Intramural Virtual Electrodes During Defibrillation Shocks in Left Ventricular Wall Assessed by Optical Mapping of Membrane Potential

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Background—It is believed that defibrillation is due to shock-induced changes of transmembrane potential ($\Delta V_m$) in the bulk of ventricular myocardium (so-called virtual electrodes), but experimental proof of this hypothesis is absent. Here, intramural shock-induced $\Delta V_m$ were measured for the first time in isolated preparations of left ventricle (LV) by an optical mapping technique.

Methods and Results—LV preparations were excised from porcine hearts ($n=9$) and perfused through a coronary artery. Rectangular shocks (duration 10 ms, field strength $E \approx 2$ to 50 V/cm) were applied across the wall during the action potential plateau by 2 large electrodes. Shock-induced $\Delta V_m$ were measured on the transmural wall surface with a 16×16 photodiode array (resolution 1.2 mm/diode). Whereas weak shocks ($E \approx 2$ V/cm) induced negligible $\Delta V_m$ in the wall middle, stronger shocks produced intramural $\Delta V_m$ of 2 types. (1) Shocks with $E>4$ V/cm produced both positive and negative intramural $\Delta V_m$ that changed their sign on changing shock polarity, possibly reflecting large-scale nonuniformities in the tissue structure; the $\Delta V_m$ patterns were asymmetrical, with $\Delta V_m < \Delta V_m >$. (2) Shocks with $E>34$ V/cm produced predominantly negative $\Delta V_m$ across the whole transmural surface, independent of the shock polarity. These relatively uniform polarizations could be a result of microscopic discontinuities in tissue structure.

Conclusions—Strong defibrillation shocks induce $\Delta V_m$ in the intramural layers of LV. During action potential plateau, intramural $\Delta V_m$ are typically asymmetrical ($\Delta V_m < \Delta V_m >$) and become globally negative during very strong shocks. (Circulation. 2002;106:1007-1014.)

Key Words: arrhythmia • defibrillation • excitation • mapping

Electrical shocks are commonly used to interrupt ventricular fibrillation, yet key aspects of the interaction between shocks and cardiac tissue are not well understood. In particular, it is not well known how shocks cause changes of transmembrane potential ($\Delta V_m$) that are essential for defibrillation. The classic cable theory predicts that shock-induced $\Delta V_m$ decay rapidly with distance from the tissue-bath interface so that no $\Delta V_m$ should be present beyond a few electrotonic space constants from the wall surface, leaving the bulk of the left ventricle (LV) unaffected by a shock. To explain defibrillation, it has been suggested that intramural $\Delta V_m$ are induced by resistive discontinuities in the tissue structure, i.e., changing fiber orientation, or nonuniformity in the shock electrical field. Whether shocks indeed induce intramural $\Delta V_m$ remains unknown. Recent optical mapping studies provided important new information about shock-induced $\Delta V_m$ in the heart, but these studies were limited to measurements from the heart surface. Therefore, the goal of the present study was to measure shock-induced $\Delta V_m$ in the intramural layers of the LV. For this purpose, we used isolated coronary-perfused preparations of LV wall excised from pig hearts. Shocks of variable strengths were applied across the wall, and shock-induced $\Delta V_m$ were measured optically on the transmural surface.

Methods

Preparations of LV Wall

Pigs of either sex weighing 20 to 25 kg (Animal Resource Program, University of Alabama at Birmingham) were anesthetized and operated on in accordance with the American Heart Association guidelines for the care and use of animals, as described elsewhere. Hearts were stopped by injection of 10 mmol/L KCl solution, removed, and placed in cold (≈5°C) Tyrode solution (see below) with 10 mmol/L KCl. The LV was excised, and a branch of the left anterior descending coronary artery was cannulated. To visualize the area of perfusion, a green food-coloring dye was injected into the artery. A preparation containing the perfused area was excised with a sharp razor blade. The approximate location and orientation of the excised tissue are schematically shown in Figure 1A.
The preparation length and width were \( \approx 4 \) and 2 cm, respectively. On one side of the preparation, a straight cut was made through the ventricular wall at the edge of the perfused area. Only those preparations that exhibited uniform perfusion across the transmural section were used for experiments. The preparations were placed into a tissue bath with a glass window for optical mapping (Figure 1B) and arterially perfused with Tyrode solution (in mmol/L: 129 NaCl, 4.5 KCl, 1.3 CaCl\(_2\), 1 MgCl\(_2\), 1 NaH\(_2\)PO\(_4\), 25 NaHCO\(_3\), 5 glucose) that was gassed with a mixture of 95% O\(_2\) and 5% CO\(_2\) (36°C). To avoid motion artifacts, solution was supplemented with 15 mmol/L of an electromechanical uncoupler, 2,3-butanedione monoxime (BDM). The solution was delivered to the preparations by a roller pump at a pressure of 40 to 50 mm Hg. The preparations were immersed in the solution. Two types of boundary conditions (BCs) for electrical current flow at the transmural surface were used: impermeable BCs when preparations were prepared by cutting with a hand saw, and permeable BCs when preparations were shifted \( \approx 2 \) mm away from the glass.

Preparations were paced at a cycle length of 500 ms via an electrode placed at a preparation edge. Rectangular shocks (duration 10 ms) were applied via 2 large mesh electrodes (dimensions 5×2.5 cm\(^2\)) located at opposite ends of the tissue bath. In the absence of preparations, such electrodes produced a uniform electrical field. Shock strength in the bath was measured by a bipolar electrode (wire diameter 50 \( \mu \)m, interelectrode distance 1 mm) glued to the glass window near the mapping area (Figure 1B).

**Optical Mapping of Shock-Induced \( \Delta V_m \)**

Preparations were stained by the \( V_m \)-sensitive dye di-4-anepps (Molecular Probes). As shown in Figure 1C, tissue fluorescence was excited at 540±10 nm with a 250-W tungsten-halogen lamp (Oriel). The emitted fluorescence was measured at >610 nm with a photographic lens (Nikon, 50 mm), a 16×16 photodiode array (Hamamatsu), and a data acquisition system described previously.\(^{12-13}\) Measurements were performed at a sampling rate of 5 kHz/channel and a spatial resolution of 1.2 mm/diode.

Shocks with a strength of 2 to 50 V/cm were applied during the action potential (AP) plateau, at which shock-induced \( \Delta V_m \) are not masked by the flow of sodium current. \( \Delta V_m \) were measured as the difference between a linear-regression fit of the plateau before shock application and the \( V_m \) level at the shock end (Figure 1D); \( \Delta V_m \) were normalized by the AP amplitude (APA). The AP duration (APD\(_{50}\)) was measured as a time interval between 50% levels of AP upstroke and the repolarization phase. Data were expressed as mean±SD. Tissue structure was examined in 5-\( \mu \)m-thick transmural slices of formalin-fixed myocardium that were stained for collagen by the picrosirius red technique.\(^{14}\)

**Results**

Experiments were performed in 5 preparations with impermeable BCs and 4 preparations with permeable BCs. Wall thickness measured in the preparation center was 1.6±0.3 cm (\( n=9 \)). Figure 2A illustrates an outline of a preparation, a schematic of tissue chamber, preparation, and shock electrodes. Shocks were applied 50±6 ms after AP upstrokes measured at the preparation center. Optical mapping revealed that depending on the shock strength, 3 different types of shock-induced \( \Delta V_m \) patterns were produced.

**Effects of Weak Shocks on Intramural \( \Delta V_m \) and APD**

Figures 2C through 2E illustrate the effects of the weakest shocks (E\( \approx 2 \) V/cm) on intramural \( V_m \) with impermeable BCs. Figure 2C displays optical recordings obtained in control and with 2.0 and \( -1.9 \) V/cm shocks; Figure 2D displays corresponding isopotential \( \Delta V_m \) maps. The 2-V/cm shock induced positive \( \Delta V_m \) at the tissue edge facing the cathode and negative \( \Delta V_m \) at the edge facing the anode. With the \( -1.9 \)-V/cm shock, the polarization pattern was reversed. In both cases, maximal \( \Delta V^+ \) and \( \Delta V^- \) were achieved at the wall edges, and there was a relatively gradual transition in \( \Delta V_m \) magnitude between the edges. A local \( \Delta V_m \) increase in the wall middle at distance (\( x \)) \( \approx 7 \) mm (Figure 2E) was too small (<6% APA) to be considered a virtual electrode.

The lower plot in Figure 2E illustrates shock-induced changes in APD\(_{50}\) (\( \Delta \)APD\(_{50}\)) at different locations across the wall. APD\(_{50}\) was prolonged at sites of maximal \( \Delta V_m \), whereas at sites of maximal \( \Delta V_m \), APD\(_{50}\) was either not changed or was slightly prolonged.

**Effects of Intermediate Shocks**

Figure 3 shows the effects of \( \approx 9 \)-V/cm shocks, which differed from the effects of the weaker shocks in 4 main aspects. (1) The stronger shocks produced localized increases of \( \Delta V^+ \) and \( \Delta V^- \) inside the wall. One such region with \( \Delta V_m \), \( \approx 30\% \) APA was created just under the epicardium in the...
lower left part of the mapping area (site 13). With the
−8.8-V/cm shock (Figure 3B, bottom map), this area was
positively polarized, and it was surrounded by an area of
negative polarization. Such isolated polarization, as well as
the overall nonuniform distribution of ΔV_m across the wall,
clearly indicated the presence of intramural virtual electrodes.
(2) The ΔV_m induced by the stronger shocks were asymmet-
crical. Thus, ΔV_m was larger than ΔV_m at the same sites
(Figure 3A, traces 1 and 11), and the area occupied by ΔV_m
was larger than the area of ΔV_m (Figure 3B). (3) Quite
unexpectedly, negative ΔV_m extended toward the cathode
side of the preparation, such as in the lower part of the top
map in Figure 3B. Close inspection of the V_m recordings from
this area (Figure 3A, site 12) revealed that there was an initial
positive deflection of V_m at the edge of the wall (red trace),
but ΔV_m became negative toward the end of the shock, likely
because of electrotonic interaction with the adjacent area of
large negative ΔV_m (site 13). (4) Stronger shocks prolonged
APD at all sites across the wall for both ΔV_m and ΔAPD50.

Effects of Strong Shocks
Increasing the shock strength further produced ΔV_m patterns
of the third type illustrated in Figure 4. Shocks with E=28
V/cm produced predominantly negative ΔV_m across the wall
where either minor ΔV_m (<10% APA) were present (Figure
4B, top map) or the whole transmural surface was negatively
polarized (bottom map). The degree of APD prolongation

Figure 2. Effects of weak shocks on intramural V_m (impermeable BCs). A, Outline of preparation and shock electrodes.
stim indicates stimulation; epi, epicardium; and endo, endocardium. B, Isochronal map of activation spread. C, Optical recordings of V_m and shock (E) in control and with 2.0- and −1.9-V/cm shocks. Numbers correspond to photodiodes indicated in Figure 2D. D, Isopotential maps of ΔV_m distribution for 2 shocks at t=9 ms after shock onset. E, Spatial profiles of shock-induced ΔV_m and ΔAPD50.

Figure 3. Effects of intermediate shocks on intramural V_m. A, V_m recordings in control and with 9.1- and −8.8-V/cm shocks. B, Isopotential maps of shock-induced ΔV_m.
was similar for shocks of both polarities and was not dependent on the local value of $\Delta V_m$ (Figure 4C).

Intramural virtual electrodes were observed in all 5 LV preparations with impermeable BCs. The specific $\Delta V_m$ patterns differed between preparations, but all of them exhibited intramural $\Delta V_m$ of the 2 types described above. The transition between different $\Delta V_m$ patterns on increasing shock strength is illustrated in Figure 5, with panel A depicting maximal and minimal $\Delta V_m$, panel B showing areas occupied by $\Delta V_m^+$ and $\Delta V_m^-$, and panel C showing profiles of $\Delta V_m$ across preparations ($n=5$). Plots in panels A and B can be separated into 3 areas. Shocks with $E \approx 2$ V/cm produced both $\Delta V_m^+$ and $\Delta V_m^-$ of similar magnitudes. Shocks stronger than $\approx 4$ V/cm induced asymmetrical $\Delta V_m$ with $\Delta V_m^+ > \Delta V_m^-$. Shocks with $E > \approx 34$ V/cm induced predominantly negative polarizations.

Figure 6 shows the effects of shocks on APD$_{50}$ averaged across mapping area (A) and relative standard deviation of APD$_{50}$ (B) in 5 preparations. Application of shocks caused prolongation of average APD$_{50}$ in a dose-dependent manner, whereas the variability of APD$_{50}$ was slightly decreased. This decrease in the APD standard deviation reflects increased uniformity of $\Delta$APD$_{50}$, distribution illustrated in Figure 4C.

Role of BCs
To evaluate the role of BCs in formation of virtual electrodes, $\Delta V_m$ were measured in 4 preparations with permeable BCs. In 2 of these preparations, measurements were subsequently repeated with impermeable BCs. Figure 7 compares $\Delta V_m$ maps measured with 2 BCs in the same preparation at different shock strengths. It demonstrates that both localized virtual electrodes (A and B) and globally negative $\Delta V_m$ (C) were observed with both BCs. Moreover, $\Delta V_m$ patterns and shock dependence of maximal and minimal $\Delta V_m$ (D) were qualitatively similar to those observed with impermeable BCs.

Tissue structure was examined in 2 pig hearts. Figure 8 illustrates the gross morphology (A) and microscopic structure (B) of thin transmural slices stained with picrosirius red. These images demonstrate the existence of both macroscopic structural nonuniformities related to blood vessels and changing orientation of fiber bundles (Figure 8A) as well as microscopic discontinuities related to collagen septa (bright lines in Figure 8B). The large blood vessels such as shown in Figure 8A were not present on the transmural surface during optical mapping, but they could be located close enough beneath the surface to produce subepicardial $\Delta V_m$ such as exemplified in Figure 3B.

Discussion
This work presents the first optical measurements of shock-induced intramural $\Delta V_m$ in the LV wall. The most important findings of this work are as follows: (1) shocks of sufficient...
Both positive and negative shocks of moderate strength were strongly nonuniform.

Induced by shocks in the bulk of the LV, which makes prolongation for both positive and negative transmural surface; and (3) strong shocks induced APD prolongation for both positive and negative tissue structure. 4,16 The other explains electrical field (activating function) of cardiac critical mass. It has long been believed that successful defibrillation of the heart depends on \( V_m \) changes in a “critical mass” of cardiac tissue, 15 which postulates the presence of intramural \( \Delta V_m \) in the bulk of ventricular muscle. However, the experimental evidence supporting this hypothesis was lacking. Moreover, there is contradictory evidence from the classic linear cable model that indicates that no intramural \( \Delta V_m \) should be induced by shocks in the bulk of the LV, 1 which makes experimental verification especially important. The present study presents the first experimental evidence that defibrillation shocks indeed induce \( \Delta V_m \) in the deep layers of LV wall.

Two types of \( \Delta V_m \) were observed. Intramural \( \Delta V_m \) induced by shocks of moderate strength were strongly nonuniform. Both positive and negative \( \Delta V_m \) were observed, and their sign was changed on changing the shock polarity (Figure 3). \( \Delta V_m \) induced by stronger shocks were predominantly negative, independent of shock polarity (Figure 4). This is an unexpected finding that is in apparent contradiction to the concept that shocks should produce polarizations of both signs, reflecting inflow of current into the intracellular space at some locations and outflow at other locations.

There are 2 main mechanisms by which the electrical field can change \( V_m \) far from shock electrodes or the tissue-bath interface. One mechanism relates \( \Delta V_m \) to a nonuniform electrical field (“activating function”). 3,16 The other explains \( \Delta V_m \) by nonuniform tissue structure, eg, resistive discontinuities 1,2 or fiber rotation. 3 Separating these 2 mechanisms in a tissue with restricted extracellular space can be difficult, because \( \Delta V_m \) caused by tissue nonuniformities will inevitably lead to nonuniformities in the extracellular field. Therefore, the existence of field nonuniformities does not necessarily mean that they are the cause of \( \Delta V_m \). This would be the case if the field nonuniformity was not related to the tissue structure, eg, if it were due to electrode geometry and/or BCs. This was not the case in the present study, because the bath electrical field was uniform without preparations. Therefore, it is more likely that intramural \( \Delta V_m \) were due to nonuniform tissue structure.

It is also likely that 2 different types of \( \Delta V_m \) were due to different structural factors. The isolated areas of positive or negative \( \Delta V_m \) induced by shocks of moderate strength were probably caused by relatively large-scale nonuniformities such as fiber rotation, 3 variation in the surface-to-volume ratio, 17 or blood vessels. Owing to light integration from some depth, both surface and subsurface tissue nonuniformities could contribute to \( \Delta V_m \). With impermeable BCs, uneven tissue surface could lead to current flow across the transmural surface at some locations, which could also cause \( \Delta V_m \). This factor, however, should not play the primary role, because qualitatively similar results were obtained with permeable BCs. As expected from \( \Delta V_m \) produced by large nonuniformities, they changed their sign with changing shock polarity.

The nature of the second type of intramural \( \Delta V_m \) is more difficult to explain. We suggest that uniformly negative \( \Delta V_m \) are due to microscopic discontinuities in the tissue structure. Myocardium contains discontinuities of multiple types, including blood vessels, collagen septa (Figure 8B), and intercellular clefts. 18 Intramural cells are organized into relatively sparsely interconnected bundles 19 and layers. 20 The basic features of \( \Delta V_m \) in such structures can be inferred from the shock response of a cell strand or an intercellular cleft. Optical measurements indicate that shocks invariably induce
both positive and negative $\Delta V_m$ at the opposite sides of such structures. However, if these polarizations are measured on a macroscopic scale (1.2 mm in the present study) that exceeds structure dimensions and the electrotonic space constant ($\equiv 0.3$ to 0.5 mm$^2$), then the negative and positive polarizations should be averaged out. Signal averaging is likely to be augmented by light contributions from deeper cell layers. In a system with a linear $V_m$ response, the net result would be a zero or negligible macroscopic polarization. This can explain the absence of detectable virtual electrodes during weak shocks when $\Delta V_m$ were nearly symmetrical. Stronger shocks, however, induce nonlinear $\Delta V_m$ with a strong negative bias ($\Delta V_m^{-}$) during AP plateau. Because of this asymmetry, macroscopic measurements of $\Delta V_m$ produced by small discontinuities should yield only negative $\Delta V_m$. This can explain the present observations of globally negative $\Delta V_m$ and that the maximal magnitude of hyperpolarization was much smaller than that measured at microscopic resolution in cell cultures.

**Effects of Shocks on APD**

Shock-induced APD prolongation or shortening have been reported previously, and both are considered important factors in defibrillation. In the present work, APD shortening was observed only rarely at sites of $\Delta V_m$ during the weakest shocks. More typically, APD was prolonged at sites of both $\Delta V_m^+$ and $\Delta V_m^-$, and this effect became more prominent with increasing shock strength and $\Delta V_m$ becoming more negative. This finding appears counterintuitive, because negative polarization reflects a withdrawal of positive charge from the intracellular space, and therefore, it should be followed by faster repolarization. The explanation for this paradox might be related to the same factor that we think is responsible for the globally negative $\Delta V_m$, ie, microscopically discontinuous tissue structure. Indeed, if shock-induced $\Delta V_m$ are due to microscopic structures such as cell bundles and layers, then the areas of $\Delta V_m^+$ and $\Delta V_m^-$ at the edges of these structures are in close proximity to each other. Strong negative polarization is expected to reactivate ionic channels in cardiac membrane and reset these myocytes to their resting states. After a shock, these cells will become excited by depolarization spreading from the areas of $\Delta V_m^-$ and generate new action potentials. During macroscopic measurements, the combined duration of new and preceding action potentials will be interpreted as APD prolongation. Thus, the observed characteristics of both shock-induced $\Delta V_m$ patterns and APD changes indicate that microscopic discontinuities in the tissue...
channel blockers, however, the effect of BDM is expected to be only quantitative, without radical changes in $\Delta V_m$ patterns. Finally, the present study was limited to $\Delta V_m$ measurements during the AP plateau. Because $V_m$ responses are different during repolarization and diastole, the magnitude and pattern of intramural $\Delta V_m$ induced by shocks during these AP phases will be different, but this effect will not change the existence of intramural virtual electrodes.

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References

20. Le Grice IJ, Smaill BH, Chai LZ, et al. Laminar structure of the heart: ventricular myocyte arrangement and connective tissue architecture in the dog. Am J Physiol. 1995;35:H571–H582.
21. Fast VG, Rohr S, Gillis AM, et al. Activation of cardiac tissue by extracellular electrical shocks: formation of “secondary sources” at inter-
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