Mechanism of Ventricular Defibrillation
The Role of Tissue Geometry in the Changes in Transmembrane Potential in Patterned Myocyte Cultures

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Background—The geometry of the myocardium may influence changes in transmembrane potential (ΔVm) during defibrillation. To test this hypothesis, specific nonlinear structures (bifurcations, expansions, and curved strands or “bends”) were created in patterned cultures of neonatal rat myocytes.

Methods and Results—Extracellular field stimuli (EFS; 7 to 11 V/cm field strength) were applied parallel to the strands. Changes in Vm were measured with microscopic resolution using optical mapping techniques. In bifurcations, EFS produced 2 ΔVm maxima (so-called secondary sources) at the shoulder of each limb that were separated by a decrease of either hyperpolarization or depolarization at the insertion of the stem strand. In expansions, EFS produced a significant decrease in ΔVm at the insertion site of the expansion compared with the ΔVm maxima measured at the lateral borders. In 50% of experiments, tertiary sources of opposite polarity appeared in the strand due to local electrotonic currents. New action potentials were propagated from the sites of ΔVm maxima located at the lateral borders of the expansions. In bends, the strand oriented in parallel to the field dominated electrotonically and partially cancelled the sources produced by the perpendicular segment.

Conclusions—In electrically well-connected nonlinear structures, EFS produced changes in Vm at resistive boundaries that were determined by the electrotonic interaction between sources of different, direction-dependent strength. In addition, the interaction between localized secondary sources at nonlinear boundaries generated local current circuits, which gave rise to further changes in Vm (tertiary sources). (Circulation. 2000;101:2438-2445.)

Key Words: defibrillation ▪ potentials ▪ myocytes ▪ mapping

Extracellular electrical shocks are widely used for the treatment of ventricular tachyarrhythmias. The extracellular current flow induced by defibrillation shocks is believed to produce changes in the membrane potential (ΔVm); these changes then interrupt reentrant circuits by prolonging ventricular refractoriness and/or by producing new excitation changes then interrupt reentrant circuits by prolonging ventricular refractoriness and/or by producing new excitation changes then interrupt reentrant circuits by prolonging ventricular refractoriness and/or by producing new excitation changes then interrupt reentrant circuits by prolonging ventricular refractoriness and/or by producing new excitation.

The spacing between sites showing membrane responses of opposite polarity can be small (in the range of 30 to 100 μm). Several mechanisms likely explain the formation of circumscribed ΔVm. In simple representations of cardiac tissue (“continuous linear cables”), ΔVm is confined to the region adjacent to the shock electrodes. This region, which extends over 3 length constants, has been termed the “near field” or “polar region,” and the resulting ΔVm, the “primary source.” Secondary sources, ie, ΔVm far (>2 to 3 mm) from the shock electrodes, may occur at sites where the current flowing during a shock is forced to locally redistribute between the intra- and extracellular compartments. This redistribution may be due to localized changes in electrical cell-to-cell coupling, changes in the geometrical arrangement of cardiac cell strands or fibers, local inhomogeneities in the resistance of the extracellular space, or anisotropy-dependent changes in the ratio of extra-to intracellular resistance (“the bidomain effect”).

We recently introduced an experimental model of patterned neonatal rat myocyte cultures to test the effect of electrical shocks on cardiac cellular networks. Although this model cannot mimic all the properties of cardiac tissue in situ, dense cell strands can be grown in predefined patterns similar to those that occur in vivo, and ΔVm can be optically recorded in cardiac myocytes with high spatial resolution. We have shown that secondary sources are created at the borders of linear cell strands. Moreover, the minimal distance separating electrically well-connected cells must be >100 to 200 μm for excitatory secondary sources to occur. In the present study, we created nonlinear structures, using the patterned growth technique, to test the hypothesis that the changes in Vm and the formation of new excitation waves during extracellular field stimuli (EFS) are related to tissue geometry and to the orientation of cell strands within the electrical field.
Methods

Preparation of Patterned Cell Cultures

Cell monolayers with specific growth patterns were produced on glass coverslips as previously described.8,11 Examples of growth patterns used in these experiments, which represent tissue geometry encountered in vivo, are shown in Figure 1B. The first pattern, “bifurcations,” consisted of a stem strand (width, 100 μm; length, 1 mm) dividing into 2 parallel strands of the same width and length. The second pattern, “expansions,” consisted of small strands (width, 40 to 300 μm; length, 2 to 10 mm) emerging into a large area abruptly. The third pattern, “bends,” consisted of a strand (width, 50 to 100 μm) bent at an angle of 90°. Each leg in the bend was 1 mm in length. Measurements were performed after 3 to 8 days in culture. During experiments, cells were superfused with Hanks balanced salt solution (GIBCO) at a temperature of 34°C.8,10

Optical Recordings of ΔVm

Transmembrane potential changes were measured from the change in fluorescence of the voltage-sensitive dye RH-237 (Molecular Probes, 1.5 to 2 mol/L). The optical mapping system has been described previously in detail.12 Photocurrents from 96 photodiodes were converted to voltages, amplified, multiplexed, and digitized with 12-bit resolution and a sampling rate of 25 kHz per channel.

Application of EFS

Cells were stimulated at a cycle length of 500 ms via a bipolar electrode that was positioned >1 mm from each recording site. This minimal distance was selected to avoid interference with steady-state propagation (ie, the virtual electrode effect). EFS (field strength, 6 to 12 V/cm; duration, 8 ms; truncated exponential pulses) were delivered using a custom-built device and were applied via a pair of platinum-plate electrodes positioned at opposite ends of the bath (Figure 1A). The defibrillator was triggered by the stimulus, and produced EFS at preselected times during the cardiac cycle. In experiments involving EFS application during phase 2 of the action potential, the delay between the stimulus and the EFS was 20 ms. The field strength was homogeneous and linear throughout the bath.8 In 3 experiments, the field gradient was linearly correlated (r=1.0; P<0.001) to the output voltage of the defibrillator over a range of 25 to 100 V. A total of 2 to 4 shocks of opposite polarity were applied at each measuring site, and 1 to 4 measuring sites were selected per culture dish. To avoid phototoxicity, the light-exposed areas between measuring sites did not overlap.

Data Analysis

The action potential amplitude (APA) was defined as the difference in fluorescence intensity measured before the onset of the action potential and immediately after the action potential reached the plateau. The change in fluorescence induced by the EFS was determined as the difference between light intensities measured 1 ms before and 4 ms after the onset of the EFS. Shock-induced ΔVm was expressed as a change in fluorescence intensity relative to the APA (ΔVm/APA) in percent (Figure 1C).8 At an average APA of 100 mV,1.0 APA translates directly into mV. Local activation times were determined at 50% of the APA using linear interpolation between the nearest sampling points. Activation maps and isopotential maps illustrating the EFS-induced changes in ΔVm/APA were constructed using linear interpolation between the diodes.8

Statistical Analysis

Data are expressed as mean±SD. Differences were compared using the 2-tailed paired or unpaired t tests, where appropriate. Differences were considered significant if P<0.05.

Results

Effects of EFS at Cell Strand Bifurcations

Bifurcating cardiac trabecula or strands are found in many cardiac regions, including trabeculated parts of the atria, ventricular subendocardium, the Purkinje network, and surviving myocardium of infarct scars. In 6 experiments, EFS were applied in parallel to the strands at both polarities (ie, the stem of the bifurcation either faced the anode or the cathode). The secondary sources generated by the EFS (7 V/cm) are shown in Figure 2. Activation maps were recorded in each experiment. The activation map depicted in Figure 1B illustrates a relatively smooth pattern of isochrones across the bifurcation, with local crowding of isochrones at the site of the bifurcation due to current-to-load mismatch.12 Inhomogeneous gap junction expression would be expected to lead to more localized crowding and irregularly shaped isochrones.8,10

Figures 2C and 2D depict the ΔVm created by EFS of either polarity. If the stem of the bifurcation was directed toward the anode, the bifurcation was hyperpolarized; if the stem of the bifurcation faced the cathode, the bifurcation was depolarized. Two maxima were located at the shoulder of each limb, and they were separated by a dip in the hyperpolarization or depolarization at the insertion of the strand. The greatest ΔVm produced by an EFS applied during the early plateau phase of the action potential was consistently found at the 2 shoulders of the bifurcation. The action potential...
amplitude (APA) amounted to $-137 \pm 41\%$ (corresponding to $-137 \text{ mV}$ for an APA of $100 \text{ mV}$) if the bifurcation stem faced the anode, and $52 \pm 28\%$ if the stem faced the cathode (normalized to $7 \text{ V/cm}$ of electrical field strength).

The geometry of a bifurcation can be considered a combination of 2 geometries: (1) a bifurcation point, where the stem emerges into a short segment directed perpendicular to the electrical field, and (2) 2 short segments perpendicular to the electrical field after the bifurcation, which form a turn or a bend and are subsequently connected to 2 strands running in parallel to the electrical field. To separate the effects of these different geometric factors, experiments were carried out to evaluate the effect of strand insertion (abrupt geometrical expansion) alone and the effect of strand bending.

Effects of EFS at Abrupt Tissue Expansions
Tissue structures with features similar to abrupt expansions occur at Purkinje-fiber muscle junctions and in midmural ventricular layers. The corresponding isopotential maps together with the superimposed isochronal maps are illustrated in Figure 3 for 2 expansions with a strand width of 40 $\mu\text{m}$ (A and B) or of 270 $\mu\text{m}$ (C and D). EFS at a field strength of 9 to 11 $\text{V/cm}$ were applied in parallel to the direction of each strand 20 ms after the pacing stimulus. In A and C, the beginning of the cell strand was directed toward the anode, and in B and D, it was toward the cathode. In all panels, the isochronal maps show local slowing and curving of propagation at the transition from the strand to the large area due to local current-to-load mismatch. The regular shape of the isochronal lines indicates the absence of major localized inhomogeneities in gap junction coupling.

The colored isopotential maps show marked hyperpolarization at the border delimiting the large area, beginning at both sides lateral to the insertion of the strand, if the strand faced the anode, and marked depolarization if the strand was directed toward the cathode. Similar to observations made...

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**Figure 2.** Effect of EFS on bifurcating strands. A, Phase contrast image of bifurcation (magnification, $\times 20$). Width of each strand is 100 $\mu\text{m}$. B, Shaded background illustrates bifurcation (magnification, $\times 10$), and superimposed grid denotes position of photodiode array. Area of each square is $60 \times 60 \mu\text{m}^2$. Isochronal map (isochron interval, 500 $\mu\text{s}$) shows action potential propagation from main stem into 2 bifurcating branches. C, Isopotential map of $\Delta V_m/\text{APA}$ (in %) induced by EFS with field strength of 7 $\text{V/cm}$ applied parallel to strand (see arrow). Background shading outlines bifurcation. Each color represents isopotential step of 10%. + indicates anode; − indicates cathode. EFS were applied 20 ms after the pacing stimulus. D, Isopotential map induced by EFS of opposite polarity (field strength, 7 $\text{V/cm}$). Format corresponds to that shown in C.
previously in linear strands, hyperpolarization exceeded depolarization when EFS were applied during the plateau phase of the action potential.\textsuperscript{8} In addition to the secondary sources observed at either side of the strand insertion, distinct sources of opposite polarity appeared in the main strand (60 to 100 \(\mu\)m from the strand insertion). The maxima of these sources were located within the recording field in 50\% of the experiments. Sources of opposite polarity (Figures 3C and 3D) were also observed in the large tissue area.

At the site of the insertion into the strand, \(\Delta V_m\) was minimal. Figure 4 shows the dependency of the relative change in the strength of the shock-induced secondary source along the border on the width of the inserting strand. As shown previously, a large source results if a tissue border is oriented perpendicular to the direction of the electrical field,\textsuperscript{8} whereas no secondary source is expected to occur in a long strand directed parallel to the electrical field if the observation site is located further than \(\approx 3\) space constants or \(> 1\) mm from the end of the strand. The geometries illustrated in Figure 3 represent a combination of these 2 shapes and orientations. Increasing strand width created an increasing dip between the sources at the lateral borders because increasing the width of the strand increased the electrotonic separation of the perpendicular boundaries at either side of the insertion. Figure 3 also offers an explanation for the \(\Delta V_m\) observed in the strand. In all experiments, the “dip” in source strength was maximal at the site of the insertion of the strand (corresponding to the minimum shown in Figure 4). From this location, \(V_m\) gradually changed in the opposite direction, toward both the strand and the large tissue area. These sources are likely to be caused by the loops of local electrotonic current created by the gradients between the secondary sources located at the tissue border (see Discussion).
The excitatory effect of the EFS was tested by applying the shocks after the repolarization of the preceding action potential. In Figure 5, the excitatory effect of the EFS was compared with normal propagation in an abrupt tissue expansion with a strand width of 40 μm. The small strand width was selected to produce marked current-to-load mismatch and unidirectional propagation block\textsuperscript{12} at the insertion into the large area (Figure 5B). In contrast, the EFS applied with a field directed in parallel to the strand produced, in accordance with the potential distribution shown in Figure 3, a secondary source, with depolarization eliciting new propagated action potentials from the 2 sources located beyond and lateral to the insertion point of the strand.

**Effects of EFS Nonlinear, Bending Strands**

To further evaluate the effects of EFS on ΔV\textsubscript{m} in branching strands, neonatal rat myocytes were grown in a pattern that consisted of one strand segment oriented in parallel and the other oriented perpendicular to the electrical field. The joint between the 2 parts consisted of a 90° bend or shoulder (Figure 6B). Figures 6C and 6D illustrate the distribution of ΔV\textsubscript{m} generated by an EFS of 7 V/cm at the 90° transition between the linear strand parts. If the outer border, which was perpendicular to the field, faced the cathode (Figure 6C), depolarization dominated in the whole region of the bend. A large hyperpolarization prevailed if this same site faced the anode (Figure 6D).

Two features of source distribution were consistently observed. First, hyperpolarization or depolarization, according to field direction, was maximal in the bend and extended toward the strand part that was oriented perpendicular to the field. No source of opposite polarity was observed in the perpendicular segment at the inner border of the strand within the imaged field. This differs from purely linear strands directed perpendicular to an electrical field in which a polarity change is a consistent finding.\textsuperscript{8} In 6 experiments, the EFS (field strength, 8.5±0.9 V/cm) applied during the early plateau phase of the action potential produced a maximal ΔV\textsubscript{m} of −103±40%APA if the parallel segment faced the anode and 59±8%APA if the parallel segment faced the cathode. In 3 experiments, a depolarizing EFS applied 500 ms after the previous pacing stimulus generated a new excitation wave, which originated at the site of maximal depolarization at the bend itself and propagated into the 2 limbs.

**Discussion**

In this study, we showed that EFS create secondary and tertiary membrane potential sources at nonlinear tissue struc-
atures (bifurcating cell strands, abrupt expansions, and bending strands). These sources differ from the $\Delta V_m$ observed in simple, linear structures. One important difference relates to the observation that the magnitude of a secondary source created at a strand border depends on its orientation in the field created by the EFS. A nonlinear border (e.g., a bend) can be viewed as a connection of 2 linear segments. Consequently, at the connection site, 2 sources of different magnitude are expected to interact electrotonically, and the larger source is expected to dominate over the smaller. A semiquantitative estimation of this interaction can be derived from linear cable theory. If an EFS of a strength of $E$ is applied in parallel to a linear tissue segment of length $D$ and a nonvariable length constant $\lambda$, then $\Delta V_m$ along the distance $(d)$ from one segment end to the other is given by the following equation:

$$\Delta V_m(d) = E \times \lambda \times \frac{\sinh(d/\lambda)}{\cosh(D/2\lambda)}$$

where $d=0$ corresponds to the middle of the segment, and $-d$ and $d$ correspond to the ends of the segment. The graphic presentation of this equation in Figure 7 provides an explanation of the effects of the nonlinear boundaries presented in Results. Figure 7 shows that the $\Delta V_m$ (envelope curve), which is always present at the segment borders, starts to decrease and the voltage profile becomes linear if the length $(D)$ decreases beyond $\approx 2\lambda$. This decrease of $\Delta V_m$ and the change in shape of the profiles is due to the mutual interaction of sources of opposite polarity as length $(D)$ decreases.

The quantitative extrapolation of the source computation in Figure 7 to our results is limited by the fact that the asymmetry between hyperpolarization and depolarization is not considered (Figures 2 and 3). Qualitatively, however, Figure 6 predicts that rotating a strand segment of length $L$ and width $W$ in an electrical field will change the dominating boundaries. If the segment is aligned in parallel to the field, $L$ corresponds to $D$ in the equation, and the segment ends are the dominating boundaries. After rotating by $90^\circ$, $W$ will correspond to $D$, and the lateral boundaries will dominate. Therefore, with a strand of $W=80$ $\mu$m $\ll \lambda$, $\Delta V_m$ will decrease with rotation. Connecting 2 segments of different
orientations (eg, bends; Figures 5 and 6), will lead to an electrotonic interaction between the secondary sources formed by these 2 individual parts, and the larger source formed by the component oriented in parallel to the field will electrotonically dominate. This explains the observations illustrated in Figures 2 and 5: no source of opposite polarity is present in the segment oriented perpendicular to the strand. This observation is consistently made in linear strands.8

Sources in nonlinear structures were also observed at sites remote from the borders (Figure 3, asterisks). These tertiary sources were likely caused by the circumscribed secondary sources located at the tissue borders. The gradients in Vm between the localized secondary sources at either side of the strand insertion would be expected to induce loops of electrotonic current flow. Consequently, changes in Vm will be observed at the sites where these currents cross the cell membranes. Several arguments favor this hypothesis. (1) A tertiary source caused by the electrotonic current flow from or to the secondary sources located lateral to the strand insertion is expected to be of opposite polarity. This was a consistent observation in our experiments. (2) Impulse propagation at the location of these tertiary sources (main tissue strand) was continuous. Therefore, source generation by a locally inhomogeneous expression of gap junction could be excluded as an alternative explanation. Moreover, such resistive barriers would have produced a source of the same polarity as the secondary source. (3) A source created by electrotonic interaction with a remote tissue discontinuity was unlikely because no discontinuity was present within a distance of >2 mm (Figure 1B).

The present study confirms previous observations that EFS applied during diastole induce symmetrical changes in Vm, whereas shocks applied during the plateau phase of the action potential induce large hyperpolarizations and relatively smaller depolarizations.5,8 Although shocks falling into the plateau phase are unlikely to elicit propagating waves, they can induce hyperpolarization, reactivation of the sodium current, and new action potentials, which locally prolong the refractory state.3,4,16 Therefore, they may exert an important defibrillatory effect. Further studies will be necessary to elucidate the exact nature of ΔVm at the level of membrane channels and the consequence for electrotonic interaction in discontinuous tissue structures.

The interference of cardiac structure with EFS that produce changes in Vm remote from the shock electrodes may either prolong the action potential or create new excitation waves. In isolated, single cardiac cells, such sources of opposite polarity are created at the cell poles.17 In well-coupled cardiac cellular networks, no secondary sources are observed at cell borders, but the sources are mainly determined by the more macroscopic tissue boundaries.8 Large secondary sources with the ability to create new excitation waves are only formed if the resistive (“gap junction-free”) separation of cells is >100 to 200 μm.10 It is, therefore, unlikely that the cell borders in adult hearts in vivo will create significant secondary sources unless an extreme degree of anisotropy is reached. Nonlinear, trabeculated, and bifurcating structures are found physiologically in the atria and in tissue surviving from myocardial infarction.13,14 The present results suggest that such structures can produce secondary and tertiary sources and that these structures may form predictive sites for the exertion of a defibrillatory effect. Furthermore, structural remodeling associated with myocardial fibrosis in other diseases may significantly enhance the density of secondary and tertiary sources in both the atrium and ventricle.

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