^{***}	-68.8 ± 8.6 ^{***}	-59.3 ± 12.3 ^{***}	-

Group data represented as mean ± SEM.

* p < .05,

** p < .001 vs. control (0 V/cm shock)

p < .05 cathodal vs. anodal shock for same tissue and voltage

§ p < .05 right vs. left chamber for same polarity