Mechanisms of shock-induced arrhythmogenesis during acute global ischemia

YUANNA CHENG, KENT A. MOWREY, VLADIMIR NIKOLSKI, PATRICK J. TCHOU, AND IGOR R. EFIMOV

Department of Cardiovascular Medicine, Cleveland Clinic Foundation, Cleveland 44195; and Department of Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio 44106

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Cheng, Yuanna, Kent A. Mowrey, Vladimir Nikolski, Patrick J. Tchou, and Igor R. Efimov. Mechanisms of shock-induced arrhythmogenesis during acute global ischemia. Am J Physiol Heart Circ Physiol 282: H2141-H2151, 2002—Little is known about the mechanisms of vulnerability and defibrillation under ischemic conditions. We investigated these mechanisms in 18 Langendorff-perfused rabbit hearts during 75% reduced-flow ischemia. Electrical activity was optically mapped from the anterior epicardium during right ventricular shocks applied at various phases of the cardiac cycle while the excitation-contraction decoupler 2,3-butanedione monoxime (BDM; 15 mM) was used to suppress motion artifacts caused by contraction of the ischemic tissue. Repolarization was preserved and negative changes in preshock transmembrane potential. The success or failure of the shock is determined by this shock-induced polarization pattern. The induction of a shock-induced reentrant arrhythmia is via a virtual electrode-induced phase singularity mechanism (VEIPS). However, nearly all of these studies investigated defibrillation/vulnerability in the normal, nonischemic myocardium. Defibrillation/vulnerability under ischemic conditions was not intensively studied despite the fact that up to 70% of implantable cardioverter defibrillator patients have some form of coronary artery disease (21, 53). Behrens et al. (3) presented evidence of increased vulnerability during external shocks associated with increased heterogeneity of ventricular repolarization in acute global ischemia. However, the defibrillation threshold was not affected by acute myocardial ischemia in their study. Holley and Knisley (24) characterized the response of the ischemic tissue to electric shock and reported observations of virtual electrodes under ischemic conditions. However, the role of virtual electrodes in defibrillation failure and shock-induced vulnerability in ischemia remains unclear.

Our goal was to investigate shock-induced vulnerability and defibrillation under the conditions of acute global ischemia produced by reduced flow in the Langendorff-perfused rabbit heart using voltage-sensitive dye and imaging techniques.

UNCOVERING THE MECHANISM by which strong electric shocks extinguish life-threatening arrhythmias has been challenging researchers for many years since its discovery in 1899 (34). The last decade of research has resulted in a significant improvement of our understanding of the basic mechanisms of defibrillation summarized in the virtual electrode polarization hypothesis of defibrillation, which offers new insight into the effects of the electric shocks on the myocardium (15). This success was primarily due to earlier theoretical and experimental advancements that resulted in the formulation of the bidomain formalism of cardiac syncytium (19, 28, 44) and the fast fluorescent mapping of electrical activity in the heart (10). The virtual electrode polarization hypothesis of defibrillation (15) is based on numerous theoretical (18, 39, 41, 43) and experimental (6, 13, 14, 20, 26, 29, 50) studies. According to this hypothesis, a shock induces both positive and negative changes in preshock transmembrane potential. The success or failure of the shock is determined by this shock-induced polarization pattern. The induction of a shock-induced reentrant arrhythmia is via a virtual electrode-induced phase singularity mechanism (VEIPS). However, nearly all of these studies investigated defibrillation/vulnerability in the normal, nonischemic myocardium. Defibrillation/vulnerability under ischemic conditions was not intensively studied despite the fact that up to 70% of implantable cardioverter defibrillator patients have some form of coronary artery disease (21, 53). Behrens et al. (3) presented evidence of increased vulnerability during external shocks associated with increased heterogeneity of ventricular repolarization in acute global ischemia. However, the defibrillation threshold was not affected by acute myocardial ischemia in their study. Holley and Knisley (24) characterized the response of the ischemic tissue to electric shock and reported observations of virtual electrodes under ischemic conditions. However, the role of virtual electrodes in defibrillation failure and shock-induced vulnerability in ischemia remains unclear.

Our goal was to investigate shock-induced vulnerability and defibrillation under the conditions of acute global ischemia produced by reduced flow in the Langendorff-perfused rabbit heart using voltage-sensitive dye and imaging techniques.

Address for reprint requests and other correspondence: Y. Cheng, Dept. of Cardiovascular Medicine, Desk FF1-06, Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44195 (E-mail: chengy@ccf.org).

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METHODS

Experimental preparation. Langendorff-perfused hearts (n = 18) from young rabbits (age: 60 ± 4.7 days) were used in the present study. A detailed description of the heart preparation has been previously published (12–14). Briefly, the rabbit was anesthetized with nembutal, and the heart was then removed and placed on a modified Langendorff apparatus for retrograde perfusion. A custom-made 10-mm platinum coil electrode (Guidant) was inserted into the right ventricular cavity through the pulmonary artery. A second similar electrode was positioned in the bath 1–2 cm above and 1–2 cm behind the heart. The heart was stained with a gradual injection of 550 μl of stock 1.25 mg/ml solution of the voltage-sensitive dye di-4-ANEPPS (Molecular Probes) in DMSO (Fisher Scientific) over 10–15 min. The excitation-conversion decoupler 2,3-butanedione monoxime (BDM; Fisher Scientific) (22) was added to the perfusate of the following composition (in mM): 15 BDM, 128.2 NaCl, 1.3 CaCl2, 4.7 KCl, 1.05 MgCl2, 1.19 NaH2PO4, 25 NaHCO3, and 11 glucose.

Global acute ischemia was produced by rapid reduction of the flow rate by 75% from 20 ml/min in control to 5 ml/min. Ischemia-induced electrophysiological changes were continuously monitored with electrograms and optical imaging. A relatively steady state of action potential (AP) duration (APD) was reached between 20 and 30 min of flow reduction in the hearts. Therefore, data collection was performed after 30 min of flow reduction during ischemia, and data were compared with control data collected at the normal flow rate of 20 ml/min.

Optical mapping. The fluorescence was excited by a direct current-powered light source at 520 nm by a 16 × 16 element square matrix of photodiodes coupled to a computerized data conditioning and acquisition system. Data were filtered at 1 kHz and sampled at a rate of 1,894 frames/s, yielding a temporal resolution of 528 μs. The field of view was 16 × 16 mm in all experiments.

Optical APs were recorded before, during, and after the application of shocks. Typical data scans were 1–2 s and included the last basic beat AP, the onset of the next AP, and the shock-induced response. A single lead electrocardiogram (ECG) was recorded using two Ag/AgCl probes placed ~1 cm from the right and left side walls of the glass chamber relative to a Ag ground placed at the bottom of the chamber. This lead configuration produced an ECG qualitatively similar to the lead I of the body surface ECG.

Experimental protocol. The heart was positioned in a temperature-controlled, water-jacketed glass chamber with the anterior wall facing the optical apparatus. Figures 1–3 show the typical fields of view (square line, 16 × 16 mm) seen by the photodiode array. The heart was paced at a basic cycle length (CL) of 300 ms by electrical stimuli of twice the diastolic pacing threshold strength and 2-ms stimulus duration from the apex of the heart. Truncated exponential monophasic shocks of 8 ms in duration were delivered from a 150-μF capacitor defibrillator (HVS-02, Ventritex) between the two electrodes described above. In 13 of 18 experiments, shocks with ±100-V strength were applied at various phases of the cardiac cycle. A shock of 100 V was chosen because in our previous study we reported the shock-induced vulnerability in the structurally normal heart and demonstrated that 100-V shock could induce 100% of arrhythmias when applied at the certain phases of APD (52). We extended this investigation under ischemic conditions in this study. Triggering of shocks at variable coupling intervals from the last pacing stimulus was performed via a custom pacing program embedded into the data acquisition and analysis program, as previously described (14).

Classification of shock-induced arrhythmia. In some cases, no extrasystole resulted from the shock. Occurrence of one or more extrasystole was defined as shock-induced arrhythmia, which can be further divided into nonsustained or sustained ones. Nonsustained shock-induced arrhythmia was defined as an arrhythmia that self-terminated in <1 min. Sustained shock-induced arrhythmia was defined as an arrhythmia that lasted >1 min and had a CL < 160 ms that required a defibrillation rescue shock to terminate it.

Time constants of cellular response to shock. A set of experiments was conducted in 5 of 18 rabbits to quantify the transmembrane response to a defibrillation shock; the cellular time constant (τ) was calculated during delivery of the
shock in control and during the ischemia. Monophasic shocks (8 ms in duration, 150 μF) of ±100, ±130, ±160, ±190, and ±220 V were applied at 25%, 50%, and 75% of the APD. The polarizations were approximated for both control and ischemia with single-exponential fits using the Levenberg-Marquardt method (33). To automate the processing of large amounts of data, a custom program was developed using Microsoft Visual C++ to automatically analyze the data with the ability for manual review and correction. The τ was calculated only in the virtual electrode areas away from the shock electrode because electroporation created near the shock electrode (1) would contaminate the cellular response, and the virtual electrode areas have been shown to provide the substrate for shock-induced arrhythmogenesis and defibrillation failure (6, 14). Because the shock electrode in this study was always inserted in the right ventricular cavity and seen at the left edge of field of view (see Figs. 1–3), τ was calculated from optical traces at the right side of field of view only. To more accurately and objectively define the areas of virtual electrodes at a distance from the shock electrode and thus those traces accepted for analysis, the following exclusion criteria were followed: 1) The virtual electrode polarization area directly above the shock electrode was not included in the analysis to avoid contamination of cellular responses by electroporation; 2) To improve the fidelity of τ measurement, only strong transmembrane responses to the shock (amplitude > 10 mV) were considered; 3) Also to improve fidelity of τ measurement, only traces with a signal-to-noise ratio above 75 were considered. A total of 33,291 individual responses to a total of 300 shocks (150 shocks in control and 150 shocks in ischemia) were included in the analysis: 18,169 during control and 15,122 during ischemia from five hearts.

Data analysis and visualization. The signal analysis software programs used in this study have been previously described (14). These programs automatically calculated from all 256 optical recordings maps of activation (37), repolarization (16), and APD (52). Briefly, activation, repolarization, and APD were calculated as follows: For each optical channel, we defined depolarization (activation) time as the time difference between the pacing stimulus and the maximum of the first derivative of the AP upstroke. Assuming the base potential as 0% and maximum potential as 100%, we defined repolarization time as the time difference between the pacing stimulus and the time when an optical signal repolarized to the 10% level. APD was calculated as the difference between repolarization and depolarization times. Thus the changes in resting potential and AP amplitude (APA) brought by global ischemia will not affect calculated time maps of activation, repolarization, and APD. Because APD was calculated using the AP at 90% repolarization (APD90) definition described above, all APD90 values will be referred to as APD throughout this report. For transmem-
brane potential voltage maps, we assigned 0% for the resting potential and 100% for the maximum APA and expressed all voltage maps relative to the last basic beat for each individual channel (%APA). Activation time (AT) was subtracted from coupling interval (CI), defined as the time difference between the stimulus and the shock application, to calculate the percent APD in each channel at which the shock was applied according to the following formula: %APD = (CI – AT)/APD × 100. The vulnerable window was defined by excluding the coupling interval at which arrhythmia incidence was significantly (P < 0.05) below 50% (52). Dispersion of repolarization was defined as the difference between the shortest and longest repolarization times across the field of view (3). The gradient of the transmembrane potential and its derivative was calculated using a five-point algorithm similar to that used in our previous report to calculate the conduction velocity (37). An isoelectric window was calculated from the field of view as a delay between the start of the shock application and the upstroke of the first shock-induced response.

Figure 1 illustrates the three main types of analysis used in our study. Figure 1A, shows a photograph of the heart superimposed with an isochronal map (orange map) of post-shock activation during arrhythmogenesis. The time scale starts at the time of shock withdrawal. This approach is limited by its two-dimensional nature, being able to represent only the single sweep of a wavefront across the field of view. Therefore, a three-dimensional stack plots was also used to visualize multiple rotations of reentry of the post-shock activation. To build such a plot, each of the shock-induced response from 256 optical channels was low-pass filtered at 50 Hz, differentiated, and normalized relative to the last basic beat response [(dV/dt)max] from the same recording site. The horizontal x-y plane represents the field of view and the vertical z dimension corresponds to time, increasing from top to bottom. With the use of IDL software (Research Systems; Boulder, Co), three-dimensional volumes were built by stacking the two-dimensional plots of normalized derivatives of optical recordings. An isosurface was then constructed using a density threshold value of 30%. The stack plot shown in Fig. 1B visualizes the first two reentrant beats of the arrhythmia, one of which is also shown in Fig. 1A as a conventional isochronal map. Arrhythmogenesis was better understood with maps of transmembrane potential at the end of the shock, shown at the top of the stack plot. This map revealed the virtual electrode polarization developed by the shock. In this case, the right ventricle was positively polarized relative to the preshock potential, whereas the left ventricle was negatively polarized.

**Statistical analysis.** Group data were expressed as mean values ± SD. Statistical comparisons were performed using paired or unpaired t-tests. Differences were considered significant when P < 0.05.

**RESULTS**

**Effects of acute global ischemia on the electrical activity.** Figure 2 shows a photograph of the experimental preparation used in the present study (A) and representative optical recordings of APs during different phases of acute ischemia (B). During ischemia, APD progressively shortened and became more triangular than that in control recordings. Figure 2C shows reduction of the mean APD during ischemia over the time. Shown data represent an average of 256 optical recordings collected from the preparation shown in Fig. 2A. Note that after 25–30 min of ischemia, APD reduction more or less stabilized.

Figure 3 shows maps of activation, repolarization, and APD during control and ischemia from another representative preparation. The activation pattern remained similar with only a slight slowing of the conduction in ischemia compared with that in control. The average conduction time across the filed of view (minimum AT subtracted from maximum AT) was 34.1 ± 9.1 and 36.8 ± 10.2 ms (P = 0.061, n = 13 rabbits) in control and after 30 min of ischemia, respectively. In contrast, repolarization underwent significant changes during acute ischemia. The average repolarization time was significantly reduced. Repolarization in control was 232 ± 16 ms. Repolarization after 30 min of ischemia was 186 ± 30 ms (P < 0.01, n = 13 rabbits). Dispersion of repolarization increased (P < 0.01, 45 ± 17 ms in control and 73 ± 28 ms in ischemia, n = 13 rabbits). The repolarization pattern became highly heterogeneous, as can be easily seen in the repolarization and APD maps in Fig. 3. Furthermore, in all hearts (n = 13), we found a significant (P < 0.01) reduction of APDsp: 176 ± 9 ms in control and 129 ± 26 ms after 30 min of ischemia.

These findings confirm previous observations during acute ischemia in various species (3, 11, 38, 40, 46, 47).

Shock-induced vulnerability is enhanced by acute ischemia. Figure 4 shows the incidence of shock-induced arrhythmia provoked by ±100-V, 8-ms monophasic shocks delivered at various phases of APD. In this case, we considered all types of arrhythmia, including sustained and nonsustained. In control, arrhythmia incidence was observed mainly during the T wave (APD > 50%), whereas in ischemia it was evident at any phase of APD. The width of vulnerable window increased from 30–90% of APD in control to ~10 to 100% of APD in ischemia. Average incidence was 36% and 61% in control and ischemia, respectively (P < 0.01). These data summarized a total of 307 and 152 shocks from 13 hearts under control and ischemic con-
ditions, respectively. Noteworthy, out of all shock-induced arrhythmias, average incidence of sustained arrhythmias were significantly different: 16% and 53% in control and ischemia, respectively \((P < 0.01)\). Thus in control in this preparation, arrhythmias were primarily self-terminating, lasting <1 min. In contrast, in ischemia, arrhythmias were primarily sustained and required defibrillation.

**Virtual electrode polarization and deexcitation in acute ischemia.** Figure 5 shows contour maps of the transmembrane voltage at the end of \(-100\)-V, 8-ms shocks applied at 15%, 40%, and 60% of APD juxtaposed with isochronal maps of postshock activation under control and ischemic conditions. All maps were recorded from the same field of view in the representative heart shown in Fig. 2. A \(-100\)-V cathodal shock produced the virtual electrode polarization pattern with an area of positive polarization near the shock electrode (virtual cathode) and adjacent area of negative polarization (virtual anode). No significant qualitative differences were evident in the virtual electrode polarization patterns at the end of the shock between control and ischemia applied at the same phase of APD.

However, there was a difference in the occurrence of the postshock break excitation. In control, as we have recently described (52), a shock applied during the absolute refractory period (Fig. 5, top left: 15% of APD) failed to produce deexcitation in the area of negative polarization (virtual anode). This was due to the all-or-none repolarization effect (30, 49). The term deexcitation is used to describe the situation in which the negative polarization is strong enough to abolish the AP and restore excitability. In most cases, the lack of an extensive deexcited region precluded break excitation from occurring. In contrast, shocks applied late in the APD (Fig. 5, top right: 60% of APD) deexcited a large region of the heart. This deexcitation provided the substrate for formation of a wide wavefront of break excitation, which resulted in a reentrant arrhythmia via VEIPS. Shocks applied during the plateau phase (Fig. 5, top middle: 40% of APD) were able to deexcite a small region at the apex (lower middle part of the field of view), providing a substrate for a small wavefront of break excitation. This wavefront barely survived propagating in a fractioned fashion between nondeexcited regions.

In ischemia, shock-induced deexcitation appeared at any phase of APD and occupied a larger area available for postshock break excitation. As evident from activation maps, break excitation was induced at any phase of shock application and resulted in arrhythmia.

Figure 6 further illustrates the genesis of wavefront of break excitation. We recently suggested that the wavefront of break excitation arises when two conditions are met: 1) sufficient deexcitation is produced (6), and 2) a critical transmembrane voltage gradient at the boundary between virtual anode and virtual cathode is reached (7). Figure 6 shows the distribution of the amplitude of the transmembrane voltage gradient immediately after the shock (within 0.5 ms). Figure 6 shows the same data as in Fig. 5. As seen in Fig. 6, top, both the control and ischemia transmembrane voltage gradients reached similar amplitudes in the same area between the virtual anode and virtual cathode. Yet wavefronts of break excitation (Fig. 6, bottom) were not...
generated in all cases. Under control conditions, a decrease in the prematurity of shock application resulted in a decrease of the width of the wavefront until no wavefront was generated (control: 15% of APD). No significant delay was observed between shock withdrawal and wavefront generation, which remained within 20 ms. In contrast, in ischemia, a wavefront was generated at any phase. However, its first appearance was delayed with a decrease in the prematurity of shock application from 12 to 53 ms. The large isoelectric window (53 ms), defined as a delay between shock and epicardial excitation, could be the result of a delayed breakthrough of a intramural propagation of virtual electrode-induced scroll waves that occurs inside of the bulk of the myocardium.

There was also a difference in the kinetics of the responses to the shocks. Figure 7 shows the time constants of shock-induced responses for different shock polarities and strengths and times of application. Virtual cathode polarization (depolarizing cellular responses) was consistently faster in control compared with ischemia for almost all strengths and times of application. However, statistical significance was not reached for the virtual anode area (hyperpolarizing cellular responses).

Fig. 6. Relation between postshock transmembrane voltage gradient and wavefront origin. A: postshock distribution of the absolute amplitude of the transmembrane voltage gradient (black band). B: distribution of $\frac{dV_m}{dt}$ normalized to the $(dV_m/dt)_{\text{max}}$ of the last basic AP upstroke recorded at the same location (gray band). Numbers (in ms) represent the time elapsed after shock withdrawal. A and B show the analysis of the same data presented in Fig. 5.

Fig. 7. Summary of cellular responses to the monophasic shocks applied at the different phases of APD. Shocks at the intensities of $\pm 100$, $\pm 130$, $\pm 160$, $\pm 190$, and $\pm 220$ V were delivered at 25%, 50%, and 75% of the APD. $\tau$, Cellular time constant. *Significant difference ($P < 0.05$).
Observation of defibrillation failure during ischemia due to self-defibrillation: evidence of isoelectric window. In a majority of hearts (9 of 13) under ischemic conditions, mechanisms of shock-induced arrhythmogenesis appeared similar to that under control conditions. Namely, arrhythmia was induced by the VEIPS mechanism (14). Figure 8 illustrates a representative example. This record was obtained from the same heart as in Fig. 1. Notice that the time scale is the same as in the stack plot of Fig. 1. Comparison of Figs 1 and 8 illustrates similarities between the two types of arrhythmogenesis under control and ischemic conditions: both are reentrant waves rotating in a counterclockwise direction. However, this comparison also illustrates significant differences. Under ischemic conditions, the pathway was significantly more tortuous and discontinuous compared with control. This was due to shortening of APD, significant dispersion of repolarization, and local deceleration of conduction, which caused a higher degree of fractionation of conduction wavefronts.

However, in several hearts (4 of 13), in addition to the virtual electrode-induced phase singularity mechanism of arrhythmogenesis, there were also defibrillation failures. These cannot be explained by this mechanism, because of a significant isoelectric window or delay between the shock and epicardial excitation during failed defibrillation (5). Interestingly, this type of defibrillation failure was observed in ischemic hearts only after a number of applied shocks, which could potentially have caused additional damage to the heart. Repeated attempts to rescue these hearts led to an acceleration of refibrillation with a shortening of the isoelectric window (339 ± 189 ms in average). These four hearts could not be rescued, and the experiments were eventually terminated.

Figure 9 illustrates optical recordings from one of these four hearts. The trace in Fig. 9A shows the fibrillation terminated by a biphasic rescue shock (17) followed by self-refibrillation. No pacing stimulus was applied after rescue shock. An isoelectric window of
450 ms is clearly seen in the optical trace. Such extended delay before self-refibrillation cannot be explained by a delay of break excitation in the bulk of myocardium to the epicardium. The seven expanded superimposed traces in Fig. 9B show the first and second beats after the isoelectric widow. They were taken from the depolarized regions shown in Fig. 10 marked as 1–7. The first postshock beat (Fig. 10, left) appeared near the left ventricular apex and spread normally in the right side of the field of view but significantly slower in the left side of the field of view (next to the shock electrode and shown as a circled region). Maps of repolarization and APD50 revealed that the area of slow conduction was also an area of delayed repolarization. The second beat (Fig. 10, middle) revealed that the conduction delay in this area doubled. Maps of repolarization and APD50 showed that this small area was significantly depolarized (optical traces from this region are shown in Fig. 9B) and remained unexcitable when the third beat arrived. A stack plot (Fig. 10, right) shows that the third wavefront fractionated at this island of depolarized tissue (red arrow in the stack plot) and formed reentry, which rapidly deteriorated into fibrillation.

DISCUSSION

In the present study, we confirmed that global acute ischemia produces a significant reduction of APD and increase of dispersion of repolarization in the whole rabbit heart, which is in agreement with findings from others (3, 11, 38, 40, 46, 47). Also in an agreement with the observations by Behrens et al. (3) during externally delivered shocks, we demonstrated that ischemia resulted in a significant increase of vulnerability to shock-induced arrhythmogenesis during internal defibrillation-strength shocks. This was evident from widening of the vulnerable window (from 30–90% of APD in control to −10 to 100% of APD in ischemia) and increased propensity of sustained arrhythmias (16% in control vs. 53% in ischemia).

Besides ventricular repolarization heterogeneity as an important contributing factor, we speculate that an enhanced susceptibility to deexcitation due to ischemia might also contribute to such dramatic difference in vulnerability. Normally, deexcitation during the absolute refractory period is of an all-or-nothing nature, whereas during the relative refractory period it is of a gradual nature (23, 49, 52). Only the latter provides the substrate for arrhythmogenesis, whereas the former is antiarrhythmic. That explains the vulnerable window in the normal condition. Ischemia makes it possible to deexcite cells in a gradual fashion during nearly the entire refractory period, which leads to a possibility of arrhythmogenesis at any coupling interval of shock application in ischemic hearts. However, additional studies are required to explore this hypothesis at a cellular level.

Our data indicate (see Figs. 5, 6, and 8) that, during ischemia, a VEIPS mechanism is responsible for shock-induced arrhythmogenesis in the majority of cases: break excitation wavefronts were produced at the areas of maximum gradient between the virtual cathode and virtual anode as during control. However, these wavefronts had much more tortuous pathways and increased instability due to increased dispersion of activation and repolarization. These instabilities lead to wavefront fractionation and development of ventricular fibrillation.

The virtual electrode polarization pattern remained grossly similar in ischemia compared with that in control (Fig. 5). However, there was a different effect of ischemia on depolarizing and hyperpolarizing cellular responses (τ+/τ_), with τ+ being increased by ischemia and τ− unaltered. We speculate that increased τ+ in
ischemia might be related to an increase in intracellular resistance caused by gap junction uncoupling (32, 36, 25, 51). The resultant slowing conduction of wavefront of activation is considered a risk factor for arrhythmogenesis, which may contribute to defibrillation failure of electric shock. However, the exact cellular mechanism of the difference in \( \tau_r/\tau_w \) during ischemia remains unknown. Further pharmacological studies are required to explore the ionic currents involved.

Furthermore, we observed persistent self-defibrillation in four hearts after a number of shocks. The presence of a significant isoelectric window (339 ± 189 ms) indicates that the defibrillation was not due to shock-induced break excitation. We observed that in these hearts, conduction in the area adjacent to the shock electrode was significantly slowed, which contributed to postshock wave fractionation and reentry, and deteriorated into fibrillation (Fig. 10). The presence of large isoelectric windows (>300 ms) observed only under ischemic conditions and only in four hearts cannot be explained by intramural propagation of virtual electrode-induced scroll waves as under normal and some ischemic condition (Fig. 6, bottom left), which was <60 ms (8). It is noteworthy that this type of failure was not observed until a certain number of shocks were delivered to the ischemic heart. We never observed this type of failure and large (>60 ms) isoelectric windows in nonischemic hearts. This suggests that shock-induced damage superimposed with ischemia provides the substrate for either a focal or reentrant mechanism of arrhythmogenesis (or both) not directly related to shock-induced break excitation. Additional studies are required to elucidate the exact mechanisms of this type of defibrillation failure.

**Study limitations.** First, shock-induced arrhythmias are complex three-dimensional phenomena. Therefore, the two-dimensional mapping technique used in this study provides only limited insights into the mechanism. For example, our ability to reveal the mechanism of the isoelectric window is limited. Nevertheless, our data strongly indicate that the observed phenomenon can be extended to the three-dimensional situation. Unfortunately, no experimental technique is available at present to assess the three-dimensional map of electrical activity. Computer simulations may provide the missing link.

Second, we used the excitation-contraction uncoupler BDM in this study. On one hand, BDM is known to have an antifibrillatory effect (27, 35), and the protective effect of BDM from ischemia-reperfusion injury is also well known (2, 4, 45). These effects of BDM could bias our estimates of vulnerable window toward smaller degree. However, even though these protective effects of BDM exist, our study indicates that fibrillation was readily inducible under ischemia, whereas it was not readily inducible under control conditions. On the other hand, BDM is a general phosphatase acting on numerous proteins (9, 31, 42). It is reported that BDM has various other effects. For example, a recent report suggests that the Na/Ca exchanger is also inhibited by BDM by unknown mechanisms other than phosphatase activity (48). Thus caution should be taken in interpretation of present data from this study.

Third, the present study was performed under experimental constraints. During ischemia, the vulnerable window and the value of \( \tau \) were determined over a period of time during which there were ongoing changing electrophysiological states of the ventricular myocardium. It is very difficult, if not impossible, to keep myocardial ischemia at equilibrium over time. Measurements were begun 30 min after the onset of ischemia, because the APD duration was significantly altered at this time and tended toward a quasi equilibrium for the subsequent period of time. We measured the vulnerable window and \( \tau \) in a separate set of experiments in an attempt to reduce the time of data collection during ischemia to a minimum.

Finally, in the present study, we investigated the effects of global rather than regional ischemia. Global ischemia affects the heart in a more homogeneous way compared with that of regional ischemia, such as acute myocardial infarction, which occurs in a distinct territory of the heart. This may contribute to discrepancies in the exact pattern of virtual electrodes to that of global ischemia. We are presently working on a regional ischemia/infarct model to address this limitation.

In conclusion, in the present study, we confirmed in our model a well-known effect of ischemia resulting in dramatic reduction of the APD along with increased dispersion of repolarization. We observed the widened vulnerable window and increased severity of arrhythmias during ischemia. The shock-induced virtual electrode polarization pattern remains similar between control and ischemia. In ischemia, depolarizing response time constants increased, whereas hyperpolarizing response time constants were unaltered. A VEIPS mechanism was responsible for shock-induced arrhythmogenesis during ischemia in the majority of cases. However, in ischemia, virtual electrode-induced wavefronts of break excitation had much more tortuous pathways in the three-dimensional myocardium and increased instability leading to wavefront fractionation. The increased propensity to shock-induced arrhythmias in ischemia is due to the increased dispersion of repolarization and altered deexcitation. We further demonstrated that in some cases, persistent self-reinitiating arrhythmia occurred after an isoelectric window under acute ischemic conditions after rescue shocks first terminated the arrhythmia. This was probably due to either a focal or reentrant mechanism of arrhythmogenesis (or both) accelerated by discontinuous conduction and wavefront fractionation, which was not directly related to shock-induced break excitation.

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SHOCK-INDUCED ARRHYTHMOGENESIS IN ISCHEMIA

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