Intramural Virtual Electrodes in Ventricular Wall
Effects on Epicardial Polarizations

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Background—Intramural virtual electrodes (IVEs) are believed to play an important role in defibrillation, but their existence in intact myocardium remains unproven. Here, IVEs were detected by use of optical recordings of shock-induced transmembrane potential (V_m) changes (ΔV_m) measured from the intact epicardial heart surface.

Methods and Results—To detect IVEs, isolated porcine left ventricles were sequentially stained with a V_m-sensitive dye by 2 methods: (1) surface staining (SS) and (2) global staining (GS) via coronary perfusion. Shocks (2 to 50 V/cm) were applied across the ventricular wall in an epicardial-to-endocardial direction during the action potential plateau via transparent mesh electrodes, and shock-induced ΔV_m were measured optically from the same epicardial locations after SS and GS. Optical recordings revealed significant differences between ΔV_m of 2 types that became more prominent with increasing shock strength: (1) for weak shocks, SS-ΔV_m were larger and faster than GS-ΔV_m; (2) for intermediate shocks, cathodal GS-ΔV_m became multiphasic, whereas SS-ΔV_m remained monophasic; and (3) for strong shocks, cathodal GS-ΔV_m became uniformly negative, whereas SS-ΔV_m typically remained positive. The radical differences in the shape and polarity of SS and GS polarizations can be explained by the contribution of subepicardial IVEs to optical signals. Histological examination revealed a dense network of collagen septa in the subepicardium, which could form the IVE substrate.

Conclusions—Intramural virtual electrodes are reflected in optical measurements of shock-induced ΔV_m on the intact epicardial surface. These IVEs could be a result of microscopic resistive discontinuities formed by collagen septa. (Circulation. 2004;109:2340-2356.)

Key Words: arrhythmia • defibrillation • excitation • mapping • ventricles

The current theory of defibrillation is based on the assumption that shocks produce changes of transmembrane potential (ΔV_m) in the majority of myocardium, but whether ΔV_m occur in the intramural tissue layers is not well known. In a recent study, optical mapping was used to measure shock-induced ΔV_m (“virtual electrodes”) on the transmural surface in isolated wedge preparations of the left ventricular (LV) wall. It was found that shocks induced widespread ΔV_m across the transmural surface, which could possibly account for defibrillation. However, the extrapolation of these findings to the intact myocardium is limited by the differences between electrical properties of the cut transmural surface in wedge preparations and the intact LV wall. Therefore, the goal of the present study was to examine whether intramural virtual electrodes (IVEs) exist in the intact myocardium. It is hypothesized that, because of integration of optical signals from different tissue depths, intramural virtual electrodes can be detected in optical recordings of ΔV_m measured from the intact LV epicardium. This hypothesis was examined in isolated coronary-perfused LV preparations that were stained with a V_m-sensitive dye by 2 different methods: (1) by staining only the epicardial tissue layers and (2) by global tissue staining (GS) via coronary perfusion. Radically different shock-induced V_m responses were observed on the epicardium of the same preparations stained by the 2 techniques. These differences provide evidence for the existence of subepicardial intramural virtual electrodes in the intact LV wall.

Methods
Porcine LV preparations (length, ~4.5 cm; width, ~2 cm; thickness, ~1.8 cm) were obtained as described previously. Preparations were stained with the dye di-4-ANEPPS twice: first by surface staining (SS) and then GS. In the first method, preparations were immersed in 20-μmol/L dye solution for ~1 hour. Stained preparations were placed in a tissue bath with a glass window for optical mapping (Figure 1A) and perfused for the rest of experiment through a coronary artery with Tyrode’s solution containing 15 mmol/L of 2,3-butanedione monoxime. After completion of optical measurements (see below) in SS tissue, the preparations were stained globally by arterial injection of a 5-mL bolus of 20-μmol/L dye solution, and measurements were repeated.

Preparations were paced at a 500-ms interval with a bipolar electrode. To induce action potentials (APs) with a small delay, a 1.5- to 2-V/cm shock (duration=5 ms) was applied across the LV wall in the epicardium-to-endocardium direction via 2 transparent mesh electrodes. A series of test shocks (duration=10 ms) with
Results

Shock-induced $\Delta V_m$ were measured in 7 LV preparations. In each preparation, there were significant differences in $\Delta V_m$ waveforms measured after SS and GS, and these differences became more prominent with increasing shock strength. Activation patterns were not substantially different (not shown).

$\Delta V_m$ Produced by Weak Shocks

Figure 2 shows $\Delta V_m$ produced by 3-V/cm shocks in SS and then GS tissue. In measurements of both types, cathodal shocks produced only positive $\Delta V_m$ (B), and anodal shocks produced only negative $\Delta V_m$ (C). Whereas the SS and GS polarizations were qualitatively similar, there were significant differences in their magnitudes and time courses, with SS-$\Delta V_m$ being substantially larger and faster than corresponding GS-$\Delta V_m$ (D and E).

Similar $\Delta V_m$ were observed in all preparations (n=7). All responses to weak shocks had the same simple monophasic shape, but SS-$\Delta V_m$ were significantly larger and faster than GS-$\Delta V_m$. For cathodal shocks with $E=4.7\pm1.6$ V/cm, SS-$\Delta V_m$ and GS-$\Delta V_m$ averaged over the mapping area were 54$\pm20\%$ and 16.4$\pm10\%$ APA (P<0.001), respectively; a large difference was also observed during anodal shocks. The time constant of exponential fit ($R^2=0.96\pm0.05$) during weakest cathodal shocks ($E=2.9\pm0.3$ V/cm) was $\approx23\%$ larger for GS-$\Delta V_m$ than for SS-$\Delta V_m$ (2.7$\pm0.4$ versus 2.2$\pm0.7$ ms, n=5, P<0.05). An even larger difference ($\approx52\%$) between SS and GS time constants was measured during anodal shocks.

$\Delta V_m$ Induced by Intermediate-Strength Shocks

Increasing shock strength caused significant changes in the $\Delta V_m$ shape. Figure 3 demonstrates the effects of $\approx14$-V/cm shocks. During cathodal shocks, SS-$\Delta V_m$ remained positive and, in most cases, monophasic (C). In contrast, the majority of GS-$\Delta V_m$ became biphasic, whereby an initial $V_m$ rise was followed by a decline (traces 1, 2, 4, and 5). Moreover, the polarity of GS-$\Delta V_m$ could change from positive to negative during the shock (trace 5). $\Delta V_m$ maps (A) showed that SS-$\Delta V_m$ were always larger than GS-$\Delta V_m$.

During anodal shocks (B and D), both SS- and GS-$\Delta V_m$ remained negative. The SS-$\Delta V_m$ became biphasic, whereby initial $V_m$ decline was followed by $V_m$ increase (D). The GS-$\Delta V_m$ either remained monophasic (not shown) or became slightly biphasic. $\Delta V_m$ maps (B) show that the SS-$\Delta V_m$ were larger than GS-$\Delta V_m$ at the shock beginning ($t=1$ ms), but these differences attenuated toward the shock end.

Similar findings were obtained for shocks with $E=16\pm5$ V/cm in 7 preparations. In SS tissue, cathodal $\Delta V_m$ were always positive, and anodal $\Delta V_m$ were always negative. In GS tissue, anodal $\Delta V_m$ were always negative, whereas cathodal $\Delta V_m$ could change their polarity from positive to negative during the shocks. In most cases, SS-$\Delta V_m$ were larger than GS-$\Delta V_m$. Thus, average cathodal SS- and GS-$\Delta V_m$ were 21$\pm16\%$ and 4$\pm4\%$ APA (P<0.001) at shock beginning and 44$\pm25\%$ and $-9\pm14\%$ APA (P<0.001) at shock end. For anodal shocks, large differences between average SS- and
GS-$\Delta V_m$ were observed at the shock beginning ($P<0.001$), but they became closer at the end ($P=\text{NS}$).

The threshold for occurrence of biphasic negative $\Delta V_m$ was lower in SS than in GS measurements. The threshold was determined as the field strength when $\Delta V_m$ at the shock end became more positive than $\Delta V_m$ in the middle (t=5 ms). During anodal shocks, this threshold was 9±3 and 16±5 V/cm ($n=7$, $P<0.01$) for SS- and GS-$\Delta V_m$, respectively.

$\Delta V_m$ Induced by Strong Shocks
Further increase of shock strength resulted in more radical differences between SS and GS polarizations. Figure 4 illustrates the effects of $E=39$ V/cm shocks. The GS-$\Delta V_m$ became negative and biphasic during both cathodal and anodal shocks (C and D). In contrast, the shape of SS-$\Delta V_m$ was different for different shock polarities and different locations. During the anodal shock (D), SS-$\Delta V_m$ were strongly biphasic, with a rapid rise after the initial decline. The SS-$\Delta V_m$ map (B) was uniformly negative at the shock onset but, because of rapid $V_m$ rise, became predominantly positive at the shock end.

During the cathodal shock (C), SS-$\Delta V_m$ were either positive monophasic (traces 3 to 5) or biphasic with changing polarity (traces 1 and 2). The SS-$\Delta V_m$ maps (A) showed both positive and negative polarizations at the shock beginning but uniformly positive polarization at the shock end.

Qualitatively similar results were obtained in all 7 preparations in response to shocks with $E=38\pm6$ V/cm. Shocks of both polarities produced mostly negative bipolar GS-$\Delta V_m$, whereas SS-$\Delta V_m$ were of several waveform types, described above.

Figure 5 presents data on maximal and minimal $\Delta V_m$ measured over the entire mapping area in all preparations after SS and GS. Maximal and minimal $\Delta V_m$ were achieved during cathodal and anodal shocks, respectively. In general, SS-$\Delta V_m$ were larger than GS-$\Delta V_m$. At the shock beginning,
the largest maximal and minimal SS-$\Delta V_m$ were $\approx 50\%$ and $-100\%$ APA (A), respectively, whereas corresponding GS-$\Delta V_m$ did not exceed $25\%$ and $-70\%$ APA (B). Large differences were also measured at the shock end, when largest maximal and minimal SS-$\Delta V_m$ were $\approx 100\%$ and $-150\%$ APA (C), respectively, whereas corresponding GS-$\Delta V_m$ were $\approx 50\%$ and $-75\%$ APA (D). The maximal SS-$\Delta V_m$ remained positive for all shocks (A and C). In contrast, maximal GS-$\Delta V_m$ became negative for shocks stronger than $\approx 30$ V/cm (B and D).

**Depth of Dye Staining**

Figure 6 illustrates distributions of di-4-ANEPPS in subepicardial regions of SS and GS preparations. In SS preparations (A, left), dye fluorescence was confined to a narrow subepicardial layer. The average profile of fluorescence intensity (right) reveals a nearly exponential decay, with intensity decreasing by $80\%$ within $0.23$ mm from the epicardium. From all SS preparations ($n=5$), the average distance of $80\%$ fluorescence decay was $0.25\pm0.06$ mm. In the GS preparations ($n=4$), dye distributions were nearly uniform (B).

**Subepicardial Collagen Distribution**

Figure 7 presents fluorescence images of collagen distribution in subepicardial regions of LV at different magnifications. A dense layer of collagen with a thickness of 50 to 100 $\mu$m can be seen at the epicardium (C). From this layer, collagen protrudes into the subepicardial muscle layers.
Below this region, there are multiple collagen septa, some of them reaching a length of \( \approx 1 \) mm, oriented primarily parallel to the epicardium (A and B). At some locations (A, upper right), however, a change in collagen septum orientation could occur. In addition to collagen septa, there are discontinuities related to blood vessels of varying diameters. Qualitatively similar collagen distribution was observed in 2 LV preparations.

**Discussion**

This work examines the existence of intramural virtual electrodes by comparing optical \( \Delta V_m \) signals measured from intact LV wall after SS and GS with a \( V_m \)-sensitive dye. It was found that \( \Delta V_m \) of 2 types measured from the same locations exhibited the following differences: (1) for weak shock, SS-\( \Delta V_m \) were larger and faster than GS-\( \Delta V_m \); (2) for intermediate shocks, cathodal GS-\( \Delta V_m \) became multiphasic, whereas SS-\( \Delta V_m \) remained monophasic; and (3) for strong shocks, cathodal GS-\( \Delta V_m \) became uniformly negative, whereas SS-\( \Delta V_m \) typically remained positive. The radical differences between \( \Delta V_m \) of 2 kinds reflect the existence of intramural virtual electrodes.

**Source of Optical Signals**

It is known that optical signals measured from cardiac tissue represent a weighted average of contributions from tissue layers at different depths. The depth of signal collection is determined by a combination of multiple factors, including

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*Figure 4. Optical \( \Delta V_m \) produced by strong shocks. A, Maps of \( \Delta V_m \) induced by cathodal shocks with \( E=39 \) V/cm. B, Maps of \( \Delta V_m \) induced by anodal shocks. C and D, Selected optical recordings of \( \Delta V_m \) after SS (blue traces) and GS (red traces).*
light absorption and scattering, wavelengths of excitation and emission light, spatial resolution, etc. Direct experimental determination of the collection depth is difficult, and most reliable estimates were obtained with mathematical models. Simulations in a Monte Carlo model of light propagation estimated that 80% of fluorescent photons measured over an area of 1 mm in diameter originate from a depth of \( \approx 1.2 \) mm; a similar estimate was obtained with a diffusion approximation of light propagation.

Measurements of transmural dye distribution performed here indicate that optical signals measured from SS tissue originate from a much thinner tissue layer. In such tissue, an 80% decrease in dye fluorescence was observed at a depth of \( \approx 0.25 \) mm. Assuming no light absorption or scattering, this value would represent the collection depth of optical signals in SS preparations. Because of significant light absorption and scattering by the tissue, however, the collection depth is expected to be smaller than that and, therefore, much smaller than the depth of signal collection in GS preparations.

**Intramural Virtual Electrodes**

Electrical shocks are believed to produce intramural virtual electrodes that play an important role in defibrillation, but their existence is still debatable. A recent study found that shocks produced widespread \( \Delta V_m \) on the transmural surface of isolated LV wall, which could potentially account for defibrillation. Extrapolation of this finding to the intact LV wall, however, is limited by the differences in boundary conditions existing at the cut tissue surface from those inside the intact LV. Therefore, the present study examined the existence of intramural \( \Delta V_m \) in the intact myocardium.

To evaluate \( \Delta V_m \) generated in the deep tissue layers, optical signals were measured twice from the same locations, first after dye staining on the tissue surface and then after GS via coronary perfusion. Because of different collection depths, the difference between signals of 2 types reflects the contribution of deep cell layers to shock-induced \( \Delta V_m \). Analysis of these signals revealed both quantitative and
qualitative differences between SS- and GS-/H9004 Vm observed at different shock strengths. During weak shocks, there were differences in magnitudes and time constants of SS- and GS-/H9004 Vm (Figure 2). Such differences can be explained by the classic cable theory, according to which the /H9004 Vm magnitude rapidly decays and the time constant increases with the distance from the tissue-bath interface.5 Therefore, depth signal averaging should produce smaller and slower /H9004 Vm in GS tissue than SS tissue, which corresponds to the present observations.

During stronger shocks, profound differences in the shape of SS and GS responses were found. One such difference was observed during shocks of moderate strength when cathodal GS-/H9004 Vm became biphasic, whereas SS-/H9004 Vm at the same locations remained monophasic (Figure 3C). Moreover, at some locations, GS-/H9004 Vm even changed their polarity from positive to negative toward the shock end. Such changes in the GS responses and their differences from SS responses cannot be explained by the continuous cable theory. The only plausible explanation is that they are a result of intramural virtual anodes formed under the epicardial surface.

The most radical differences between GS- and SS-/H9004 Vm were observed during the strongest shocks when cathodal SS-/H9004 Vm remained mostly positive but GS-/H9004 Vm became uniformly negative (Figures 4C and 5C). Such negative cathodal GS-/H9004 Vm indicate 2 things: (1) that there are intramural virtual anodes and (2) that these virtual anodes became much stronger than the primary positive polarizations produced at the epicardium-bath interface. The latter is not possible in a system with linear membrane response, in which primary polarizations are always larger than secondary polarizations.6 The reason for the dominance of virtual anodes is the negative asymmetry in the membrane response during the AP plateau, when negative polarizations are much larger than positive polarizations.7,8 It should be added that even cathodal SS-/H9004 Vm exhibited negative polarizations at some locations, indicating that there were intramural virtual anodes immediately under the epicardial surface (within <250 μm).

Epicardial GS-/H9004 Vm showed important similarities with /H9004 Vm measured previously from the transmural cut surface.1 Thus, in both cases, cathodal polarizations became uniformly negative at E≈30 V/cm; maximal positive and negative /H9004 Vm were comparable as well. These findings are in contrast to epicardial measurements in rabbit hearts, in which only positive /H9004 Vm were measured on the cathodal side of the hearts.9 The difference between the results of this and the present study could be because of differences in animal species and differences in shock strengths.

**Mechanism of Intramural Virtual Electrodes**

In general, intramural virtual electrodes can arise via 2 different mechanisms, one dependent on spatial gradients of the shock field10,11 and the other dependent on spatial gradients in resistive tissue properties.6,12 It is highly unlikely that there were significant gradients of electrical field over a relatively small epicardial mapping area faced with a much larger plate electrode oriented parallel to the epicardium. Therefore, the most likely reason for intramural /H9004 Vm was nonuniform resistive tissue properties.

Spatial gradients in tissue resistivity can result from different anatomic structures of cardiac muscle, including fiber rotation, microscopic tissue discontinuities such as collagen septa, and blood vessels. Most likely, IVEs measured here were not a result of fiber rotation, because it occurs predominantly in the plane parallel to the epicardial surface.12 Therefore, these IVEs were most likely related to microscopic tissue discontinuities. Histological examination of subepicar-
dial tissue layers revealed a dense network of collagen septa and blood vessels (Figure 7). These collagen septa could have lengths of up to ≈1 mm, and they were oriented primarily parallel to the epicardial surface, which is similar to the distribution of collagen septa in the LV of human hearts. Such collagen septa create an efficient substrate for IVE formation, with positive and negative ΔV_m produced at opposite septa sides. Because of the negative asymmetry of V_m response, spatial averaging of optical signals from microscopic IVE results in negative ΔV_m observed during cathodal shocks.

ΔV_m Waveforms
Shock-induced ΔV_m had complex shapes that varied with shock strength. On the qualitative level, all waveforms can be explained by spatial and temporal summation of V_m responses of 3 basic types. The ΔV_m of the first type are passive ΔV_m that have a monophasic exponential shape, similar to ΔV_m observed during the weakest shocks (Figure 2). The ΔV_m of the second type are asymmetrical, with negative ΔV_m exceeding positive ΔV_m. Responses of this type have been described in various cardiac preparations. Summation of linear surface ΔV_m with asymmetrical intramural ΔV_m can explain the biphasic shape of positive GS-ΔV_m observed during cathodal shocks of moderate strength (Figure 3C). The negative ΔV_m asymmetry is also an important factor in the explanation of why GS-ΔV_m were uniformly negative during both anodal and cathodal strongest shocks (Figure 4). In addition to linear and asymmetrical ΔV_m, a prominent role was played by V_m responses of the third type, characterized by a positive shift of V_m after an initial negative polarization. The ΔV_m of this type were previously described in cell cultures. Recently, it was found that the occurrence of such ΔV_m was paralleled by cell uptake of the membrane-impermeable dye propidium iodide in the areas of negative but not positive ΔV_m, indicating that such biphasic ΔV_m are the result of electroportation at sites of negative polarization. Membrane electroportation was implicated in the detrimental effects of shocks, such as loss of excitability and mechanical function, generation of postshock arrhythmias, and failure of defibrillation at very strong shocks. In the present work, signs of electroportation were observed in both SS and GS measurements, but the threshold shock strength for their occurrence was significantly lower in SS than GS recordings, indicating that SS measurements provide a more sensitive indicator of detrimental shock effects.

Implications and Limitations
The demonstration of intramural virtual electrodes in the intact LV wall validates the current theory of defibrillation, which postulates that the electrical field causes V_m changes in a critical mass of myocardium. Whereas the existence of IVEs was proven here for a relatively thin subepicardial tissue layer, the fact that the same intramural tissue structure is found in much deeper tissue layers indicates that similar IVEs should be produced by shocks there as well.

Acknowledgment
This study was supported by National Institutes of Health grant HL-67748.

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Circulation. 2004;109:2349-2356; originally published online April 26, 2004;
doi: 10.1161/01.CIR.0000127962.74368.D9

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
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