High-resolution optical mapping of intramural virtual electrodes in porcine left ventricular wall

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Abstract

Objective: It is believed that shock-induced intramural virtual electrodes (IVE) play a critical role in defibrillation. IVE were recently demonstrated in the porcine left ventricle (LV), but their origin remains unknown. Macroscopic optical mapping showed that strong shocks induce IVE of only one polarity, which contradicts theoretical predictions. It is hypothesized that IVE have a microscopic origin and that microscopic positive and negative IVE are spatially averaged during macroscopic optical mapping. This hypothesis was examined by mapping \( V_m \) responses at the transmural LV surface with increased optical resolution.

Methods: Rectangular shocks (strength=2–48 V/cm; duration=10 ms) were applied across isolated coronary-perfused porcine LV preparations \((n=7)\) during the action potential plateau and diastole. Shock-induced \( V_m \) responses were measured at low resolution (LR; 1.2 mm/diode) and high resolution (HR; 0.11 mm/diode).

Results: During plateau shocks with strength \( \geq 20 \) V/cm, LR recordings demonstrated only negative \( \Delta V_m \) extending to the cathodal preparation edge. In contrast, HR recordings from this area as well as from intramural locations revealed both positive and negative \( \Delta V_m \) at all shock strengths. During diastolic shocks, only positive polarizations were observed at LR, but both positive and negative polarizations were detected at HR. In areas of negative polarization, large activation delays were found at HR, whereas LR recordings at these locations demonstrated fast activation.

Conclusions: High- and low-resolution optical mapping produced radically different patterns of shock-induced polarization and activation. The occurrence of positive and negative polarizations during plateau and diastolic shocks at high but not low resolution provides evidence for microscopic nature of IVE in LV wall.

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1. Introduction

It is generally agreed that defibrillation requires induction of intramural virtual electrodes (IVE) by electrical shocks in the bulk of myocardium. Shock-induced IVE were recently demonstrated in isolated left ventricular (LV) wall \([1,2]\), but their nature remains unknown and some of their properties contradict the established concepts of shock–tissue interaction. Because shocks are applied in the extracellular space, they should produce both positive and negative \( V_m \) changes (\( \Delta V_m \)) reflecting inflow of current into the intracellular space at some locations and outflow at other locations. All existing mathematical models of defibrillation behave in this fashion \([3–8]\). In contrast, strong shocks applied during the action potential (AP) plateau in LV preparations...
induced only negative $\Delta V_m$ that extended to the cathodal edge of the preparation [1], and shocks applied during the diastole induced only positive $\Delta V_m$ [2]. It is hypothesized that these discrepancies between theory and experiments are explained by the microscopic nature of IVE. According to this concept, shocks produce microscopic polarizations of both signs but, because of spatial averaging and nonlinear membrane response [9,10], $\Delta V_m$ of only one sign is measured by macroscopic optical mapping. If this is true, then optical measurements with higher spatial resolution should reveal positive $\Delta V_m$ induced by strong shocks during AP plateau and negative $\Delta V_m$ during diastole. To test this prediction, we compared shock-induced $V_m$ responses measured at different spatial resolutions on the transmural surface of isolated porcine LV preparations.

2. Materials and methods

2.1. Isolated preparations of left ventricle

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85–23, revised 1996). LV preparations were isolated from pigs weighing 20–25 kg of either sex as described previously [1,2]. The preparations had a length of ~4.5 cm and a transmural thickness of ~1.6 cm. They were placed into a tissue bath (Fig. 1A), which contained a glass window at the bottom for optical mapping, and arterially perfused (pressure=40–50 mm Hg) 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), which was gassed with a mixture of 95% O$_2$ and 5% CO$_2$ (36°C). To avoid motion artifacts, the solution was supplemented with 15 mmol/l of electroneutral uncoupling agent 2,3-butanedione monoxime (BDM). Optical measurements started 1 h after tissue isolation.

Preparations were paced at a cycle length of 500 ms via a bipolar electrode placed on the epicardial surface (Fig. 1A). Rectangular shocks with a duration of 10 ms and a strength of ~3, 10, 20, or 40 V/cm were applied during diastole and the AP plateau in the epicardial-to-endocardial direction using two mesh electrodes with dimensions of 6×1 cm$^2$ (Fig. 1A,B). Shock strength was measured by a small bipolar electrode glued to the glass window near the mapping area (Fig. 1A,B). When measuring effects of shocks during the AP plateau, APs were elicited by 1.5–2 V/cm shocks with a duration of 5 ms. The delay between the diastolic and plateau shocks was ~35 ms. A pause of 3–5 min was allowed for tissue recovery after ≥20-V/cm shocks. To check for measurements reproducibility, each series of measurements was completed by applying a shock of the initial strength. These comparisons showed that polarization and activation maps were highly reproducible and that they were not affected by strong shocks.

2.2. Optical mapping of $V_m$

Preparations were stained with the $V_m$-sensitive dye di-4-ANEPPS (Molecular Probes) by injection of 5 ml bolus of 10 μmol/l dye solution into a bubble trap. Two systems were used for optical mapping of $V_m$ at low and high resolutions. The general design of both low resolution (LR) and high resolution (HR) systems is schematically shown in Fig. 1A. The LR system was built as previously described [11]. Excitation light (520±30 nm) from a 250-W tungsten–halogen lamp (Oriel) was focused on the preparation by a photographic lens (Nikkor, 50 mm, f1.4). The emitted fluorescence was collected by the same lens and measured.
at >610 nm. Optical magnification was 0.85×, which corresponded to a spatial resolution of ~1.2 mm/diode. High-resolution (HR) mapping was performed using an optical system based on an inverted microscope (Axiovert 135TV, Zeiss) with 10× objective (Fluar, Zeiss), which corresponded to spatial resolution of 0.11 mm/diode. Excitation light (530±20 nm) was provided by a 200-W Hg/Xe lamp (Opti Quip). Emitted fluorescence was measured at >615 nm. In both optical systems, fluorescence changes were measured using a 16×16 photodiode array (Hamamatsu) and a data acquisition system described elsewhere [9,12] at a sampling rate of 4 kHz/channel.

Experiments were performed in seven LV preparations. In three preparations, both LR and HR measurements were carried out. In these experiments, LR measurements were performed first when the whole transmural surface was mapped (Fig. 1B). After that, a preparation and the tissue chamber were quickly transferred to the HR system. HR mapping was performed in two areas: the epicardial edge of the preparation and the preparation center, both located along the central line of the LR mapping window (Fig. 1B). In the remaining four preparations, either only LR or HR measurements were performed (n=2 each).

A shock-induced $\Delta V_m$ during the AP plateau was measured as the difference between a linear regression fit of the plateau during a 10-ms interval prior to shock application and the $V_m$ level 9 ms after the shock onset; the $\Delta V_m$ was normalized by the AP amplitude (Fig. 1C). Local activation times were determined at 50% of AP upstroke. For diastolic shocks, the interval between the moments of first and last activations was defined as the time of transmural activation.

Data were expressed as mean±S.D. Differences were compared using a two-tailed nonpaired t-test. Results were considered statistically significant if $p<0.05$.

3. Results

Experiments were performed in seven LV preparations. Wall thickness measured in the preparation center was 16.4±2.1 mm. The delay between AP upstrokes and plateau shocks was 35±3 ms. Optical mapping revealed that shock-induced $V_m$ responses and activation patterns change significantly with the changing of optical resolution.

3.1. Shock-induced $V_m$ responses at the epicardial edge

3.1.1. Effects of shocks on $V_m$ during the AP plateau

Fig. 2 compares the effects of weak shocks ($\approx 4$ V/cm) on $V_m$ measured at different optical magnifications near the epicardial edge of the same preparation. At low resolution, small negative $\Delta V_m$ [<20% action potential amplitude (APA)] were measured near the epicardium when it faced the positive shock electrode (“anodal” shock, panel A) and small positive $\Delta V_m$ were measured when it faced the negative shock electrode (“cathodal” shock, panel B). $\Delta V_m$ waveforms of both polarities exhibited a simple monophasic shape. At high resolution, subepicardial $\Delta V_m$ had a similar shape, but they became significantly larger in magnitude. The largest $\Delta V_m$ induced by anodal and cathodal shocks increased to −49 and 40% APA, respectively, at 10× magnification (panels C and D). It should be noted that the averaging of HR optical traces shown in panels C and D would produce signals with larger $\Delta V_m$ amplitudes than the LR signals from site 1.
shown in panels A and B. This indicates that additional factors besides optical magnification determined the magnitude of optical signals (see Discussion).

Fig. 3 demonstrates the effects of stronger shocks with $E = 21$ V/cm. As before, anodal polarizations were negative at all optical resolutions (panels A and C). Cathodal polarizations measured at different resolutions, however, revealed radical differences. Whereas LR-$\Delta V_m$ became negative (panel B), which is consistent with previous observations [1,2], HR-$\Delta V_m$ remained predominantly positive (panel D). Optical resolution also affected the shape of $\Delta V_m$. The LR-$\Delta V_m$ were monotonic (panels A and B), but HR-$\Delta V_m$ during both anodal and cathodal shocks were predominantly nonmonotonic where an initial negative or positive $V_m$ response was followed by a $V_m$ rise or decline, respectively (panels C and D, traces 1–5).

Effects of strong shocks with $E = 40$ V/cm on $V_m$ are shown in Fig. 4. During cathodal shocks, only negative $\Delta V_m$ were measured at low resolution (panel B), but both positive and negative $\Delta V_m$ were detected at high resolution (panel D). The majority of HR-$\Delta V_m$ had a nonmonotonic shape with initial positive $V_m$ deflection followed by a

![Figure 3](image3.png)

Fig. 3. Effects of plateau shocks with $E = 21$ V/cm on $V_m$ measured at different optical resolutions. (A) and (B) Isopotential maps of $\Delta V_m$ and optical recordings measured at 0.85× magnification. (C) and (D) Optical measurements at 10× magnification. Other designations are the same as in Fig. 2.

![Figure 4](image4.png)

Fig. 4. Effects of plateau shocks with $E = 40$ V/cm on $V_m$ measured at different optical resolution. (A) and (B) Isopotential maps of $\Delta V_m$ and optical recordings measured at 0.85× magnification. (C) and (D) Optical measurements at 10× magnification. Other designations are the same as in Fig. 2.
rapid $V_m$ decline (panel D, traces 1–4). During anodal shocks, negative $\Delta V_m$ were recorded at both LR and HR (panels A and C), similar to those measured at weaker shocks (Fig. 3).

Fig. 5 presents data on maximal (panel A) and minimal (panel B) $\Delta V_m$ induced by cathodal and anodal shocks, respectively, in five preparations. Two main differences between LR- and HR-$\Delta V_m$ can be seen. First, the maximal LR-$\Delta V_m$ became negative at shock strength $\geq 20$ V/cm, whereas maximal HR-$\Delta V_m$ remained positive for all shock strengths (panel A). Second, magnitudes of maximal and minimal HR-$\Delta V_m$ were significantly larger than that of LR-$\Delta V_m$ (panels A and B).

### 3.1.2. Effects of shocks on $V_m$ during diastole

Effects of shocks during the diastole were studied in three preparations. Fig. 6 compares optical traces and activation patterns measured at low and high resolutions during anodal shocks of different strengths. Only positive $V_m$ changes fused with AP upstrokes were measured at LR during anodal shocks with $E = 6$ V/cm (panel A, trace 1, 0.85×). The upstroke occurred immediately after the shock onset resulting in small activation times. The anodal upstroke had a biphasic shape with two rising phases of $V_m$ at the shock onset and shock end, and a negative deflection during the shock. Similar $V_m$ responses were measured at LR during stronger anodal shocks (panels B, C, and D, traces 1, 0.85×).

HR recordings obtained from the same epicardial locations revealed different $V_m$ responses. The main difference was that anodal shocks initially produced negative $\Delta V_m$ that were followed by AP upstrokes. The duration of the negative polarization and the upstroke delay were short during the weakest 6-V/cm shock (panel A, traces 1–4, 10×), but they became significantly larger as shock strength increased (panels B, C, and D, traces 1–4, 10×). During the strongest shocks (panels C and D, traces 1–4, 10×), $V_m$ remained negative and AP upstrokes occurred after the shock end resulting in activation delays of >10 ms.

Differences between $V_m$ responses measured at LR and HR during anodal shocks resulted in radically different activation patterns. At LR, epicardial activation appeared as relatively uniform and rapid with activation delays of <0.2 ms for all shock strengths (panels A, B, and C, 0.85×). Measured at HR however, activation of corresponding area was highly nonuniform with increasingly large activation delays (panels A, B, and C, 10×).

During cathodal shocks, shock-induced polarization and activation patterns measured at low and high resolutions were similar (not shown). Both LR and HR recordings exhibited only positive $\Delta V_m$ fused with AP upstrokes. The upstroke shape was somewhat different at shocks with $E \geq 10$ V/cm when LR upstrokes became biphasic and HR upstrokes remained monophasic. No significant activation delays were measured at both LR and HR.

### 3.2. Shock-induced $V_m$ responses in LV center

#### 3.2.1. Effects of shocks on $V_m$ during the AP plateau

LR and HR $V_m$ responses measured from the central LV region were qualitatively similar to those measured at the epicardial edge. As before, LR measurements of plateau $\Delta V_m$ demonstrated only negative polarizations during strong shocks of both polarities (Fig. 4, panels A and B). At HR, shock of one polarity produced both positive and negative $\Delta V_m$ (Fig. 7, panel A), and shock of another polarity produced only negative $\Delta V_m$ (panel C).

Panels B and D present the summary of maximal and minimal $\Delta V_m$ measured at different optical resolutions. Similar to epicardial polarizations, maximal LR-$\Delta V_m$ became negative at shocks with $E \geq 20$ V/cm, but maximal HR-$\Delta V_m$ remained positive for all shock strengths (panel B). Magnitudes of maximal and minimal HR-$\Delta V_m$ were typically larger than those of LR-$\Delta V_m$.

#### 3.2.2. Effects of shocks on $V_m$ during diastole

Effects of diastolic shocks on $V_m$ in the LV center measured at low resolution are presented in Fig. 6. Only positive $\Delta V_m$ fused with AP upstrokes were observed at all
shock strengths. Shocks with $E = \approx 6$ V/cm produced predominantly monophasic upstrokes (panel A, traces 2–3, 0.85×), and shocks with $E = \approx 21$ V/cm produced biphasic upstrokes (panels B and C, traces 2–4, 0.85×). All these shocks resulted in rapid tissue activation (panels A, B, and C, 0.85×). Very strong shocks with $E = \approx 42$ V/cm produced complex AP upstrokes with three rising $V_m$ phases (panel D, traces 2–4, 0.85×) and delayed transmural activation (Panel D, 0.85×), which is consistent with previous observations [2].

As with epicardial recordings, HR measurements revealed negative $\Delta V_m$ followed by AP upstrokes, however these negative $\Delta V_m$ were distinctly detectable only at strong shocks with $E = \approx 20$ V/cm. The magnitude and the duration of initial negative $\Delta V_m$ increased with increasing shock strength and became extremely large at shocks with $E = \approx 40$ V/cm (Fig. 7, panel E, traces 1–2). The magnitude of negative $\Delta V_m$ induced by diastolic shock was paralleled with magnitude of negative $\Delta V_m$ induced by plateau shocks (compare traces 1–4 in panels C and E). The shape of AP upstrokes also changed with increasing shock strength. AP upstrokes were predominantly monophasic at shocks with $E = \approx 5$ V/cm. Shocks with $E = \approx 5$ to 20 V/cm induced both monophasic and biphasic upstrokes. The monophasic upstrokes typically were associated with positive $\Delta V_m$ measured during AP plateau, whereas biphasic upstrokes were paralleled with negative plateau $\Delta V_m$, which is consistent with previous observations [2]. Shocks with $E = \geq 40$ V/cm produced biphasic or triphasic upstrokes (Fig. 7, panel E). During strong shocks with $E = \geq 25$ V/cm, activation of areas exhibiting negative $\Delta V_m$ was delayed until shock end (Fig. 7, panel E).

It should be noted that, contrary to shock responses, AP upstrokes and activation patterns during regular pacing did
not exhibit obvious radical differences between low- and high-resolution measurements that could be attributed to discontinuous tissue structure (data not shown).

4. Discussion

In this study, high-resolution optical mapping was used to examine the hypothesis that electrical shocks produce microscopic intramural virtual electrodes in the LV wall. The main findings are as follows: (1) the resolution of optical mapping strongly affects patterns of shock-induced polarization and activation; (2) during strong plateau shocks, only negative $\Delta V_m$ were observed at LR, but both positive and negative $\Delta V_m$ were registered at HR; (3) during diastolic shocks, HR measurements detected negative $\Delta V_m$ preceding AP upstrokes, whereas such $\Delta V_m$ were absent in LR recordings; (4) large activation delays were found at HR in the areas of negative $\Delta V_m$, whereas LR recordings at these locations demonstrated fast activation. These results support the hypothesis of the microscopic nature of intramural virtual electrodes.

4.1. Microscopic virtual electrodes

Defibrillation requires that electrical shocks cause $V_m$ changes or “virtual electrodes” in intramural tissue layers [13,14]. Shock-induced IVE were demonstrated in isolated preparations of the left ventricular wall [1,2], but their mechanism remains unknown. Studies in mathematical models indicate that virtual electrodes can occur via two main mechanisms, one dependent on a nonuniform electric field [4,15] and the other dependent on nonuniform tissue structure [3,6,7]. A common feature of both mechanisms is that shocks induce both positive and negative polarizations that change their sign when shock polarity is changed. In contrast, experiments in the LV myocardium demonstrated that strong shocks applied during the AP
plateau induced only negative $\Delta V_m$ [1], and shocks applied during diastole induced only positive $\Delta V_m$ [2]. Results of low-resolution optical measurements obtained in this work are consistent with these findings.

The most striking feature of these IVE is that they have a negative sign even at the cathodal edge of the wall where large positive polarizations are expected due to the presence of a tissue boundary. To explain this paradox, it was proposed that shocks produce microscopic IVE of both signs, but they are spatially averaged during macroscopic optical mapping [1]. Combined with a negatively biased membrane response [9,16], such spatial averaging can result in globally negative polarizations. The logical test of this hypothesis is mapping at a higher spatial resolution, which was accomplished in this work.

Results of these experiments support the microscopic hypothesis of IVE. Thus, high-resolution measurements during cathodal shocks revealed positive $\Delta V_m$ immediately under the epicardium and negative $\Delta V_m$ in a close proximity (Figs. 3 and 4). In addition, areas of positive and negative polarizations were observed in the middle of the LV wall where only negative $\Delta V_m$ were measured at LR during strong shocks (Fig. 7). When shocks were applied in diastole, HR measurements detected negative polarizations preceding AP upstrokes, whereas they were absent in LR recordings.

The reason for the radical differences between low- and high-resolution measurements of $\Delta V_m$ is likely to be the discontinuous microscopic structure of the myocardium. Histological studies showed that LV myocardium consists of microscopic muscle bundles and layers separated by collagen septa [17]. Such structures should cause positive and negative $\Delta V_m$ at their opposite sides. During optical mapping, these signals are spatially averaged. It is clear that the effect of spatial averaging is less pronounced in HR measurements compared to LR measurements.

Optical mapping of $V_m$ has recently become a very important tool in studies of shock effects and mechanisms of defibrillation in the heart [15,18–20]. The fact that optical resolution may so strongly affect $\Delta V_m$ recordings should be taken into account in the interpretation of such measurements.

Although HR measurements provided evidence for the existence of microscopic IVE, they did not delineate their exact distribution pattern. IVE caused by recurrent structural discontinuities are expected to create an alternating pattern of positive and negative $\Delta V_m$ [3,6]. The fact that such $\Delta V_m$ were not observed even at HR suggests that anatomical structures responsible for IVE have dimensions comparable or smaller than the scale of HR measurements. In addition, patterns of microscopic $\Delta V_m$ distribution might be smoothed by strong light scattering in cardiac tissue, as well as by light integration from the tissue depth [21,22]. These two factors may also possibly explain the fact that simple spatial summation of HR signals did not produce the LR signals measured from corresponding locations. A more definitive explanation of these effects requires creation of a comprehensive mathematical model incorporating discontinuous tissue structure, nonlinear membrane kinetics, and properties of light propagation in the cardiac muscle.

### 4.2. Shock-induced tissue activation

According to the “excitatory” hypothesis [13], defibrillation is caused by direct excitation of the majority of excitable myocardium by electrical shock. In this study, LR measurements demonstrated that shocks applied during the diastole cause direct and rapid activation of the ventricular bulk supporting this hypothesis, which is consistent with results of a previous study [2]. HR measurements, however, revealed that this was true only for relatively weak shocks. During stronger shocks, large local activation delays were detected at HR in those areas where LR recordings showed uniform and rapid activation. These data indicate that the process of shock-induced activation can be highly nonuniform at the microscopic level. It involves formation of IVE with closely adjacent regions of positive and negative $\Delta V_m$. Local activation is determined by the interaction between these polarizations, which is different for different shock strengths.

During weaker shocks, negative polarizations in the areas of virtual anodes are relatively small, and they can be overcome by depolarization advancing from virtual cathodes. This result in rapid local activation similar to that observed in cell cultures with small intercellular clefts for comparable shock strengths [23]. In such a case, activation times measured at high and low resolutions should be comparable, which was true for shocks with $E = 5$ V/cm in this study (Fig. 6A). The similarity of activation times at different spatial scales is the evidence of the distributed nature of shock-induced tissue excitation.

Stronger shocks, however, produce large negative polarizations in the areas of virtual anodes that might overcome the positive influence of depolarization fronts advancing from virtual cathodes, thus preventing these areas from activation. They become activated after the shock end resulting in locally delayed activation. Only small areas of delayed activation were detected at moderate shock strengths (Fig. 6C), but as shock strength was increased further, areas of delayed activation became larger. For the strongest shocks, areas of delayed activation became as large that they were noticeable at the LR as well (Fig. 6D), which is consistent with previous findings [2]. Delayed activation in the areas of virtual anodes was shown to play an important role in shock-induced arrhythmias and defibrillation failure [19,24,25]. In these studies, shocks applied during relative refractoriness produced nonuniform polarization patterns resulting in singularity points and reentry. This work indicates that delayed activation may also occur in the areas with restored excitability, which may contribute to arrhythmogenic effects of shock applied during fibrillation.

Contrary to shock-induced activation, activation maps during regular did not show radical activation differences between low- and high-resolution measurements. Also, the
shape of AP upstrokes was not qualitatively different. This suggests that regular impulse propagation might be less sensitive to the presence of discontinuities than shock-induced activation. This corresponds to the results of modeling and experimental studies showing that moderate discontinuities did not produce noticeable changes in the upstroke shape measured even on subcellular scale [26,27]. Such effects as well as local conduction slowing or acceleration should become even less prominent when spatial averaging is involved, as was the case in this study even at high resolution. On the other side, shock-induced activation is expected to be more sensitive to discontinuities, because (1) shocks produce $\Delta V_m$ of opposite signs at discontinuities, which have different effects on afferent $V_m$ evolution, and (2) $\Delta V_m$ are nonlinear, which prevents cancellation of positive and negative $\Delta V_m$ upon spatial averaging. A more complete explanation of these differences might be obtained by comparing the effects of discontinuities on impulse propagation and shock-induced activation in a computer model.

4.3. Limitations

Several limitations of this experimental model related to boundary conditions at the transmural surface and the use of electromechanical uncoupler, BDM, were discussed previously [1]. Another limitation of this work is related to the fact that optical signals are affected by light integration from the tissue depth and light scattering [21,22]. These factors limit the ability of determining the exact geometry of shock-induced IVE, as well as their correspondence with the microscopic tissue structure.

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