Optical Mapping of Arrhythmias Induced by Strong Electrical Shocks in Myocyte Cultures

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Abstract—Strong electrical shocks can induce arrhythmias, which might explain why shocks fail to defibrillate. In this work, the localization of arrhythmia source and the relationship with local changes of transmembrane potential ($V_m$) were determined in geometrically defined cell cultures using optical mapping technique. Uniform-field shocks with strength (E) of 10 to 50 V/cm were applied across cell strands with width of 0.2 and 0.8 mm. The threshold for arrhythmia induction was dependent on the strand width: in the 0.8- and 0.2-mm strands, arrhythmias were induced at $E \geq 20.6 \pm 1.8$ V/cm (n = 8) and $E \geq 30.3 \pm 1.8$ V/cm (n = 8), respectively. At the same shock strength, the arrhythmia rate and duration were larger in the wider strands. During shocks that induced arrhythmias, the $V_m$ waveforms on the anodal side revealed a positive $V_m$ shift that followed the initial large hyperpolarization and postshock elevation of the diastolic $V_m$. These $V_m$ changes were absent during failed shocks. To determine the localization of the arrhythmia source, arrhythmias were induced in narrow cell strands containing regions of local expansion. Optical mapping of the first extrabeat with a coupling interval of 315 ± 60 ms revealed that in the majority of cases (9 out of 13) the source of arrhythmias was localized in the areas of shock-induced hyperpolarization. Thus, (1) induction of postshock arrhythmias, their rate, and their duration strongly depend on the tissue structure; (2) arrhythmia induction coincides with the appearance of a positive $V_m$ shift in the areas of hyperpolarization; and (3) the source of postshock arrhythmias is located in the areas of shock-induced hyperpolarization. (Circ Res. 2002;90:664-670.)

Key Words: defibrillation □ imaging □ fluorescent dyes □ RH-237

The dependence of defibrillation success on the shock strength follows a bell-shaped curve whereby the likelihood of defibrillation first increases and then decreases with increasing shock strength.¹ The failure of defibrillation at higher shock intensities may be related to induction of arrhythmias.²-⁴ Such postshock arrhythmias were demonstrated and studied in dog,⁵-⁷ pig,⁸ and rabbit hearts,⁹ as well as in isolated cells and cell cultures,¹⁰-¹⁴ but important properties of these arrhythmias, such as localization of the arrhythmia source and relationship to shock-induced transmembrane potential ($V_m$) changes ($\Delta V_m$), remain unknown. Recent studies utilizing the optical mapping technique revealed that the effects of shocks on $V_m$ in the heart are highly nonuniform¹⁵-¹⁷ and strongly dependent on the tissue structure.¹⁸-²¹ Shocks produce areas of both positive and negative $V_m$ changes in different parts of the heart. Because the effects of shocks on myocardium are due to shock-induced $V_m$ changes, it can be suggested that initiation of postshock arrhythmias is also dependent on the tissue structure. From this, 2 questions follow: (1) what is the localization of the arrhythmia source in multicellular cardiac tissue? And, (2) what type of shock-induced $\Delta V_m$ cause arrhythmias? If initiation of postshock arrhythmias is restricted to certain areas of the heart and associated with a specific type of $\Delta V_m$, this might help to design defibrillation electrodes in such a way as to minimize detrimental effects of shocks. To address these questions, postshock arrhythmias were investigated in cultured cell monolayers with defined geometry produced using the technique of patterned cell growth.²² The spatio-temporal changes of $V_m$ during and after strong defibrillation shocks were measured using a voltage-sensitive dye and an optical mapping technique.²⁰,²³

Materials and Methods

Cell Cultures

Cell cultures with defined geometry were produced using patterned growth substrates as described previously.²⁰,²¹ The growth pattern consisted of cell strands attached to a rectangular-shaped cell region. Two types of strands were grown: (1) linear cell strands with the width of 0.2 and 0.8 mm and (2) narrow strands with a width of 0.2 mm containing a square area of expansion with a width of 0.8 or 1 mm (Figure 1). Dissociated cells were obtained from hearts of 2-day-old neonatal Wistar rats (Harlan, Chicago, Ill). The care and handling of animals were conducted in accordance with the guidelines of the AHA. The dispersed cells were suspended in medium UltraCulture (BioWhittaker) supplemented with 2 μg/mL vitamin B12, 0.1 mmol/L bromodeoxyuridine, and antibiotics. The cultures were incubated at 37°C in a humidified atmosphere containing 4% CO₂. The medium was exchanged on the next day and every second day after that. In order to stimulate cell beating, on the first 2 medium
The area of cell growth is shown in black and the area with no cell attachment in white. A spatially uniform electrical field (E) was produced in the bath using 2 platinum plate electrodes (anode “+” and cathode “−”). Two types of cell monolayers were grown: the first (A), consisted of linear cell strands with the width of 0.2 and 0.8 mm; the second (B), consisted of narrow strands (width=0.2 mm) containing an area of local expansion (width=0.8 mm).

Optical Measurements of \( V_m \)

Measurements were performed between the fourth and the sixth day in culture. Cells were transferred into an experimental chamber and stained for 5 minutes with 2.5 \( \mu \)mol/L of voltage-sensitive dye RH-237 (Molecular Probes). The dye fluorescence changes were measured using a 16×16 photodiode array (Hamamatsu) and a microscopic mapping system described previously. With 10× and 20× objectives used in this study, the spatial resolution (center-to-center interdiode distance) was 110 and 55 \( \mu \)m, respectively, and the sampling rate was 10 kHz/ch. To limit dye bleaching and phototoxicity, the duration of optical recordings was limited to 500 ms. To monitor the response of cell cultures to shocks over a longer period of time, cell motion was recorded in transmitted light for a period of 5 seconds.

The cells were paced at an interval of 500 ms with stimuli 1.2 times stronger than the diastolic threshold. Rectangular uniform-field shocks with duration of 10 ms and strength (E) of \( \sim10, 20, 30, 40, \) and 50 V/cm were applied across cell strands during the early plateau phase of action potential (AP) via 2 large platinum electrodes. The field strength was measured in the bath using a bipolar silver electrode (wire diameter=0.1 mm, interelectrode distance=1.1 mm). Delivery of shocks was synchronized with stimulation pulses so that the delay between AP upstroke and the shock onset was 10 to 20 ms. Between shocks, 3 minutes were allowed for cell recovery. Signals were normalized by the action potential amplitude (APA). A shock-induced \( \Delta V_m \) was measured as the difference between a linear fit of the plateau phase and the magnitude of the shock response \( 5 \) ms after the shock onset. Activation times were determined at the 50%-level of AP upstroke.

To determine whether shocks affected cell morphology, phase-contrast images of cells were taken at 50× magnification before and after shocks using a CCD videocamera and a frame-grabber. To test whether strong shocks induced membrane electroporation, \( ^{10,24} \) cell uptake of fluorescent dye Lucifer Yellow (Sigma) was investigated. The dye was applied at a concentration of 2.5 \( \mu \)mol/L for 2 minutes, then it was washed away, and fluorescent images (excitation filter 480/40 nm, emission filter 535/50 nm) were taken at 10× or 50× magnification. In each preparation, dye uptake was measured 4 times: (1) without shocks; (2) after applying a series of three \( \sim10-V/cm \) shocks with interval of 30 seconds during the first minute of dye exposure; (3) after application of a series of three \( \sim50-V/cm \) shocks; and (4) without shocks again.

Data were expressed as mean±SD. Differences were compared using the 2-tailed paired \( t \) test. All statistical probability values are expressed for differences from control conditions. Results were considered statistically significant if \( P<0.05 \).

Results

Dependence of Arrhythmia Induction on the Strand Width

Figure 2 illustrates induction of arrhythmias by electrical shocks in 2 linear cell strands with width of 0.2 and 0.8 mm (Figure 2A). Both strands were paced simultaneously by an electrode located between the strands above the measuring area. Uniform-field shocks were applied across the strands 20 ms after a paced beat, and the occurrence of postshock arrhythmias was assessed using measurements of cell motion during a 5-second interval. As shown in Figure 2B, a shock with a strength of 10 V/cm failed to induce arrhythmias in both narrow and wide strands. A 22-V/cm shock induced 1 extrabeat with a cycle length (CL) of 362 ms in the wide strand but no arrhythmia in the narrow strand. A 32-V/cm shock induced a short run of fast arrhythmia (duration=2.2 seconds, average CL=224 ms) in the wide strand and 1...
extrabeat (CL=390 ms) in the narrow strand. Further increase of the shock strength to 43 and 54 V/cm increased the arrhythmia rate and duration in both strands. With all shocks, the arrhythmia rate was faster and the duration was longer in the wide strand than in the narrow strand.

Similar findings were obtained in a total of 8 pairs of narrow and wide strands. Shocks with E=9.3±1.2 V/cm failed to induce arrhythmias in strands of both types. In the 0.8-mm strands, arrhythmias were induced by shocks with E≥20.6±1.8 V/cm. In the 0.2-mm strands, arrhythmias were induced at E≥30.3±1.8 V/cm. The duration and the average cycle length of arrhythmias measured within the 5-second interval (Figure 2C) increased progressively with increasing the shock strength. Both parameters were significantly larger in the wide strands in comparison to the narrow strands. Repetitive shocks of the same strength produced arrhythmias of similar duration and rate. Thus, when shocks with a strength of 30.6±1.0 V/cm were applied twice in 5 strand pairs, the first shocks produced arrhythmias with durations of 1.16±0.5 and 1.96±0.4 seconds in 0.2-mm and 0.8-mm strands, respectively, and the second shocks produced arrhythmias with durations of 1.47±0.3 (NS) and 2.12±0.3 seconds (NS), respectively.

Arrhythmia Induction and Shock-Induced \( V_m \) Changes

To determine the roles of \( V_m \) changes in the generation of postshock arrhythmias, shock-induced \( \Delta V_m \)s were measured during shocks that failed or succeeded to induce arrhythmias in both 0.2- and 0.8-mm wide cell strands.

Figure 3 illustrates the effects of 2 shocks in an 0.2-mm strand. Activation spread along the strand was smooth with an average conduction velocity of 38 cm/s (not shown). Shocks with a strength of 21 and 32 V/cm that either failed or induced an arrhythmia were applied approximately 16 ms after AP upstrokes. Figure 3A shows an isopotential map of \( \Delta V_m \) distribution induced by the 21-V/cm shock. Figure 3B compares the initial portions of corresponding action potentials from selected photodiodes and Figures 3C and 3D display whole \( V_m \) traces.

As shown in Figure 3A, the 21-V/cm shock induced positive \( \Delta V_m \)s at the cathodal side of the strand and negative \( \Delta V_m \)s at the anodal side. The \( \Delta V_m \) magnitudes were maximal at the strand edges, and there was a gradual transition between the edges. The \( \Delta V_m \) distribution was uniform and strongly asymmetric: maximal \( \Delta V_m \) was approximately twice as large as maximal \( \Delta V_m \) and \( \Delta V_m \) occupied a much larger area than \( \Delta V_m \). During the shock (Figure 3B, thin traces), the \( \Delta V_m \) waveforms (traces 1 to 3) were monotonically increasing. \( V_m \) first decreased and then reached a plateau after approximately 5 ms. The \( \Delta V_m \) waveform (trace 5) was nearly monotonically increasing but it reached a plateau faster, within 1 ms after the shock onset. At the intermediate location (trace 4), the shock induced first a positive deflection that was later followed by a return to the baseline. Such \( \Delta V_m \)s were previously described as asymmetric \( \Delta V_m \)s of type II.\footnote{Figure 3C compares \( V_m \) recordings obtained during a 500-ms interval without a shock (gray traces) and with the 21-V/cm shock (black traces). The repolarization and diastolic phases of the action potentials were distorted by the motion artifact and photobleaching, which made it difficult to estimate the effect of shocks on diastolic \( V_m \) from a single recording. However, the direct comparison of control and shock recordings indicated that the shock did affect the diastolic \( V_m \).

The \( \Delta V_m \) waveforms produced by the 32-V/cm shock that induced an arrhythmia (Figure 3B, thick traces) exhibited a major difference from the failed shock. In this case, the \( \Delta V_m \) traces were non-monotonic: the initial large hyperpolarization (traces 1 to 3) was followed by a return of \( V_m \) to more positive levels. The magnitude of this shift at the anodal strand edge calculated as the difference between the end-shock and the peak \( \Delta V_m \) was \( \sim \)109% APA. As a result, \( \Delta V_m \) at the end of the shock became smaller than the one induced by the weaker, 21-V/cm shock. Positive \( V_m \) shift at sites of \( \Delta V_m \) was paralleled by a similar shift at the site of \( \Delta V_m \) (trace 5), resulting in non-monotonic \( \Delta V_m \) waveforms as well. Such non-monotonic \( \Delta V_m \)s were previously described as nonlinear \( \Delta V_m \)s of type III.\footnote{Figure 3D indicates that the shock caused elevation of the diastolic \( V_m \) by \( \sim \)38% APA (versus \( \sim \)3.4% APA for 21-V/cm shock). The spatial distribution of \( \Delta V_m \) in the middle of the shock pulse (not shown) was qualitatively similar to the one observed during the weaker shock (Figure 3A).

In the wide strands, the \( \Delta V_m \)s during the shocks that failed or induced arrhythmias reproduced the essential features of \( V_m \) responses observed in the narrow strands. Figure 4 illustrates the effects of 2 shocks with strength of 12 V/cm
Similar results were obtained in a total of six 0.2-mm strands and six 0.8-mm strands. In the 0.2-mm strands, positive $V_m$ shift and diastolic elevation were negligible at 22±1.3-V/cm shocks (6.3±7% APA and 4.4±12% APA, respectively) and present at 32±2.8-V/cm shocks (82.4±25% APA and 46.5±12% APA [both $P<0.001$], respectively). In the 0.8-mm strands, these values were negligible at 10.9±1.6-V/cm shocks (2.7±5% APA and 4.3±7% APA, respectively) and present at 20±1.5-V/cm shocks (63.5±20% APA and 25.6±9% APA, respectively). The shock strengths for the occurrence of positive $V_m$ shift and diastolic elevation correspond to the thresholds for arrhythmia induction in respective cell strands (Figure 2). Shocks caused no long-term changes in conduction velocity. In wide strands, velocity was 25.3±1.7 cm/s before shocks and 25.2±1.9 cm/s (n=6, NS) 3 minutes after 39.2±1.8-V/cm shocks, respectively, indicating that shocks produced no long-lasting changes in cell excitability.

**Localization of the Arrhythmia Source**

The finding that the occurrence of arrhythmias in wide strands was paralleled with the specific changes in the negative, but not in the positive shock-induced $\Delta V_m$ waveforms (Figure 4), suggests that the arrhythmias were related to shock-induced hyperpolarization. If this is the case, then arrhythmias in the wide strands should originate from areas that are negatively polarized by the shocks. It was difficult to examine this hypothesis in the long linear cell strands, where the source of the arrhythmia could be located anywhere outside of the mapping area. However, because the threshold for arrhythmia induction was dependent on the strand width (Figure 2), it should be possible to restrict the localization of the arrhythmia source in strands with nonuniform width. To accomplish that, experiments were carried out in narrow cell strands containing local expansions that are shown in Figure 1B. Similar to the linear cell strands, application of strong shocks induced arrhythmias with rate and duration progressively increasing with increased shock strength. In order to avoid the influence of contraction artifact on the pattern of activation spread, the localization of the arrhythmia source was determined only during extrabeats with relatively long coupling intervals induced by the weakest shocks. The source of such arrhythmias was located within the mapping area in 13 strands. Figure 5 illustrates initiation of an arrhythmia in a strand containing an area of local expansion (Figure 5A). Similar to linear cell strands, a shock with a strength of 35 V/cm induced $\Delta V_m$ of type III, with a prominent positive shift following the initial negative polarization at the anodal side of the strand (Figure 5C, traces 1 and 2). The isochronal map of activation spread during the extrabeat (Figure 5F) revealed that the site of the earliest activation was located at the left side of the strand, which was hyperpolarized by the shock (Figures 5C and 5E). This was also apparent from close inspection of the $V_m$ traces during the extrabeat (Figure 5D). This pattern of arrhythmia initiation was observed in the majority of cases (9 out 13). In 3 cases, the source was distributed over the whole mapping area, and in one case, it was located in the depolarized area. In 4 out of 9 cases, where the source of arrhythmia was in the hyperpolarized area,
repetitive shocks of the same strength were applied. In all cases, the source of arrhythmia induced by the second shock remained at the same location.

It has been previously suggested that postshock arrhythmias are caused by membrane electroporation. To examine whether strong shocks produced electroporation in cell strands, cell uptake of dye Lucifer Yellow (LY) was investigated in 4 monolayers containing strands with local expansions. The degree of dye uptake was characterized by the level of fluorescence intensity (F) averaged over the expansion area at 10x magnification. In control conditions (no shocks given), application of LY for 2 minutes resulted in a low-level staining (F=26.7±5.5 AU, n=4). Next exposure to LY and to a series of 3 shocks with a strength of 10.5±0.2 V/cm insignificantly increased fluorescence (F=29.7±4.8 AU, NS), which can be attributed to further non-shock-related cell staining. A similar insignificant increase in fluorescence was observed after application of 53±4.1-V/cm shocks (F=32.5±4.5 AU, NS in comparison to 10-V/cm shocks), as well as after the follow-up LY exposure without shocks (F=33.2±4.9 AU, NS). In all cases, shocks did not produce spatial differences in fluorescence intensity between the cathodal and anodal sides of the strands (not shown). Also, no significant changes in fluorescence intensity following shocks were observed when images were taken at a 50x magnification. Even the strongest shocks caused no changes in cell morphology. This was examined in 6 monolayers by comparing phase-contrast images of cell strands at 50x magnification taken before and immediately after shocks with a strength of 48.1±3.2 V/cm.

**Discussion**

This study presents first spatially-resolved optical mapping of arrhythmias induced by strong electrical shocks. The most important findings of this work are that (1) the shock strength necessary for arrhythmia induction in cell strands, as well as arrhythmia rate and duration, depend on the strand width; (2) arrhythmia induction is paralleled by the appearance of a positive shift of Vm during shocks in the areas of negative Vm and with postshock elevation of the diastolic level of Vm, and (3) the source of arrhythmias with relatively long cycle lengths is localized in the areas of shock-induced hyperpolarization.

**Role of Tissue Structure in Shock-Induced Arrhythmias**

Arrhythmias induced by strong defibrillation shocks that can account for defibrillation failure in patients have been studied in whole hearts as well as in cultured cell monolayers. The occurrence of these arrhythmias has been linked to the high extracellular potential gradients created by the shocks. This concept, however, is incomplete because it does not take into account the structural aspects of the shock effects on cardiac tissue. Recent optical mapping studies have revealed that the effects of shocks on Vm in the heart are nonuniform and strongly dependent on the tissue geometry. The results of the present study indicate that initiation of postshock arrhythmias is also critically influenced by the tissue structure. Thus, the field threshold for arrhythmia induction as well as the arrhythmia rate and duration were dependent on the strand width (Figure 2). These findings correspond to the relationship between the strand width and the magnitude of shock-induced ΔVm demonstrated previously, and they emphasize the important role of the tissue structure in the effects of defibrillation shocks in the heart.

**Relationship Between Shock-Induced ΔVm and Postshock Arrhythmias**

Shock-induced Vm changes in cardiac tissue have been a subject of multiple theoretical and experimental studies. It has been previously shown and confirmed in the present study that, contrary to the classical cable model, application of
shocks during the plateau phase of action potential produces nonlinear $V_m$ changes of 2 different types. Shocks of a moderate strength induced $\Delta V_{m}$ that were characterized by asymmetric $\Delta V_{m}$ distribution, with $\Delta V_{m}$ being much larger than $\Delta V_{m}$, and simple monotonic shapes. This type of $V_m$ response (type II) was observed in different cardiac preparations including isolated guinea pig papillary muscles,10,31 cultured strands of neonatal rat myocytes,18 and isolated guinea pig myocytes. A recent study in cell cultures indicated that the negative bias in the $V_m$ response is due to an outward current flow in the areas depolarized by the shocks.20 A follow-up study provided evidence that this outward current involves L-type calcium channels.31 The other non-linear $\Delta V_{m}$ type (type III) was observed at very strong shocks. These $\Delta V_{m}$ waveforms are also asymmetric but contain a prominent time-dependent positive shift that reduced the degree of $\Delta V_{m}$ asymmetry. It was shown that this positive $V_m$ shift is due to an inward membrane current flowing in the areas of shock-induced negative $\Delta V_{m}$ indicating that it is a hyperpolarization-induced current. The nonlinear cell responses to defibrillation shocks described above are important because they modulate the magnitudes and the pattern of postshock $V_m$ distribution and, therefore, the outcome of a defibrillation attempt. They become even more important in light of the present study showing that generation of post-shock arrhythmias is paralleled by a transition between 2 types of nonlinear $\Delta V_{m}$.s. Correlation between the occurrence of type III $\Delta V_{m}$ and postshock arrhythmias indicates that these 2 phenomena are related. A possible explanation for this relationship is that these phenomena are due to the same inward current that is induced by strong shocks in the areas of strong hyperpolarization and persists after the shocks.

The nature of this inward current is not presently clear. A possible explanation is that it is caused by membrane electroporation. The occurrence of arrhythmias and of type III $\Delta V_{m}$ response was paralleled by postshock elevation of the diastolic $V_m$. A similar elevation of diastolic $V_m$ in response to strong shocks was observed in previous studies.9,10,31,33–35 In some of these studies, diastolic $V_m$ elevation was accompanied by arrhythmias,9,10,34 whereas in other studies arrhythmias were not observed,33,35 which could be due to different experimental conditions (low temperature33 or use of a electromechanical uncoupler35). Although the occurrence of membrane electroporation was not established by independent methods in these studies, the postshock diastolic $V_m$ elevation was interpreted as a sign of electroporation. Similarly, it could be suggested that electroporation was responsible for postshock arrhythmias in our experiments as well. However, measurements of dye uptake after shocks with a strength of up to 50 V/cm did not confirm this hypothesis. These data are in agreement with a previous study that reported no significant dye uptake in cell cultures at such shock strength.52 It should be noted that these data do not exclude electroporation as the underlying mechanism for arrhythmias induced by shocks weaker than 50 V/cm. It is possible that electroporation induced by such shocks was relatively short-lived, and although it caused arrhythmias, it produced only a small dye uptake, which was not sufficient for detection in a single cell layer. However, it is also possible that some inward ionic current activated at large negative $V_m$ is involved in these events.

Another explanation for postshock arrhythmias can be related to shock-induced calcium overload14 caused by impairment of the SR calcium pump46 and/or short-lived membrane electroporation. Calcium overload can cause oscillations of the intracellular calcium concentration and triggered arrhythmias via activation of transient inward current,57 whose main component is either the electrogenic Na$^+$-Ca$^{2+}$ exchange current,38,39 calcium-activated nonselective cation current, or calcium-dependent chloride current.40 Elucidation of the nature of the inward current responsible for postshock arrhythmias requires further investigation.

**Localization of the Arrhythmia Source**

An important result of this study is that the postshock arrhythmias most often originated from the areas of shock-induced hyperpolarization. It corresponds to the finding that arrhythmia induction was paralleled with changes in the shock-induced negative $\Delta V_{m}$ waveforms. Together these results indicate that shock-induced hyperpolarization was the primary factor in the generation of arrhythmias. In this respect, there is a similarity between arrhythmias and the contractile injury of cells subjected to strong shocks, which according to experiments in isolated myocytes occurred first at the cell end facing the anode.41 To explain the inward asymmetry, it was proposed that shocks induced membrane breakdown at both cell ends but the increase in the intracellular calcium concentration and cell contracture occurred only at the anodal cell end due to electroosmosis of calcium ions. Since calcium overload is responsible for postshock arrhythmias, then the electroosmosis effect might play an important role in their induction. An alternative explanation is that membrane breakdown occurs asymmetrically, only at sites of shock-induced hyperpolarization, whereas at sites of depolarization the cell membrane is protected by strong outward current, which limits the magnitude of positive $\Delta V_{m}$ to below 100 mV20 and thus prevents $V_m$ from reaching levels necessary for electroporation.

The localization of the source of postshock arrhythmias in the whole heart is not presently known. If the finding that these arrhythmias originate from the areas of shock-induced hyperpolarization holds true for whole hearts, it can provide the rationale for designing shock electrodes in such a way as to minimize the exposure of myocardium to large negative $\Delta V_{m}$.s. This would allow increasing shock energy and defibrillation efficacy without concomitant proarrhythmic shock effects.

It should be noted that this finding is limited to relatively slow arrhythmias induced by weaker shocks when coupling intervals were long enough to avoid interference with cell contractions from the previous beats. It is possible that stronger shocks can render cells inexcitable at the anodal strand edge, and therefore, the arrhythmia source can be shifted to the area less affected by the shock. This possibility needs further investigation.

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References


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